Relationship Between Insulin and Growth Hormone in Growth and Development of Rat Submandibular Glands (33832)

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In previous studies, it was found that a normal secretion of insulin was required to maintain the normal growth and histological integrity of the salivary glands as well as the growth of the body in general in immature female rats (1). These defective changes of the salivary glands in the diabetics were prevented or restored by insulin replacement therapy. Since insulin synergizes the physiological action of growth hormone (GH) by increasing amino acid transport into the tissue and accelerating their incorporation into protein, the present studies attempted to determine whether or not the administered or exogenous GH would stimulate the growth and development of the submandibular gland in diabetic rats.

Materials and Methods. Expt. 1. Weanling female Sprague-Dawley strain rats[1] were deprived of food for 24 hr. They were then injected subcutaneously with 20 mg of alloxan /100 g of body wt to induce diabetes mellitus. Rats in an additional group were injected with distilled water alone to serve as intact controls. The successfulness of induction of diabetes mellitus was judged by the presence of sugar in the urine as tested by Tes-tape[2] about 24 hr after alloxan injection. The presence of diuresis and polydipsia were also useful criteria. Animals with doubtful diabetes were not used. All animals were kept under the same environmental conditions, fed with Purina chow and supplied with tap water *ad libitum*. The diabetics were divided randomly into 5 groups, one of which served as diabetic controls. Beginning on the second day following alloxan injection, diabetic rats in the test groups were given daily subcutaneous injections of 1 unit (U) of protamine zinc insulin (PZI)[3], 50 μg of GH[4], 100 μg of GH, or 1 U of PZI plus 50 μg of GH for 14 days. Original PZI suspension (40 USP units of insulin/ml) was diluted so that the daily required doses of PZI were suspended in 0.2 ml of the vehicle.[5] The daily required doses of GH were dissolved in 0.4 ml of distilled water at pH 6. Diabetics receiving PZI treatment also were given daily contralateral injections of 0.4 ml of distilled water and those receiving GH treatment also were injected contralaterally with 0.2 ml of insulin vehicle. Intact and diabetic controls were similarly treated with both insulin vehicle and distilled water.

Expt. 2. The second experiment was conducted later to further assure whether or not the porcine GH, which was used in Expt. 1, would stimulate submandibular gland growth in hypophysectomized (hypox) rats. Female Sprague-Dawley strain rats were hypox[6] on day 21. Beginning on the sixth postoperative day, hypox rats were divided randomly into 2 groups. Each group was subdivided equally and the rats were housed in two cages. All animals were fed and watered as suggested by the Hormone Assay Laboratory. Since the source of endogenous pituitary GH was eliminated, the hypox rats in the test groups

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1 Holtzman Co., Madison, Wisconsin.

2 Eli Lilly and Company, Indianapolis, Indiana.

3 Protamine zine and Iletin, Eli Lilly and Company, Indianapolis, Indiana.

4 Raben type, 0.5 IU/1.0 mg, Nutritional Biochemicals, Cleveland, Ohio.

5 Insulin vehicle consisted of 1.6% glycerin (w/v), 0.25% phenol (w/v), 0.2% dibasic sodium phosphate (w/v) and 97.5% distilled water.

6 Hypophysectomized by the Hormone Assay Laboratory, Chicago, Illinois.

TABLE I. Influence of Insulin and Growth Hormone on Rat Submandibular Gland Growth.

