Evidence for Insulinotropic Effect From Rat Parotid Glands

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Previous observations have suggested that the salivary glands exercise a regulatory role on insulin secretion and/or glucose metabolism. We have challenged the issue by studying in unanesthetized, unrestrained rats the short- and long-term effect of selective sialoadenectomy on the animals' ability to meet an intra-arterial glucose challenge. Ten-minute intra-arterial glucose tolerance tests were carried out in chronically catheterized adult rats before and after sialoadenectomy. Eighty-five to 100 days postsurgery, the parotidectomized rats experienced a 45% reduction in plasma immunoreactive insulin output ($P < .001$) compared with the sham-operated animals; the plasma glucose levels of the test subjects remained 19% higher ($P < .001$) than those of the control group. In younger rats, similar observations were made; however, the difference in insulin and glucose responses between treatments was less than in the adult rats. Our findings suggest that the insulinotropic effect resides primarily with the parotids, and the role of the submandibular glands seems to be permissive at best. We hypothesize that parotidectomy deprives the β-cells of a humoral principle that appears to be essential for optimizing the immediate insulin response to a glucose challenge. These results suggest that the insulinotropic effect of the parotids is of particular importance when aging changes insulin secretion and action. *Diabetes* 37:441–45, 1988

The physiologic role of parotid glands is more complex than their recognized salivary function. Various investigators have suggested an endocrine relationship between the parotids and the pancreas. Bilateral enlargement of the parotid glands has been associated with diabetes mellitus; this effect was interpreted as a compensatory hyperplasia in response to decreased insulin secretion (1–5). The North American Pima Indians exhibit a high prevalence of diabetes, with 61% of the cases associated with asymptomatic parotid gland enlargement (6). In animal studies, some investigators found that induced glycosuria in intact animals could be ameliorated either by parotid duct ligation or by administration of salivary gland extract (7,8). It was also reported that parotidectomy causes a transient decrease in blood glucose and that the administration of parotid gland extract causes hyperglycemia (9,10). Parotin, a parotid protein with ubiquitous effects, was found by some investigators to increase glucose tolerance (11); however, other investigators have reported either a hypoglycemic effect or no effect (12,13). The inherent weakness in these studies is that the β-cell function was evaluated indirectly by monitoring changes in blood glucose. We know that various factors, both neural and hormonal, can have a significant effect on blood glucose that could limit the interpretability of these observations. We report in the rat the chronic effect of sialoadenectomy on the secretion of insulin in response to intra-arterial infusion of glucose.

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MATERIALS AND METHODS

Catheterization. Male Sprague-Dawley (Harlan-Sprague-Dawley, Indianapolis, IN) rats were anesthetized with methohexital sodium (5 mg/100 g body wt). The left common carotid artery was cannulated as described by Popovic and Popovic (14) with polyethylene tubing PE-50 (Clay Adams, New York). Its distal end was tunneled under the skin around the shoulder, and it was exteriorized on the back of the animal between the shoulders. The tubing was protected by inserting it inside a 30-cm-long metal coil soldered at a right angle to a 2.5 × 4-cm metal plate that was sutured atop the catheter hole onto the animals' skin. The animals were rested for 5 days before starting any experiment.

Animal maintenance. Rats were kept in raised 18 × 24 × 17-cm cages. A dark plastic lid was fitted with a slot, allowing the catheter-protecting coil to move freely with the animal. The cages were set in such a way that all manipu-

lations through the catheter could be done without the animals seeing the investigator. Catheters were flushed twice daily with sterile heparinized saline (20 U/ml) and then filled with a stronger solution (300 U/ml). The animals had access to water and laboratory chow ad libitum. Their weight and food intake were monitored daily.

Glucose tolerance test (GTT). Rats were fasted 24 h before the test. The glucose-infusion test was always conducted at 1400 h under strictly controlled and constant conditions. A 420-μl baseline blood sample was drawn at a constant rate for 3.5 min. An infusion of 78 μmol glucose/100 g body wt, dissolved in 200 μl of saline, was performed in 15 s. After the catheter was flushed and equilibrated, the first blood withdrawal was started 30 s after infusion and continued for 1.5 min at a constant rate of 5 μl/s. Blood samples collected at 6 and 10 min were drawn for 20 s at a rate of 17.5 μl/s. After withdrawal of each blood sample, an equal volume of warm saline was infused for 20 s. Plasma immunoreactive insulin (IRI) titer was determined by radioimmunoassay (RIA); glucose level was determined by the glucose oxidase method (15).

