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Hypophysis and Function of Pancreatic Islets

I. The Influence of Hypophysectomy on Insulin Secretion and Biosynthesis of Proinsulin and Insulin in Isolated Pancreatic Islets of Rats**

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Summary. Insulin secretion and biosynthesis of proinsulin and insulin were determined in isolated pancreatic islets of hypophysectomized rats. Control rats were of both same age and weight. Hypophysectomy was performed either 13 or 5 weeks prior to the investigation, the weight of the animals being either 80 or 170 g. Biosynthesis of insulin was estimated from the amounts of radioactivity incorporated into proinsulin and insulin after incubation of isolated islets at 50 or 300 mg% glucose in the presence of ^{3}H-leucine for 3 h. Islet proteins were separated on Sephadex G 50 fine. — Hypophysectomy resulted in a significant decrease of both glucose stimulated secretion and biosynthesis of insulin. It was found that this reduction was 1) more significant when compared with controls of same age 2) more marked in rats which had been hypophysectomized 13 weeks before than in rats after an interval of 5 weeks and 3) less in rats which had been hypophysectomized at a weight of 170 g than in rats in whom pituitary ablation was performed at a weight of 80 g. At basal glucose concentrations, no significant changes of both secretion and biosynthesis of insulin were apparent. The relation of radioactivity incorporated into proinsulin and insulin was unchanged under all conditions. Insulin content of the isolated islets used was found within about the same range in all rats, apart from the animals which had been hypophysectomized 13 weeks before. In islets of these rats, a reduction to 84% was observed. — Our findings may be explained by reduced sensitivity of the pancreatic B-cell to glucose and a slower rate of insulin biosynthesis after hypophysectomy.

Key words: Insulin secretion, biosynthesis of proinsulin and insulin, isolated pancreatic islets, insulin content, hypophysectomy.

The relationship between the anterior pituitary gland and carbohydrate metabolism has been investigated by many authors since the early work of 4, 5 and 22. Randle [16] described decreased insulin like activity in plasma of patients with hypopituitarism as compared with normal human controls. Insulin like activity was found to be reduced also in hypophysectomized animals [17, 2, 15].

Martin and Gagliardino [12] described a reduction of both insulin secretion and incorporation of ^{3}H-leucine into the total insulin fraction of isolated pancreatic islets of hypophysectomized rats. A substitution of short duration with growth hormone — as well as with human placental lactogen [11] — improved both parameters. Malaisse, *et al.* [9] obtained similar results regarding insulin secretion from pieces of rat pancreas.

In *in vivo* studies, on the other hand, substitution with growth hormone of considerably longer duration was necessary to correct the hormonal and metabolic defects observed after hypophysectomy: Pfeiffer [15] reported that, in dogs, more than 10 days of substitution were required to normalize reduced insulin like activity in serum. Penhos, *et al* [14[. proved that substitution with relatively low doses of growth hormone every other day for 3 weeks normalized disturbed glucose tolerance in hypophysectomized rats. Administration of large doses of growth hormone to their rats during the first week after hypophysectomy led to rapid impairment of glucose tolerance.

According to Malaisse *et al.* [9], addition of growth hormone to the incubation medium of isolated islets from normal rats did not stimulate insulin secretion, unlike corticotrophin (ACTH) and thyrotrophin [10]. In the isolated perfused rat pancreas, ACTH was also found to stimulate insulin secretion [20], whereas the results obtained in this preparation with growth hormone and human placental lactogen were conflicting [7]. On the other hand, ACTH added *in vitro* to pancreatic slices failed to stimulate incorporation of leucine into insulin whereas small positive effects were observed after addition of growth hormone [21].

Therefore, we decided to study the influence of hypophysectomy and administration of growth hormone and ACTH to rats as to the secretion and biosynthesis of both proinsulin and insulin in isolated pancreatic islets. Special emphasis should be laid on the influence of the following factors: age and weight of the rats, time of onset, doses and duration of substitution.

A description of the methods used and the results obtained after hypophysectomy will be presented in

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this first of a group of papers. The following one will deal with the effects of *in vivo* administration of growth hormone and ACTH on secretion and biosynthesis of proinsulin and insulin in isolated pancreatic islets of hypophysectomized rats.

