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Role of Insulin in Mandibular Growth and Development (41212)

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Abstract. The effects of insulin deficiency and of its correction on mandibular growth in relation to bodily growth were investigated. Diabetes mellitus was induced by a sc injection of alloxan (20 mg/100 g body wt). One group of diabetic rats received daily sc injections of protamin zinc insulin (PZI; 3 U/100 g body wt) for 38 days. A second group of diabetic rats and a group of normal animals received injections of PZI vehicle. It was found that untreated diabetes was associated with a significant retardation of mandibular growth as judged by the lengths of the rami and corpora. This retardation was prevented by the administration of PZI. Bodily growth, as judged by body weight of untreated diabetics, was also retarded. The administration of PZI entirely corrected the deficiency in mandibular growth, but only partially that of bodily growth.

It has been known for years that postnatal growth and development are accelerated by several hormones: growth hormone, thyroxine or triiodothyronine, androgens derived from either the adrenal cortices or the testes, and estrogens (1, 2). Recent studies have shown that somatomedins mediate the action of growth hormone on bone growth (3–5), that both growth hormone and insulin are required for the release and activity of somatomedins (4, 6, 7) and that the growth of the secondary cartilage of the mandibular condyle is stimulated by growth hormone or somatomedins and thyroxine, as well as by the mechanical stimulation exerted by the lateral pterygoid muscle (8–12). It has also been observed that growth is impaired in children with poorly controlled diabetes (2, 13–16). On the other hand, a role of insulin in mandibular growth has not been described. Thus, the purposes of these studies were to determine whether (i) alloxan-induced diabetes interferes with mandibular growth (in relation to bodily growth) and (ii) insulin replacement prevents any deficiency observed in alloxan-diabetic animals.

Materials and Methods. Thirty-nine Sprague–Dawley rats (Sasco, Inc., Omaha, Nebr.) were used for the experiment. Ten rats were used as normal controls and 29 were rendered diabetic by a single sc injection of 20 mg alloxan/100 g body wt. The successful induction of diabetes was judged by the detection of glucose in the urine (Tes-Tape, Eli Lilly & Co., Indianapolis, Ind.), about 24 hr after alloxan injection. Of the 29 diabetic rats, 19 were untreated and 10 received daily sc injection of protamine zinc insulin (PZI, Eli Lilly, 3 U/100 g body wt in 0.3 ml of vehicle) beginning on the day after the successful induction of diabetes. No glucosuria was observed in the PZI-treated diabetic rats during the entire period of treatment. Rats in the control and diabetic groups received an equal volume of PZI vehicle. All rats were kept under the same environmental conditions, with free access to Purina Chow and tap water. The presence of glucosuria in the untreated diabetic rats was checked twice weekly throughout the experimental period. Ten doubtful diabetic or morbibund rats in the untreated group and one unhealthy rat in the control group were eliminated; thus, 9 rats in each of these two groups and 10 PZI-treated diabetic rats survived to the 40th day of the experiment, when they were killed by ether inhalation, weighed, and autopsied. At autopsy, the mandibles were dissected out, autoclaved at 20 lb/in.^{2}, 120°, for 20 min, the soft tissue was removed with a tooth brush, and the bones were dried in an oven (Stabil-Therm, Blue M Electric Co., Blue Island, Ill.) at 40° for 72 hr. The distances

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from the posterior border of the condyle to the posterior border of the mental foramen of both mandibles of each rat (17) were measured and their average was considered the length of the mandible. Similarly the average of the distances between posterior border of the condyle and the more delineated anterior border of the mandibular foramen was considered to be the length of the mandibular rami. All measurements were performed at 40× magnification using a microscope (Bausch & Lomb) equipped with a calibrated mechanical stage under direct illumination of the lateral or medial bone surface. Since there are no definite reference points for measuring the length of the mandibular corpus, this was calculated by subtracting the length of the ramus from the mandibular length. The means of the five parameters (mandibular length, length of mandibular rami, length of mandibular corpora, final body weight, and weight gain) of the untreated diabetic rats were compared with those of the treated diabetic and of the control rats, by Student's t test.

