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STUDIES ON THE EFFECT OF RESERPINE ON THE PEROXIDASE ACTIVITY OF SUBMAXILLARY GLAND

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Abstract—In an earlier report we have described how the administration of reserpine (0.5 mg/kg) stimulates the peroxidase activity of rat and mouse submaxillary gland [R. Chakraborty, B. Mukherjee and R. N. Hati, *Life Sci.* **35**, 1913 (1984)]. Further studies have been carried out in this area. This stimulation of the enzyme activity started from 0.02 mg reserpine/kg body wt. The effect of reserpine was found in all subcellular fractions of submaxillary gland. Iodinating activity of the enzyme preparation was also increased by reserpine treatment. Kinetic studies indicate that reserpine increased the V_{max} of the enzyme without altering K_m. Protein synthesis inhibitors inhibited the increase of the enzyme activity by reserpine. Dibutyryl c-AMP, theophylline or both drugs together did not alter the peroxidase activity. The effect of reserpine was also found in experiments using slices and single cell preparations of submaxillary gland, suggesting that the drug is probably acting directly on the acinar cells.

The physiological function of the submaxillary gland peroxidase is not fully understood except that the enzyme may be involved in extrathroidal thyroid hormone formation [1] as well as in the bacteriocidal action [2]. Though many studies have been made on this enzyme [3], the effect of biogenic amines has not been studied. Reserpine, which is an established amine-depleting drug [4–6], has been used while studying the effect of amines on submaxillary peroxidase activity. We have reported earlier that the peroxidase activity of mouse and rat submaxillary gland was elevated by the administration of reserpine [7]. Reserpine is known to alter several enzyme activities in different tissues and different mechanisms have been postulated for these effects of reserpine [8–11]. It is also known that reserpine increases the concentration of acetylcholine [12, 13]. Our study has indicated that the effect of reserpine on submaxillary peroxidase activity is not mediated by the depletion of amine or increase of acetylcholine [7]. Further studies have been carried out to find out the mechanism of the action of reserpine, results of which have been incorporated in this communication.

MATERIALS AND METHODS

Chemicals

Reserpine, puromycin, cycloheximide, dibutyryl c-AMP theophylline and glucose-6-phosphate were purchased from Sigma Chemical Co. Other chemicals used were of analytical grade. ^{131}I was obtained from Bhaba Atomic Research Centre, Trombay, Bombay.

Animals

Male Swiss mice (albino) weighing 20–25 g were used. The animals were kept on laboratory diet with *ad libitum* supply of tap water. Diet was removed after the injection of reserpine.

Preparation and assay of enzymes

(a) Peroxidase. The enzyme was prepared as mentioned earlier [7]. Animals were killed by cervical dislocation. A 5% homogenate of submaxillary gland was prepared in 0.25 M sucrose and the homogenate was centrifuged at 20,000 *g* for 30 min in a Sorvall refrigerated centrifuge. The supernatant was used as enzyme preparation. Assay and the unit of enzyme activity has been described previously [7].

Iodinating activity of the enzyme preparation was measured by the slightly modified procedure of Alexander and Corcoran [14] with the following reagents in 3.0 ml reaction mixture: 50 mM acetate buffer, pH 5.0, 0.2 mM tyrosine, 0.2 mM KI, 5–7 μCi ^{131}I, 0.25 mM H_2O_2 and a suitable volume of enzyme preparation. The reaction was initiated by the addition of H_2O_2. After 20 min, the reaction was stopped by the addition of 0.2 ml of 10^{-3} M $Na_2S_2O_3$. Then 0.6 ml of 50% TCA was added and the material was passed through a Dowex H^+ resin column (5 × 0.5 cm) to remove unreacted iodide. Organic iodine was retained on the column. The column was then washed thoroughly with distilled H_2O and its radioactivity was counted in a γ-ray spectrometer. Enzyme activity was expressed as nmol iodide incorporation/min.

(b) Succinic dehydrogenase. The enzyme was assayed by the method of Singer and Kearney [15] with slight modifications. The reaction mixture contained the following reagents in 3 ml: 10 mM sodium phosphate buffer, pH 7.4, 0.5 mg bovine serum albumin, 2 mM sodium cyanide, 10 mM sodium succinate, 1 mM EDTA, 10 mM 2,6-dichlorophenolindophenol and a suitable amount of enzyme preparation. Increase of optical density was measured at 600 nm in Pye–Unicam spectrophotometer. The enzyme activity was expressed as μmol of succinate oxidized/hr.