Plasma insulin RIA. We used a modified, double-antibody procedure derived from the Novo RIA kit (Copenhagen). The incubation was carried out in 12 × 75-mm polystyrene tubes, with a total volume of 0.8 ml. An 8-h first incubation with the sample and first antibody (anti-porcine insulin guinea pig serum M8170, Novo) was done at 22°C; this was followed by an addition of [^{125}I]iodoinsulin (ICN, Irvine, CA) and a second incubation for 24 h at 4°C. The separation of bound antigen from free antigen was achieved by precipitation with a second antibody and centrifugation. Rat insulin (Novo) was used as the standard. The sensitivity of the assay was <0.15 ng/ml plasma. The intra-assay coefficients of variation of the plasma estimates at 1.1 and 6 ng/ml were 6.5 and 3.7%, respectively. The interassay coefficients of variation at the same levels were 10 and 8.8%, respectively. Each sample determination was done in triplicate. Reduction and statistical analysis of the RIA data were done according to Rodbard (16).

Sialoadenectomy and sham operation. Rats were anesthetized with methohexital sodium (5 mg/100 g body wt). The parotid or submandibular gland was removed after blunt dissection and ligation of the major blood vessels. After removal of the salivary glands, the animals were given 5% glucose drinking water for 5 days. Sham operations were performed in a similar manner, except that the glands were only gently manipulated with blunt forceps and left in place. In this study, *total sialoadenectomy* refers to the removal of the parotid and submandibular glands, which in the rat include the sublingual glands.

Effect of parotidectomy on the β-cell response. We first studied the short- and long-term effects of parotidectomy on the acute response of the β-cell. Adult (300-g) rats were chronically catheterized, kept in individual cages, and allowed complete freedom of movement. The rats were divided into four groups. Between days −15 and 0 (day 0, day of surgery), five GTTs were done at 3-day intervals by infusing 78 μmol glucose/100 g body wt through the indwelling catheter of the awake animals. The IRI titer and plasma glucose level were obtained before glucose infusion and at 2-, 6-, and 10-min postinfusion. On day 0, each group of rats was either parotidectomized, submandibularectomized, totally sialoadenectomized, or had sham operations. The GTT was repeated in each rat on postoperative days 5, 8, 12, 15, 19, 22, 26, 29, and 33. Because it is difficult to maintain the catheter patent for >40 days, two more groups of rats were either parotidectomized or had sham operations. They were catheterized 80 days later, and the GTT was carried out on days 85, 89, 92, 96, and 100.

Effect of age and parotidectomy on IRI and glucose responses after GTT. The effect of parotidectomy on the β-cell response to a glucose load was assessed in relation to the age at which the rats underwent surgery. Weanling (50–60 g, 21 days old) and adult (275–300 g, ~80 days old) male rats were parotidectomized. Control rats for each age group had sham operations. All animals were catheterized 80 days after surgery, and the responsiveness of the β-cell to the glucose challenge was tested on days 85, 89, 92, 96, and 100 as previously indicated.

Data analysis. Pooling of data from several tests was done by averaging values of several tests for each rat and then computing the mean values ± SE of all rats. In Figs. 1 and 2 the data were also expressed as insulin-to-glucose ratios (17). Statistical difference among groups was assessed by Student's *t* test for unpaired data.