Treatment and (no. of rats)	Body wt gain (g)	Submandibular gland: Wt (mg)	Submandibular gland: Wt (mg/100 g of body wt)	Submandibular gland: Ductal diameter (μ)	Submandibular gland: Acinar cell counts[f]	Parotid wt (mg)	Parotid wt (mg/100 g of body wt)	Thyroid wt (mg)	Thyroid wt (mg/100 g of body wt)	Adrenal wt (mg)	Adrenal wt (mg/100 g of body wt)
Diabetic: vehicle (8)	9 ±2.4[g]	164.8 ±6.6	295.2 ±15.8	27.7 ±0.074	95.7 ±0.63	168.3 ±24.2	293.4 ±32.7	4.4 ±0.42	7.9 ±0.79	22.2 ±0.86	39.5 ±1.10
Intact: vehicle (13)	82 ±3.4[e,h]	298.9 ±6.1[e]	232.2 ±6.1[d]	30.1 ±0.087[e]	76.7 ±0.70[e]	257.2 ±12.2[d]	199.3 ±9.4[b]	8.0 ±0.80[e]	6.2 ±0.74 NS	32.5 ±0.85[e]	25.3 ±0.85[e]
Diabetic:											
1 U Insulin (6)	43 ±4.5[e]	201.1 ±7.0[d]	227.8 ±7.8[d]	29.2 ±0.033[e]	87.7 ±1.01[e]	215.5 ±23.0 NS	241.0 ±19.3 NS	6.3 ±0.66[a]	7.1 ±0.71 NS	27.3 ±1.80[a]	30.8 ±1.70[d]
50 μg GH[i] (11)	14 ±2.4 NS	154.1 ±7.7 NS	244.4 ±6.5[c]	24.9 ±0.038[e]	96.8 ±0.42NS	185.8 ±13.0 NS	287.0 ±15.8 NS	3.2 ±0.51 NS	5.0 ±0.69[b]	20.1 ±0.83 NS	32.5 ±1.90[c]
100 μg GH (9)	4 ±2.0 NS	135.7 ±6.0[c]	264.5 ±10.1 NS	24.5 ±0.217[e]	98.5 ±1.14[a]	145.9 ±14.2 NS	281.3 ±20.1 NS	3.3 ±0.26[a]	6.5 ±0.57 NS	19.8 ±1.10 NS	38.7 ±2.20 NS
1 U Insulin + 50 μg GH (13)	55 ±2.1[e]	233.1 ±7.2[e]	228.5 ±7.6[d]	30.2 ±0.054[e]	82.0 ±0.61[e]	244.0 ±10.9[b]	237.6 ±8.4 NS	6.6 ±0.37[e]	6.5 ±0.39 NS	28.7 ±0.89[c]	28.2 ±0.98[e]

[f] Based on the no. of nuclei of acinar cells per unit area.

[g] Mean ± SE.

[h] Indicates significance of differences from diabetic controls: superscripts $a = p < 0.05$; $b = p < 0.025$; $c = p < 0.01$; $d = p < 0.005$; $e = p < 0.001$ and NS = not significant.

[i] Growth hormone, Raben type (0.5 USP units/mg).

were injected subcutaneously with relatively large doses of GH—600 μg in 0.4 ml of distilled water per rat per day for 7 days. Intact and hypox controls were similarly treated with distilled water alone. Rats having residual pituitary tissue as examined at autopsy were eliminated.

In both experiments, the body weight and the wet weight of the submandibular, parotid, thyroid, and adrenal glands were recorded at autopsy. The submandibular glands were fixed, sectioned, stained by Azan stain (*2*). The diameters of striated ducts as well as the sizes of acinar cells (inversely related to the counts of nuclei of acinar cells per unit area) of the submandibular gland were measured by the aid of linear and square eyepiece micrometers. The mean parameters of gland weights, ductal diameters, and the counts of acinar cells of submandibular glands of hormone-treated rats were compared with diabetic or hypox controls by Student's *t* test.