Material and Methods

Animals

Male albino rats of the strain FW 49 Lemgo-Kirchb.-Bib. were used. The animals had free access to food (rat pellets Altromin ®, Altrogge, Lippe, Germany) and water. A modified stereotactic, transauricular method was used for hypophysectomy [1]. After hypophysectomy the animals were kept for 1 week on a diet consisting of rat pellets soaked in water. They suffered from transitory diabetes insipidus; 5% of the animals died within these first few days. In 90% of the rats a lack of increase in weight indicated total hypophysectomy, only these animals were used. Altogether, 55 successfully hypophysectomized rats were examined; in this paper the results obtained in 17 hypophysectomized, untreated rats will be compared with those of intact controls. Control rats were either of the same age (not hypophysectomized litters) or of the same weight as the test animals at the end of the interval following hypophysectomy. Six days before the experiment proper, all animals were injected intraperitoneally with 0.5 ml phosphate buffer (0.1 M, pH 6.6) in order to permit exact comparison with the rats on substitution therapy with growth hormone or ACTH (see part II of this study). Our studies were performed in 4 groups. Table 1 shows the distribution of the animals into these 4 groups as well as their characterization.

Preparation of pancreatic islets

After decapitation pancreata of 2 or 3 rats were cut up with scissors in chilled Krebs-Ringer-bicarbonate buffer, pH 7.4, and digested with 40 mg collagenase (Serva, Heidelberg, Germany) under constant stirring at 37° C for 17—25 min [6]. After 3 washings in chilled buffer, intact and well separated islets of comparable size were collected with a Pasteur pipette under a stereomicroscope. 80—120 islets were obtained per rat pancreas.

Insulin secretion

Batches of 5 islets each were incubated in 1 ml Krebs-Ringer-bicarbonate buffer, pH 7.4, containing 0.5% bovine serum albumin and a protease inhibitor (Trasylol ®, Bayer, Leverkusen, Germany, 1000 KIU (kallikrein-inactivator-units)/ml). Incubations were carried out under a constant gas phase of $O_2 : CO_2$ (95 : 5%, v/v) at 37° C, using a metabolic shaker. Glucose concentrations were 50 and 300 mg%. After 1 hour 2 × 0.1 ml of the incubation media were taken for double determination of immunologically measurable insulin (IMI) [13]. For this immunoassay, porcine insulin (obtained from Farbwerke Hoechst AG, Germany, 1 mg ≙ 27 U) was used as standard and for labelling and production of antibodies in guinea pigs.

Insulin biosynthesis

Batches of 25 islets were incubated in the presence of 50 μCi L-leucine-4,5-^{3}H (19 Ci/mmol, Amersham) in 1 ml of Krebs-Ringer-bicarbonate buffer, pH 7.4, supplemented with bovine serum albumin (0.2%), a protease inhibitor (Trasylol ®, Bayer, Leverkusen, Germany, 1000 KIU/ml) and 17 naturally occurring amino acids (20 μg/ml, leucine excluded). Glucose was present in concentrations of 50 or 300 mg%. Incubations were carried out under a constant gas phase of $O_2 : CO_2$ (95 : 5%, v/v) for 3 h, using a metabolic shaker at 37° C. Insulin secretion was determined at the end of the incubation or every hour (see above).

After the incubation procedure, islets and incubation media were not separated from each other but examined together in order to estimate the total synthetic capacity. In several preliminary experiments, however, the incubation media and islets were studied separately: it was found that the amounts of radioactive proinsulin and insulin secreted into the medium during the 3 h of incubation were small compared with the amounts determined within the islets.

After freezing the islets and the incubation media at — 28° C, thawing and subsequent ultrasonic disintegration (15 sec), the proteins were precipitated with ice-cold trichloroacetic acid in a final concentration of 10%. The precipitate was washed with 10% trichloroacetic acid, dissolved in 0.5 ml of acetic acid (1 M) and separated on a Sephadex G 50 fine column, 0.9 × 55 cm. The column had been equilibrated with 1 M acetic acid and calibrated with albumin, proinsulin, insulin, glucagon and leucine. 1 M acetic acid was used as eluent, fractions of 1 ml were collected. UV-absorption was recorded continuously by an Uvicord-apparatus (LBK-produkter, Sweden). 100 μl of the fractions were assayed for radioactivity in a liquid scintillation counter (Tricarb, Packard), using Bray's scintillator liquid. Immunologically measurable insulin was determined in each fraction. The radioactivity peaks were pooled, lyophylized and radioactivity as well as immunoassayable insulin were determined again in aliquots of the pooled peaks. For further identification, polyacrylamide gel electrophoresis (pH 8.6, 15% gel) was used. According to Steiner and Oyer [19], the identity of the pooled peak P (see Figs. 3 and 4) with proinsulin was also demonstrated by rechromatography on the Sephadex G 50 fine column after treatment with trypsin (p-tosyl-L-phenylalanine chlormethylketone-trypsin, obtained from Serva, Heidelberg, Germany, 50 μg, 10 min, 37° C). After treatment with trypsin, most of the radioactivity was eluted in the position of insulin, giving strong reactions with antibodies to insulin.