(c) Glucose-6-phosphatase. The enzyme was assayed by the method described by Swanson [16].

The incubation mixture consisted of the following components in 0.5 ml: 20 mM glucose-6-phosphate (sodium salt), 9 mM malate buffer, pH 6.5 and a suitable amount of the enzyme preparation. The mixture was incubated at 37° for 15 min and the reaction was stopped with 25% TCA. TCA supernatant was used for inorganic phosphate (P_i) assay by the method of Ames and Duben [17]. The enzyme activity was expressed as μmol P_i liberated/hr.

Subcellular fractions

A 10% homogenate of submaxillary gland was made in 0.25 M sucrose. The homogenate was centrifuged at 500 *g* for 10 min. The pellet was suspended with an equal volume of 0.25 M sucrose and centrifuged again at 500 *g* for 10 min. The pooled supernatant was centrifuged at 10,000 *g* for 20 min to obtain the mitochondrial fraction. The supernatant was centrifuged finally at 105,000 *g* for 1 hr to obtain the microsomal fraction and the supernatant was soluble. Mitochondrial and microsomal fractions were washed twice.

Protein was estimated by the method of Lowry *et al.* [18].

RESULTS

Figure 1 depicts the dose–response curve of the effect of reserpine on the peroxidase activity of mouse submaxillary gland. The stimulation started from 0.02 mg reserpine/kg body wt and increased up to 0.5 mg reserpine/kg body wt beyond which no appreciable increase was observed.

The effect of reserpine was studied in different sub-cellular fractions. It is seen from Table 1 that the effect of reserpine persisted in all the fractions.

Submaxillary gland can catalyse the iodination reaction [19]. Iodinating activity of mouse submaxillary gland was also stimulated by the administration of reserpine (Table 2).

Lineweaver–Burk plots (Fig. 2) describe that V_{max} of the enzyme of control animals for KI as substrate was 0.83×10^2 which was increased to 1.96×10^2 after reserpine treatment of the animals. Also, V_{max} of the enzyme prepared from control and reserpine-treated animals using H_2O_2 as substrate were 0.74×10^2 and 1.67×10^2 respectively. But, K_m of the enzyme of either group of animals was not significantly altered for any substrate (1.4 mM and 1.3 mM for KI, 0.16 mM and 0.17 mM for H_2O_2).

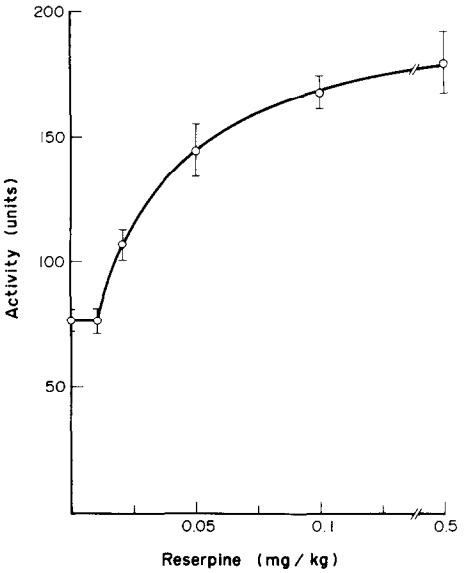

Fig. 1. Dose–response curve. Varying amounts of reserpine (as stated) have been used. Animals were killed at 22 hr after the injection of reserpine. Assay of the enzyme has been mentioned in Materials and Methods. Data were expressed as mean ± S.E. Statistical calculation was made from three different sets of experiments.

Table 3 demonstrates that cycloheximide and puromycin, two potent protein synthesis inhibitors, inhibited the reserpine-mediated increase of the enzyme activity. The treatment with these drugs did not alter the enzyme activity of normal animals to any significant extent at the same dose.

It is observed in Table 4 that treatment with dibutyryl-c-AMP, theophylline or both the drugs did not affect the peroxidase activity of mouse submaxillary gland.