Results. Expt. 1. Table I shows that alloxan-induced diabetes retarded the growth of the submandibular, parotid, thyroid, and adrenal glands and the growth of the body in general; the mean diameter of striated ducts and the size of acinar cells of submandibular glands also decreased significantly as compared to the intact controls. PZI administered to the diabetics in daily doses of 1 U/100 gm of body weight caused a significant increase in the gain of body weight and in the wet weight of submandibular, thyroid, and adrenal glands, while the wet weight of the parotid gland increased slightly but not significantly as compared to the diabetic controls. The diameters of striated ducts and the sizes of acinar cells of submandibular glands of PZI-treated diabetics also increased significantly as compared to the diabetic controls. GH given to the diabetics in daily doses of 50 μg/100 gm of body weight did not cause, in general, any significant changes in body growth and the growth of submandibular, parotid, thyroid, and adrenal glands. In fact, these mean parameters of the diabetics receiving higher dose level (100 μg) of GH even showed a slight to a significant decrease as compared to the diabetic controls. The mean diameter of striated ducts and the size of acinar cells of submandibular glands of both GH-treated groups of diabetics decreased significantly as compared to the diabetic controls. Concurrent administration of PZI and GH to the diabetics resulted in a significant increase in the gain of body weight and the mean wet weights of submandibular, parotid, thyroid, and adrenal glands, as well as an increase in the mean diameter of striated ducts and the size of acinar cells of the submandibular gland as compared to the diabetic controls. The mean absolute wet weight, ductal diameter, and acinar cell size of submandibular glands, as well as the gain of body weight of GH–PZI-treated diabetics were also significantly greater ($p<0.01$, $p<0.001$, $p<0.025$ and $p<0.05$, respectively) than those of PZI-treated diabetics. While the mean absolute wet weights of parotid, thyroid, and adrenal galnds of GH–PZI-treated rats were essentially the same as compared to those of diabetics treated with PZI alone.

The mean relative weights (gland wt to body wt ratio) of submandibular, parotid, and adrenal glands of diabetics increased significantly as compared to the intact controls. Compared with the diabetic controls, the mean relative weights of these glands of diabetics receiving PZI, GH, or their combined treatments decreased slightly or significantly.

Expt. 2 Table II shows that the gain of body weight and the mean wet weights of the submandibular and parotid glands as well as the mean diameter of striated ducts of hypox rats decreased significantly as compared to the intact controls. GH is daily doses of 600 μg/rat caused a significant increase in submandibular and parotid wet weights as well as an increase in ductal diameter of the submandibular gland in comparison with those of the hypox controls. However, the gain of body weight and the wet weights of submandibular and parotid glands in GH-treated hypox rats were less than those of intact controls.

The relative weights of submandibular glands of hypox rats were essentially the

TABLE II. Effect of Growth Hormone on Growth of Submandibular Glands in Hypophysectomized Rats.

Treatment and (no. of rats)	Body wt (g)		Submandibular gland			Parotid wt	
	Final	Gain	Wt (mg)	Wt (mg/100 g of body wt)	Diameter of striated duct (μ)	(mg)	(mg/100 g of body wt)
Hypox:[a] vehicle (7)	59.0 ± 1.7[c]	14.0 ± 1.8	153.7 ± 5.4	261.2 ± 9.0	24.8 ± 0.21	129.0 ± 8.9	217.1 ± 9.7
Intact: vehicle (10)	92.1 ± 2.2[c,f]	42.8 ± 1.2[c]	247.2 ± 6.9[c]	269.0 ± 6.6 NS	30.3 ± 0.14[c]	228.9 ± 7.4[c]	248.6 ± 5.6[c]
Hypox: 600 μg GH[g] (11)	74.4 ± 2.1[c]	30.3 ± 1.5[c]	179.7 ± 7.2[c]	241.9 ± 7.7 NS	27.2 ± 0.13[c]	173.7 ± 8.9[c]	233.4 ± 8.5 NS

[a] Hypophysectomized.
[c] Mean ± SE.
[f] Indicates significance of differences from hypophysectomized controls: superscripts $a = p < 0.025$; $b = p < 0.005$; $c = p < 0.001$; NS = not significant.
[g] Growth hormone, Raben type (0.5 USP units/mg).

same while that of the parotid gland decreased significantly as compared to the intact controls. The relative submandibular and parotid weights of GH-treated hypox rats were essentially the same as the hypox controls.