Results

Growth

Successful hypophysectomy was followed by cessation of growth in all 4 animal groups whereas non hypophysectomized litters exhibited normal growth during the period of investigation (Table 1).

Insulin secretion

Basal insulin secretion from batches each containing 5 isolated pancreatic islets at 50 mg% glucose amounted to 60—80 μU/h/5 islets. No significant differences were found between controls and hypophysectomized rats. Glucose stimulated (300 mg%) insulin secretion from each group of 5 isolated islets, on the other hand, was reduced following hypophysectomy (Fig. 1). As compared with control rats of the same age, this reduction was highly significant in all groups ($p < 0.0001$—0.001). As compared with controls of the same weight the differences, however, were not so striking ($p < 0.005$ for group I, $p < 0.05$ for

groups II, III and IV). In both cases, the maximum decrease was observed in rats of group I.

Estimations of insulin release were also carried out at hourly intervals during incubations of 25 islets for 3 h (Fig. 2). As can be seen in Fig. 2, both the amount and the rate of insulin secretion were lowered in the hypophysectomized rats as compared with normal control rats.

Table 1. *Mean weight in 4 groups of rats before and after hypophysectomy (Hx) and in control rats of both same age and weight. hx: hypophysectomized rats. non hx: intact litters (controls of some age)*

group	interval between Hx and investigation (weeks)		weight (g) at time of Hx	weight (g) at time of investigation	n
I	13	hx	79	86	6
		non hx	80	280	6
II	5	hx	76	74	3
		non hx	75	204	3
III	5	hx	80	82	4
		non hx	78	213	13
IV	5	hx	169	163	4
		non hx	171	332	10

controls of same weight:

	weight (g)	n
for group I—III	79	6
for group IV	165	6

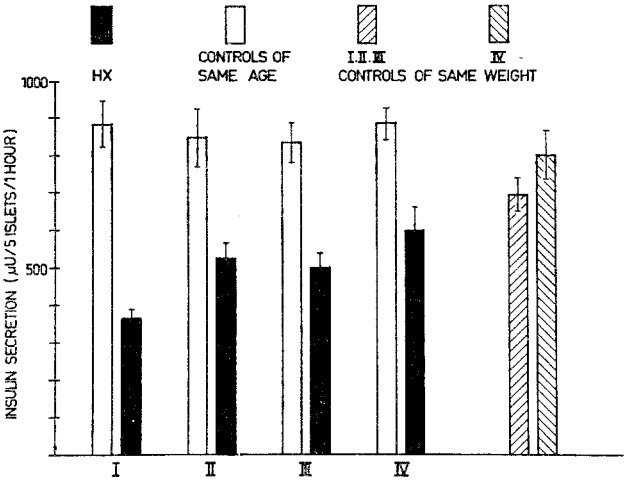

Fig. 1. Insulin secretion during incubation of 5 isolated pancreatic islets in the presence of 300 mg% glucose for 1 h. Results obtained in 4 groups (I—IV) of hypophysectomized rats (HX) and in corresponding control rats of both same age and same weight are shown. Mean ± SEM, number of experiments: n = 12 — 18

Insulin biosynthesis

Only small amounts of ^{3}H-leucine were found in the proinsulin and insulin fraction of the islets at a glucose concentration of 50 mg%. No significant variations were apparent between the individual groups.