Table 1. Effect of reserpine on the peroxidase activity in different subcellular fractions of mouse submaxillary gland

Fraction		Act/min/mg protein (mean ± S.E.)	% Stimulation	Succinic dehydrogenase* (% control value)	Glucose-6-phosphatase* (% control value)
Soluble supernatant	Normal	9.4 ± 1.0 (3)	—	13.0	11.8
	Reserpine	17.5 ± 0.9 (3)	86.2	—	—
Mitochondria	Normal	7.3 ± 1.4 (3)	—	72.4	12.8
	Reserpine	13.2 ± 1.1 (3)	80.8	—	—
Microsome	Normal	5.3 ± 1.3 (3)	—	13.0	73.0
	Reserpine	9.1 ± 1.8 (3)	71.7	—	—

Animals were killed at 22 hr after the injection of normal saline or reserpine (0.5 mg/kg s.c.). Preparation of subcellular fractions and assay of the enzymes were as mentioned in Materials and Methods. Total activities of marker enzymes in 500 *g* supernatant were used as 100% activity. Total activities of succinic dehydrogenase and glucose-6-phosphatase were 0.7 μmol succinate oxidized/hr and 45.6 μmol P_i liberated/hr respectively. Number in parentheses indicates number of determinants.

* Average value of two different experiments.

Table 2. Effect of reserpine on the iodinating activity of mouse submaxillary gland

Gr.	No. of determination	Activity/g tissue (mean ± S.E.)	% Stimulation	Act/mg protein (mean ± S.E.)	% Stimulation
Control	4	8.5 ± 1.3	—	0.62 ± 0.13	—
Reserpine	4	21.59 ± 2.8	154	1.3 ± 0.17	109.7

Animals were killed at 22 hr after the injection of normal saline or reserpine (0.5 mg/kg s.c.). Iodinating activity was measured as described in Materials and Methods.

Table 3. Effect of cycloheximide and puromycin on the reserpine-treated peroxidase activity of submaxillary gland

	Gr.	No. of determinations	Activity (units) mean ± S.E.	% Control value
1.	Normal	4	66.36 ± 12.6	100
2.	Reserpine	4	150.36 ± 18.6	226.6
3.	Reserpine + cycloheximide	4	75.12 ± 11.05	113
4.	Reserpine + puromycin	3	84 ± 9.64	126.6
5.	Cycloheximide	2	72.5*	109
6.	Puromycin	2	70.2*	106

Cycloheximide or puromycin at a dose of 50 mg/kg or 75 mg/kg respectively was injected (i.p.) twice—one 1 hr before and another 3 hr after the administration of reserpine. Animals were killed at 22 hr after the administration of reserpine (0.5 mg/kg). Enzyme was assayed as mentioned in Materials and Methods.

* Average value of two sets of experiments.

DISCUSSION

We have reported earlier that the administration of reserpine increases the peroxidase activity of mouse submaxillary gland [7]. It has been demonstrated from different laboratories that reserpine stimulates several enzyme activities in different tissues. Reserpine increases tyrosine hydroxylase activity of rat adrenal medulla [8] and this increase is due to *de novo* synthesis of the enzyme [9]. Black and Axelrod [10] have reported the increased synthesis of hepatic tyrosine transaminase by reserpine treatment [10]. They suggested that this increase of the enzyme was due to the depletion of norepinephrine content of the tissue elicited by reserpine. Reserpine also increases the monoamine oxidase activity of brain and liver [11]. Taylor *et al.* have reported an increased accumulation of glycoprotein