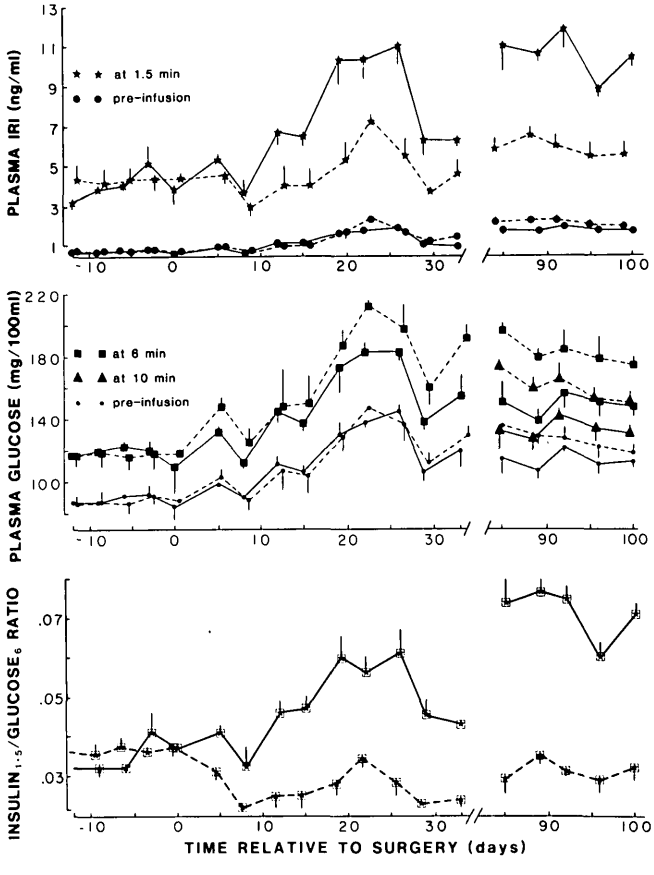

FIG. 1. Effect of parotidectomy (*dashed line*) and sham operation (*solid line*) on plasma glucose immunoreactive insulin (IRI, *top*) and plasma glucose (*middle*) responses after intra-arterial infusion of glucose in unanesthetized unrestrained adult rats. Plasma titer values are means ± SE of 4–6 rats for each glucose-infusion test performed before and after surgery. Insulin-to-glucose ratios (*bottom*) were computed from IRI data at 1.5 min (★) and from glucose titers at 6 (■) and 10 (▲) min. ●, Preinfusion ratio.