Radioactivity incorporated into proteins of the islets at 300 mg% glucose is shown in figures 3 and 4. Only minor differences were observed between the individual control groups (Fig. 3). Following hypophysectomy, radioactivities of proinsulin and insulin were reduced in all 4 groups (Fig. 4) as compared with

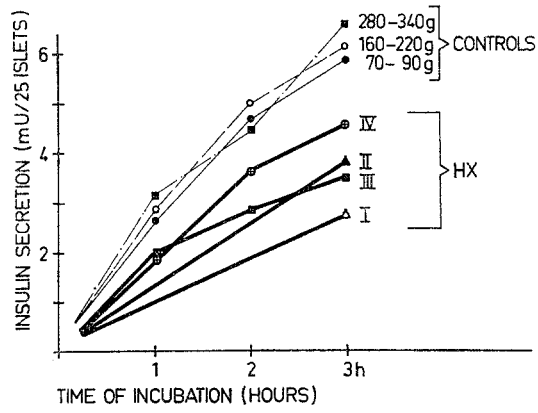

Fig. 2. Insulin secretion during incubation of 25 isolated pancreatic islets in the presence of 300 mg% glucose for 3 h. Results obtained in 4 groups (I—IV) of hypophysectomized rats and control rats of different classes of weight (70—90 g, 160—220 g, 280—340 g) are shown. The numbers of experiments (n) are identical with n in the corresponding experiments of Figs. 3 and 4

the controls. The most marked reduction was observed in group I, the smallest in group IV (to 32 and 68%, respectively). The relation of radioactivity incorporated into proinsulin and insulin did not differ significantly between controls and hypophysectomized rats. It was also essentially the same within the 4 groups of hypophysectomized animals.

Insulin content

Insulin content of the islets did not differ significantly between the individual groups of the control rats. It amounted to 48.7 ± 2.1 mU/25 islets (mean ± SEM). However, it was slightly higher in the oldest control animals. No significant changes were found after hypophysectomy in group II—IV. In group I, a reduction to 84% was observed (41.0 ± 4.6 mU/25 islets).

Discussion

Glucose stimulated insulin secretion from isolated pancreatic islets of hypophysectomized rats was found to be significantly reduced in all our groups as compared with control rats of same age. This observation primarily confirms previous findings obtained in various pancreatic preparations *in vitro* [3, 12, 9, 11]. On the other hand, in our study basal insulin secretion was not influenced by hypophysectomy. The difference

between basal and glucose stimulated secretion of insulin, however, was highly significant in all groups of hypophysectomized rats. The 2 latter findings are not in keeping with the 2 reports of Martin *et al.* [11, 12]. Statistics as well as time of incubation (1—3 h vs. 15 min) might account for these differences. From our physectomy in rats. Impairment of insulin secretion was less pronounced when hypophysectomized rats were compared with normal controls of the same weight. This observation was consistent with the finding that the rate of insulin secretion increased progressively with body weight in normal rats [8].

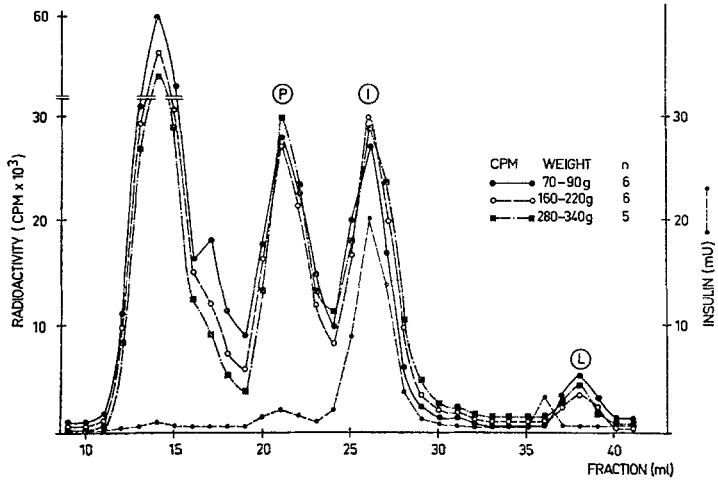

Fig. 3. Radioactivity incorporated into proinsulin (P), insulin (I) and other islet proteins (1st peak) after incubation of 25 isolated pancreatic islets from intact control rats of different weight in the presence of ^{3}H-leucine. Incubations were carried out at 300 mg% glucose for 3 h. Fractionation of islet proteins on a Sephadex G 50 fine column, 0.9 × 55 cm, 1 ml-fractions. L: free ^{3}H-leucine, n: number of experiments. In all experiments of Fig. 3 and 4 immunologically measurable insulin was determined in each fraction and in the pooled peaks. Radioactivity was compared based on the insulin content of the islets