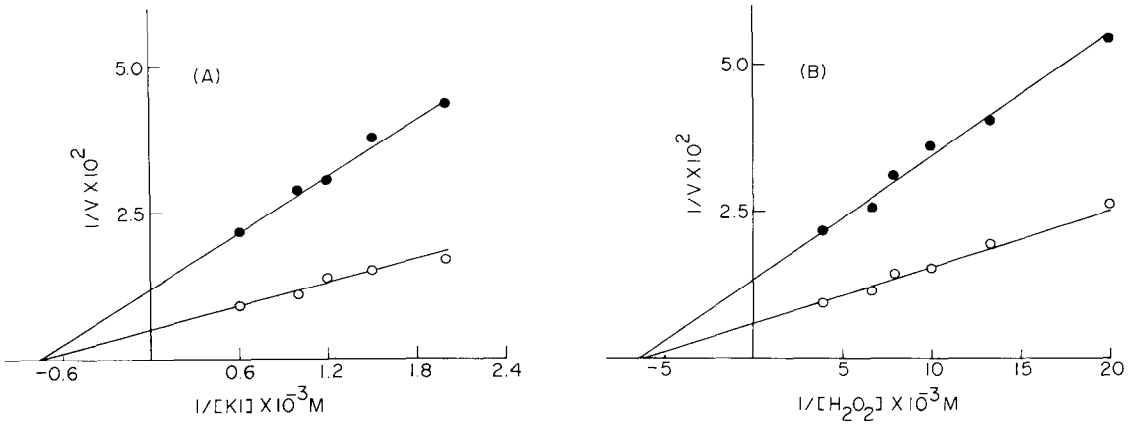

Fig. 2. Lineweaver–Burk plots. Kinetic characterizations were determined from Lineweaver–Burk plots. A—KI concentrations were varied from 0.33 to 1.66 mM with H_2O_2 concentration of 0.25 mM. B—H_2O_2 concentrations were varied from 0.05 to 0.25 mM with KI concentration of 1.66 mM. Animals were killed at 22 hr after the injection of reserpine (0.5 mg/kg s.c.). Velocity was expressed in units of enzyme activity. —●— Normal animals. —○— Reserpine-treated animals.

Table 4. Effect of dibutyryl c-AMP and theophylline on mouse submaxillary peroxidase

Gr.	No. of determinations	Activity (units) mean ± S.E.
Control	3	66.7 ± 4.4
Dibutyryl c-AMP	3	65.4 ± 1.9
Theophylline	3	66.5 ± 3.7
Theophylline + Dibutyryl c-AMP	3	64.1 ± 2.4
Reserpine	3	145.3 ± 15.05
Theophylline + Reserpine	3	142 ± 17.5

The animals were killed at 22 hr after the injection of dibutyryl c-AMP (5 mg/kg, i.p.) or reserpine (0.5 mg/kg, s.c.). Theophylline (50 mg/kg, i.p.) was injected 1 hr before dibutyryl c-AMP or reserpine.

by reserpine in rat submaxillary tissue [20]; they have shown that neither sympathetic nor parasympathetic denervation can alter the effect of reserpine. Our study has demonstrated that the effect of reserpine was neither due to the depletion of catecholamines nor due to the increase of acetylcholine [7].

The effect of reserpine on the peroxidase activity was found in all subcellular fractions of submaxillary gland (Table 1). The presence of peroxidase in different subcellular fractions of mouse submaxillary gland has been reported earlier [21]. Both mitochondria and microsomes were about 73% pure as evidenced by succinic dehydrogenase and glucose-6-phosphatase activities, the marker enzymes of mitochondria and microsomes respectively. It has been shown that some well characterized peroxidases can catalyse both iodide peroxidation and tyrosine iodination reactions [19, 22, 23]. We have reported that iodide peroxidation by submaxillary enzyme was stimulated by reserpine treatment [7]. As expected, the iodinating activity of the enzyme preparation was also increased by reserpine (Table 2). Kinetic studies reveal that reserpine treatment increased V_{max} of the enzyme for either substrate without having any significant effect on K_m (Fig. 2). This study indicates that the number of active sites of the enzyme was increased after reserpine treatment rather than an alteration of active sites of existing enzyme. The increase of the peroxidase activity by reserpine was abolished by treatment with cycloheximide and puromycin (Table 3). Kinetic studies and experiments with protein synthesis inhibitors suggest that the increase of the enzyme activity by reserpine may be due to *de novo* synthesis of the enzyme. A wide variety of hormones and neurotransmitters are known to function through the formation of c-AMP [24]. It has been reported that c-AMP is involved in the activation of tyrosine hydroxylase and ornithine decarboxylase of adrenal medulla by reserpine [25, 26]. But, in our system the effect of reserpine is not mediated, possibly through c-AMP formation, as the treatment with dibutyryl c-AMP, theophylline or both the drugs could not alter the peroxidase activity of mouse submaxillary gland (Table 4).

The effect of reserpine was found to be tissue-specific. Besides submaxillary tissue, some other tissues like stomach, parotid and thyroid are known to have strong peroxidase activities which were not altered by reserpine to any significant amount (data not shown).

Our study suggests that the stimulation of the peroxidase activity by reserpine was possibily due to the increased enzyme synthesis and this stimulation was not due to the increased formation of c-AMP.

Preliminary studies in our laboratory indicate that the effect of reserpine was also observed *in vitro* in slices and isolated cells of rat submaxillary gland. Further studies are being carried out using isolated cells of rat submaxillary gland to get more insights into the mechanism of the action of reserpine in our system.

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