Results. As shown in Table I, alloxan-induced diabetes resulted in a highly significant ($P < 0.001$) retardation of mandibular growth, as judged by the mean mandibular length and the length of the mandibular rami and of the mandibular corpora. Insulin therapy caused a significant ($P < 0.001$) reversal of this growth deficiency. Furthermore, as judged by all three parameters, the mandibular growth of the PZI-treated diabetic rats was essentially the same ($P < 0.05$) as that of the controls. The retardation of mandibular growth in the untreated diabetic rats was associated with a marked retardation of bodily growth, as judged by the loss of body weight and by the length of body and tail. Insulin-treated diabetic rats were significantly heavier and gained more weight than their untreated counterparts ($P < 0.001$ in both cases), although significantly less than the control animals ($P < 0.05$ in both cases).

Discussion. The results of these studies indicate that mandibular growth and bodily growth were retarded in the alloxan-diabetic rats and that retardation in mandibular growth was prevented by insulin

TABLE I. EFFECTS OF INSULIN DEFICIENCY AND OF ITS REPLACEMENT ON MANDIBULAR BONE LENGTH AND BODY WEIGHT OF FEMALE RATS

Treatment and No. of rats	Bone length (mm)			Body weight (g)		
	Mandibles[a]	Mandibular rami[b]	Mandibular corpora[c]	Initial	Final	Gain (+) or loss (−)
Intact: vehicle (9)	19.93 ± 0.090[d]	7.34 ± 0.072	12.59 ± 0.079	155.6 ± 1.7	364.4 ± 5.4	+208.9 ± 6.12
Diabetic: vehicle (9)	17.81 ± 0.123[e]	6.04 ± 0.042[e]	11.78 ± 0.113[e]	158.2 ± 2.2	124.8 ± 4.1[e]	−33.4 ± 3.84[e]
Diabetic: insulin[f] (10)	19.48 ± 0.269[g]	6.98 ± 0.161[g]	12.50 ± 0.127[g]	158.8 ± 3.6	342.4 ± 7.7[g,h]	+183.6 ± 8.55[g,h]

[a] Posterior border of condyle to posterior border of mental foramen.
[b] Posterior border of condyle to anterior border of mandibular foramen.
[c] Anterior border of mandibular foramen to posterior border of mental foramen.
[d] Mean ± SE.
[e] Differs from intact: $P < 0.001$.
[f] Protamin zinc insulin: 3 USP units/100 g body wt/day.
[g] Differs from diabetic: $P < 0.001$.
[h] Differs from intact: $P < 0.05$.

therapy, although insulin therapy did not entirely correct the retardation of body growth. These findings confirm and extend previous observations suggesting that insulin has a role in normal body growth, growth of tibial epiphyseal cartilage plate, and growth of endocrine and salivary glands, while growth hormone is ineffective in stimulating bodily growth in diabetic immature rats (18, 19). The decrease in the length of the mandibular rami and of the mandibular corpora in diabetic rats suggests that both the condylar endochondral growth and the growth of the mandibular corpora are interfered with (20–22).

The data do not indicate whether the action of insulin is direct or if it is mediated by that of the somatomedins, by an increased activity of the lateral pterygoid and masticatory muscles or by an increase of temporomandibular joint movements (21–24). The hypothesis of a direct effect of insulin in stimulating condylar endochondral growth is supported by the fact that none of the three groups of rats was subjected to lateral pterygoid muscle propulsion or retrusion. Furthermore, as a result of polyphagia, the diabetic rats exercised the masticatory muscles more than either the PZI-treated diabetic or the control animals. Therefore, it appears that the effectiveness of differentiation and condylar endochondral growth was decreased by insulin insufficiency.

The mechanism of this growth-promoting effect of insulin is not known. It has been reported that insulin stimulates the chondroblastic and osteoblastic synthesis of collagen and glycosaminoglycans of bone matrix (25, 26) and that insulin and proper quantity of nutrients are required for the maintenance of normal circulating level and physiological activity of the somatomedins (27, 28). It is possible that insulin may promote bone mineralization, since a decrease in duodenal calcium absorption (29, 30) and in bone calcium deposition and osteoporosis (31) have been observed in diabetic rats. Indeed, in juvenile- and adult-onset diabetic patients there is a decrease in bone mass and mineral content of the long bones, resulting in osteopenia with a concurrent increase in the excretion of urinary calcium (32, 33). The data reported herein support the concept that insulin is important in stimulating mandibular growth in the rat, although this effect does not appear to be specific, since the bodily growth was also affected by insulin deficiency.

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