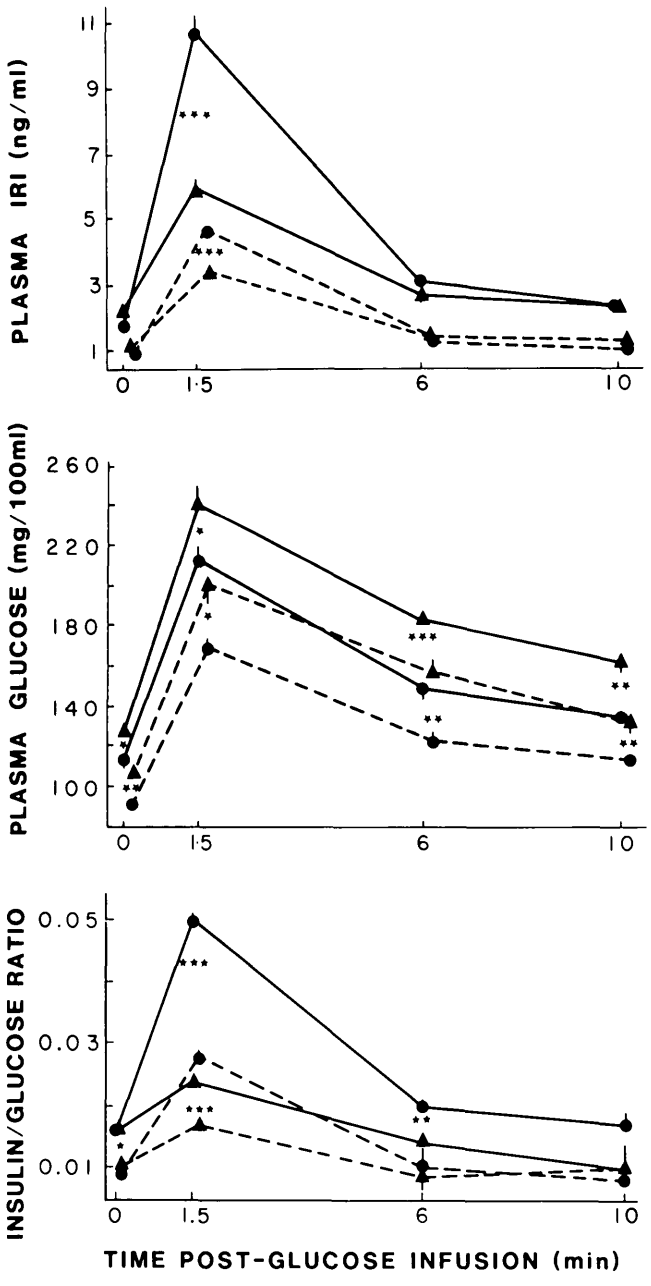

FIG. 2. Results of 10-min intra-arterial glucose tolerance test in unanesthetized unrestrained rats after parotidectomy (▲) or sham operation (●) at 21 (*dashed line*) or 80 days (adult; *solid line*) of age. Tests were done every 4 days between days 85 and 100 postsurgery by infusing 78 μmol glucose/100 g body wt. Each *point* represents mean ± SE pooled response of 6 rats between days 85 and 100. *$P < .05$, **$P < .01$, or ***$P < .001$, statistical difference between type of surgery within each age group.

RESULTS

The plasma glucose and IRI responses to individual GTTs carried out before and after surgery in sham-operated and parotidectomized rats are summarized in Fig. 1. Beginning on day 12 postsurgery, a significant increase in preinfusion plasma glucose level was paralleled by an increase in the glucose response at 6 min in both sham-operated and parotidectomized rats; similarly, IRI values before infusion and at 1.5 min followed the same pattern. Pooled preinfusion glucose titers were 90.5 ± 1.8 between days −12 and 8, and 122 ± 2.2 mg/100 ml between days 12 and 33 ($P < .001$); the corresponding preinfusion IRI titers were 0.75 ± 0.04 and 1.37 ± 0.14 ng/ml ($P < .01$).

From day 12 postsurgery, the IRI response in the sham-operated group at 1.5 min postglucose infusion showed an increase with time that also paralleled the basal glucose and IRI levels in the same group. However, a significantly different result was observed in the parotidectomized rats, which experienced a lower IRI response to the same glucose challenge in comparison with the responses of the control rats. Similarly, plasma glucose level at 6 min postinfusion paralleled the preinfusion level, but it rose more in the parotidectomized rats than it did in the control rats. A comparative display of the evolution of the glucose and IRI responses before and after surgery in the four surgery groups is presented in Fig. 3. The responses in the sham-operated and submandibularectomized groups were not different; likewise, the responses in the parotidectomized and totally sialoadenectomized rats were similar. Eighty-five to 100 days postsurgery, the glucose and IRI responses to glucose infusion stabilized to levels significantly different in the intact and the parotidectomized rats (Fig. 1). Parotidectomized rats experienced a lower IRI response at 1.5 min postglucose infusion, which was accompanied by a significant increase in plasma glucose level at 6 and 10 min. Pooled IRI responses at 1.5 min between days 85 and 100 were 10.7 ± 0.5 in the intact group and 5.9 ± 0.3 ng/ml in the parotidectomized group ($P < .001$, $n = 6$). The glucose titers were 149 ± 4.5 and 183 ± 4.5 mg/100 ml for the intact and parotidectomized rats at 6 min ($P < .001$), respectively. At 10 min, the respective glucose values were 135 ± 3.8 and 162 ± 5 mg/100 ml ($P < .01$).

Figure 2 illustrates the 10-min GTT data obtained between days 85 and 100 in intact and parotidectomized rats that underwent surgery at either 80 (adults) or 21 days of age. Rats of both age groups experienced a significant increase in fasting blood glucose titer after parotidectomy; increases were also noted within each surgery group as the rats grew older. The magnitude of the insulin response at 1.5 min was remarkably higher in the older rats, but within each age group the parotidectomized rats had a significantly lower insulin response than did the intact rats. Concomitantly, the blood glucose titers remained significantly higher at all times in the parotidectomized rats compared with the intact groups.