Discussion. The retardation of the growth and development of submandibular glands, as judged by the wet weights, the ductal diameters and the acinar cell sizes, in diabetic rats further confirmed the findings of our previous studies that normal secretion of insulin was required for normal growth and development of submandibular glands (1). The partial or incomplete restoration of the growth of salivary glands to the normal level in PZI-treated diabetics was due to the fact that the daily doses (1 U/100 gm of body wt) of PZI were below the normal requirement. This was evidenced by the previous studies which showed that in order to maintain the normal growth and histological integrity of the salivary glands, the daily doses of 2–3 U of PZI/100 g of body weight were required (1).

Of particular interest is that in the diabetics, with increase in daily doses of GH, the growth of the salivary glands proportionately decreased, while concurrent administration of GH and PZI stimulated salivary gland growth to a greater extent than those receiving PZI alone. This indicates that in absence of insulin, GH is ineffective, and that insulin is equally important as are other essential hormones in stimulating salivary gland growth. The inhibiting effect of GH on salivary gland growth in diabetics corroborated that suggested by others (3) indicating that the anabolic effect of GH required the presence of insulin. In the absence of insulin, GH exerted lipolytic and hyperglycemic or diabetogenic action. Furthermore, it was reported that in the diabetics there was an increased secretion of adrenocorticoids (4) which increased glyconeogenesis or catabolic process of protein and neutral fat which would be also unfavorable to salivary gland growth.

Since the GH used for Expt. 1 was prepared from porcine pituitary, it was pos-

sible that this Raben type GH might be ineffective in stimulating the growth of rat salivary glands. However, this did not bear out as judged by: (i) the growth of submandibular glands of GH–PZI-treated diabetics was significantly better than those of the diabetics treated with PZI alone, and (ii) the same GH preparation did stimulate the growth of salivary glands in hypox rats. The subnormal growth of the salivary glands in GH-treated hypox rats was as anticipated, since in these rats there was a decrease or cessation of the secretion of thyroxine and adrenocorticoids which, in combination with GH, were essential hormones needed to restore the normal growth of the salivary glands in female rats (5, 6). Thyroxine or GH individually was reported to stimulate salivary gland growth in nondiabetic–hypox rats (5). We also have found that adrenalectomy retarded salivary gland growth which was restored by hormonal replacement therapy (unpublished). It would be desirable to evaluate whether or not, in diabetic–hypox female rats, the growth of the salivary glands induced by GH, thyroxine, adrenocorticoid, or their combined treatments is dependent on the presence of insulin.

It was possible that the changes of nutritional conditions in diabetic rats interfered with the growth of salivary glands. However, it was observed that the diabetics ate more food than the intact controls which agreed with reports by others (7). Therefore, even if nutritional factors were involved in retarded salivary gland growth in the diabetics, it would be due to the interferences with the intermediary metabolism of carbohydrates, lipids, and proteins.

Although the body growth appears to be more sensitive to hormonal imbalance than the submandibular glands, the decrease or increase in salivary gland wet weight was, in general, parallel to the gain of body weight in diabetics of PZI-treated diabetics. Therefore, the effect of insulin described on submandibular gland growth was regarded as being nonspecific in nature.

Summary. Alloxan diabetes mellitus was induced in weanling female Sprague-Dawley strain rats. Diabetics in the test groups were given daily subcutaneous injection of 1 U of protemine zinc insulin (PZI), 50 or 100 μg of growth hormone (GH) or 1 U of PZI plus 50 μg of GH/100 g of body weight for 14 days. Diabetic and intact controls were similarly treated with vehicle. A significant retardation of the growth of salivary glands was shown in the diabetics as compared to intact controls. In the diabetics, PZI treatment resulted in a significant stimulation and GH treatment in an inhibition of the growth of the salivary glands. PZI and GH administered concurrently exhibited a synergism in stimulating salivary gland growth.

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