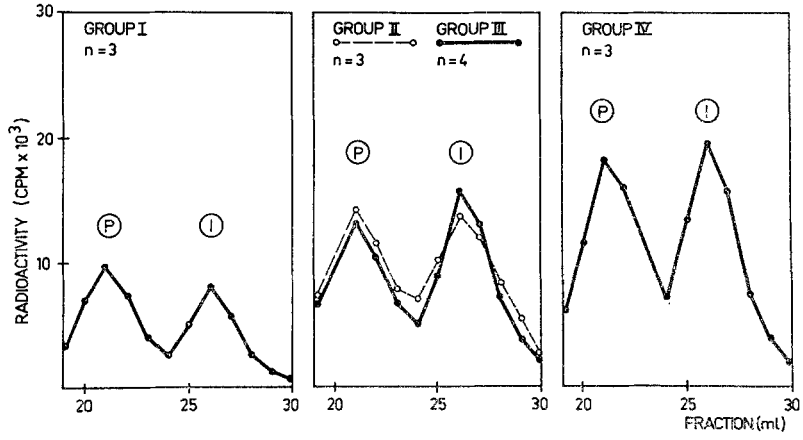

Fig. 4. Radioactivity incorporated into proinsulin (P) and insulin (I) after incubation of 25 isolated pancreatic islets from 4 groups (I—IV) of hypophysectomized rats in the presence of ^{3}H-leucine. Incubations were carried out at 300 mg% glucose for 3 h. Fractionation of islet proteins on Sephadex G 50 fine. Radioactivity was related to the insulin content of the islets. n: number of experiments

study it appears that the reduction of glucose stimulated secretion of insulin depends on the interval following hypophysectomy as well as on the stage of development at the time of hypophysectomy: The most marked reduction was observed in group I (13 weeks after hypophysectomy), the lowest in group IV (oldest rats). Penhos *et al.* [14] came to similar conclusions concerning the progressive impairment of oral glucose tolerance and the time-lag follwing hypo-

As can be concluded from the incorporation of ^{3}H-leucine into the proinsulin and insulin fraction of isolated islets in the presence of 300 mg% glucose, the synthesis of insulin was reduced after hypophysectomy. Reduction of biosynthesis appeared to be associated with impairment of insulin secretion: Maximum decrease in both processes was observed in group I, minimum in group IV (Fig. 1 and 2). However, a well-defined dependence of insulin biosynthesis on glucose

was found in all our 4 test groups, contrary to the reports of Martin [11, 12]. These authors described a lack of stimulation of insulin biosynthesis by glucose following hypophysectomy. Their conclusions derived from *in vitro* work would be difficult to reconcile with the clinical facts. The most likely explanation for this difference is that our incubations were carried out for 3 h, instead of incubating for 2 cycles of 15 min each as done by the other authors. As islets were exposed to ^{3}H-leucine for such a short period, the radioactivity measured by these authors in the total insulin extract of the islets must have been due to labelled proinsulin only [18]. Hence, no conclusions can be drawn from their data as to whether hypophysectomy or growth hormone affects conversion of proinsulin to insulin. In our experiments, the relation between radioactivity incorporated into proinsulin and insulin was found to be unchanged in all control and test groups. Thus, it is unlikely that growth hormone or any other pituitary hormone is influencing the conversion of proinsulin to insulin as such.

Insulin content estimated from batches of 25 isolated islets was found to be diminished by pituitary ablation (to 84%) only in group I, consisting of rats which had been hypophysectomized for as long as 13 weeks. In studies with isolated islets, generally islets of about the same size are collected. Taking this fact into consideration, our data correspond to those of Malaisse *et al.* [9] who found no differences between insulin content of pancreatic pieces from normal and hypophysectomized rats, provided that the insulin values were related to body weight or to the amount of pancreatic tissue.

Our findings of both reduced secretion and biosynthesis of insulin during stimulation with glucose in spite of unchanged insulin content of the islets used are consistent with the hypothesis that hypophysectomy results in a decreased sensitivity of the pancreatic B-cell to glucose and, furthermore, a slower rate of insulin biosynthesis. In the following paper it will be shown that the deficiency of growth hormone rather than that of ACTH might account for these defects.

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