The long-term effect of parotidectomy on growth and food consumption in both immature and adult rats is shown in Fig. 4; parotidectomy had no significant effect on either body weight or daily food consumption.

DISCUSSION

Our study indicates that in unanesthetized, unrestrained rats, parotidectomy chronically decreases the responsiveness of the β-cells to an acute change in blood glucose concentration. This observation suggests that parotidectomy deprives the β-cells of a parotid signal that appears to be essential for optimizing the immediate insulin response to a glucose challenge. We hypothesize that a humoral principle secreted by the parotid glands affects the pancreatic function as it modulates the sensitivity of the β-cells to blood glucose

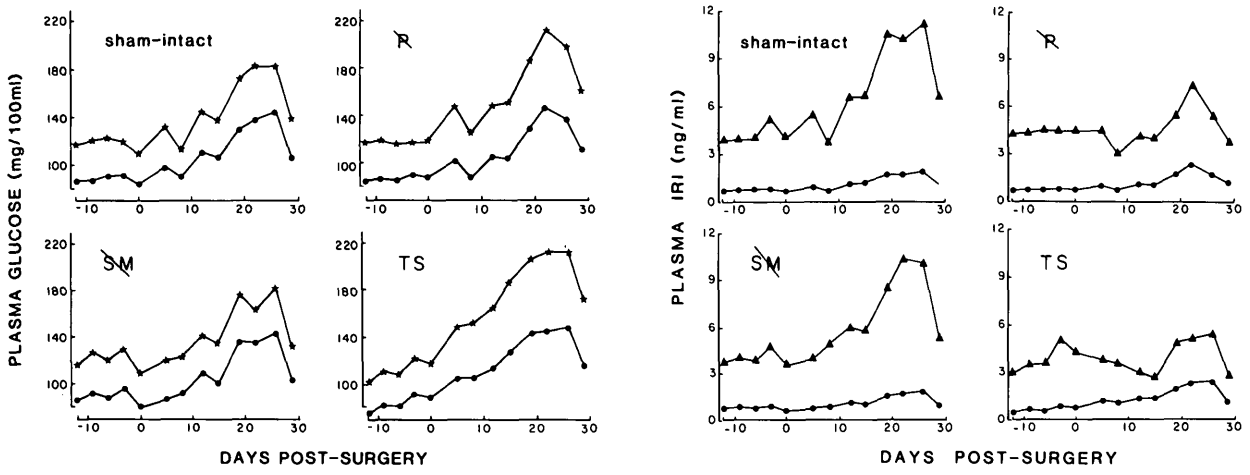

FIG. 3. Overall patterns of plasma glucose and IRI responses after intra-arterial infusion of glucose in unanesthetized adult rats subjected to 4 different operations: sham operation (sham-intact), parotidectomy (R), submandibularectomy (SM), and total sialoadenectomy (TS). Plasma titer means (n = 4–6) before glucose infusion (●) and at 1.5 (▲) or 6 (★) min postinfusion are shown for each glucose-infusion test performed before and after surgery in same rats.

and/or the insulin reserve. This insulinotropic effect appears to reside primarily with the parotids, and the role of the submandibular glands seems to be permissive at best (Fig. 3).

The influence of the parotids on the β-cells appears to last for ~10 days after removal of the glands, and it took >35 days for the pancreas to establish a new functional steady state. During the first 33 days after surgery, a transitional pattern in the glucose and insulin responses developed that was remarkably consistent at all sampling times and between all surgery groups—a progressive rise beginning on day 12 reached a peak at day 22 (Fig. 3), followed by a drop and a second rise on day 33, as shown in Fig. 1. Because IRI and glucose preinfusion titers followed this pattern in all four surgery groups, which were obtained and managed at different times, the observed changes in response probably were the consequence of the experimental conditions and/or the traumatic effect of catheterization followed by removal of the glands 15 days later.

In the rats that were catheterized 80 days after parotidectomy, a more stable response pattern seems to have been established between days 85 and 100 (Fig. 1). It is reasonable to assume that by that time, the rats had made physiological adjustments to compensate for the removal of the parotids. We therefore emphasize the results obtained between days 85 and 100, for they suggest that the observed insulinotropic effect of the parotids is not transient and the lack of effect cannot be compensated for by other physiologic mechanisms. To us, the latter observation indicates that the parotid insulinotropic effect may have physiological significance. Results in Fig. 2 indicate that in the older parotidectomized rats the GTT insulin output at 1.5 min was reduced more than it was in the younger ones. In vivo studies in rats have shown an increase in glucose intolerance with increased age and weight, particularly obesity associated with lack of physical exercise. Animals kept under those conditions exhibit higher basal blood glucose, IRI levels, and glucose-stimulated IRI response (18). Our study confirms these findings; however, whereas the older intact rats experienced a significant increase in IRI secretion after the glucose challenge, the parotidectomized counterparts lost 75% of the β-cell capacity to adapt to metabolic changes that occur in the aging process.

Several investigators have demonstrated the presence of immunoreactive and bioactive insulin-like material in various tissues, including the parotid and submandibular glands (19–26). Biosynthesis of insulin was demonstrated in these glands (22,23). From this perspective, the researcher may conclude that the decrease in circulating IRI after parotidectomy is a consequence of the removal of parotid insulin. For several reasons, we believe that this position is not tenable. First, the collective tissue concentration of extrapancreatic insulin is low, and its concentration in the parotids is estimated to be 1/7000 of that in the pancreas (22). Second, attempts to stimulate secretion of IRI from extrapancreatic sources in eviscerated rats with either arginine or glucose have been unsuccessful (27). Third, if several extrapancreatic sources of insulin exist and are significant in glucose homeostasis, the removal of one of them should trigger compensatory secretion from remaining sources. For example, in our study the submandibular glands, which have been claimed to be a possible source of insulin (23), did not show any appreciable effect on the IRI secretion, as indicated by their removal. In our opinion the physiologic role of extra-

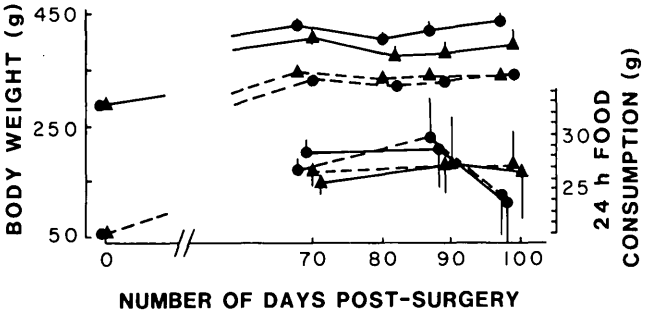

FIG. 4. Body-weight and food-consumption data of 4 groups of rats that had surgery as described in Fig. 2. No statistical difference in body weight or food consumption was found between sham-operated and parotidectomized rats in either age group.

pancreatic insulin in glucose homeostasis remains to be established. The 45% decrease in circulating IRI after parotidectomy observed in this study represents a significant departure from the normal response and suggests an impairment of β-cell function.

Insulin's collaborative function with other anabolic hormones is essential for optimal growth. We addressed the question of whether the parotidectomy-induced depression of β-cell function would affect the rat's normal growth. Because there was no significant weight difference between the intact and the parotidectomized animals in either age group 100 days after surgery, it appears that in the younger rats the availability of insulin met their anabolic needs. Furthermore, the daily consumption of laboratory chow among all groups was essentially the same and therefore does not account for the difference in insulin secretion.

In conclusion, the parotids appear to be the source of an insulinotropic principle that may be significant in maintaining the sensitivity of β-cells to circulating glucose. We have isolated a parotid hormone (PH) from porcine glands, the function of which is critical for maintaining a systemic resistance mechanism against dental decay through the activation of a dentinal fluid transport. This PH-mediated mechanism can be suppressed by a cariogenic, high-sucrose diet (28–30). Other studies have shown that poorly controlled, insulin-dependent diabetic humans and diabetic rats have a higher incidence of dental caries, which can be corrected in the rat with insulin therapy (31–33). From a systemic point of view, cariogenesis, diabetes, and PH secretion have a common denominator, glucose intolerance. Such a coincidental dependence suggests that PH may be involved in a parotid–β-cell relationship.

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