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Effects of Hypophysectomy and Growth Hormone on Cultured Islets of Langerhans of the Rat

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Summary. The effects on islet function of addition to the culture medium of rat growth hormone was studied in 4-day cultured islets of Langerhans from normal and hypophysectomised rats. In islets from hypophysectomised rats, rates of insulin release were 34% lower than in control rat islets; rates of insulin plus proinsulin and total protein biosynthesis were also lower by 48% and 16% respectively. The rates of glucose oxidation and the islet content of cyclic AMP were unchanged in islets from hypophysectomised rats but the islet content of calmodulin was decreased by 68%. The presence of rat growth hormone during the culture period restored the secretory response of hypophysectomised rat islets to that seen in control islets cultured without growth hormone but had only a marginal effect on the rate of insulin plus proinsulin biosynthesis, and no significant effect on islet calmodulin content. Glucose oxidation was increased by the presence of growth hormone during the culture period in both control (73% increase) and hypophysectomised (38% increase) rat islets. Addition of growth hormone to the culture medium also enhanced rates of insulin release and biosynthesis in control islets by 116% and 20% respectively. It is suggested that these changes arise primarily from modification of the synthesis of specific islet proteins.

Key words: Insulin release, insulin biosynthesis, growth hormone, calmodulin, cyclic AMP, islet glucose metabolism, hypophysectomy, cultured islets.

Insulin secretion is subject to acute regulation by nutrients such as glucose and amino-acids and by hormones such as glucagon. In addition B-cell secretory function is influenced chronically by experimental manipulations such as starvation [1] or hypophysectomy [2–5], and in pregnancy [6]. We have shown that the impaired insulin secretory response of islets from hypophysectomised rats persists following 4-day culture of the islets and may be reversed by the addition of growth hormone to the culture medium [2]. To investigate the mechanisms involved, we have measured other parameters of islet function in normal and hypophysectomised rat islets cultured in the absence or presence of rat growth hormone.

Materials and Methods

Islets were obtained by collagenase digestion [7] from the pancreases of male Wistar rats fed on regular rat diet (PRM, Dixon, Ware, Herts, UK). Hypophysectomised rats were purchased from Charles River Laboratories and used after 4–5 weeks from hypophysectomy. Islets were cultured for 4 days as described previously [2]. The culture medium was RPMI 1640 [8] containing glucose (6 mmol/l), penicillin (0.1 mg/ml), streptomycin (0.1 mg/ml) and 10% (v/v) inactivated calf serum (Wellcome, Beckenham, Kent, UK) and was supplemented, where stated, with rat growth hormone 1 μg/ml (National Institutes of Health, Bethesda, USA, lot GH-B-6); the pH was buffered at 7.4 with N-2-hydroxyethylpiperazine-N′-2-ethane sulphonate (Hepes) 25 mmol/l. The cultures were maintained in a metabolic incubator at 37 °C in an atmosphere of humidified air: CO_2 (95 : 5). After culture, islets carefully matched for size were incubated in bicarbonate medium [9] for measurement of various parameters as follows: for insulin release, batches of five islets were incubated for 2 h at 37 °C in bicarbonate-medium containing albumin 2 mg/ml and glucose (2 or 20 mmol/l): insulin released into the medium was measured by radioimmunoassay [10]. For measurements of insulin plus proinsulin and total islet protein synthesis, batches of five islets were incubated in bicarbonate-medium containing glucose (20 mmol/l), albumin (2 mg/ml) and {4,5-^{3}H}-leucine (4 μCi; 50 Ci/mmol) at 37 °C for 90 min. The incorporation of {^{3}H}-leucine into insulin plus proinsulin was measured using insulin-binding affinity columns and into total islet protein by trichloroacetic acid precipitation as previously described [11, 12]. Rates of glucose oxidation were measured as $^{14}CO_2$ evolution from {U-14C} glucose by batches of seven islets incubated for 2 h at 37 °C in bicarbonate-medium containing

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Table 1. The effects of hypophysectomy of the donor rats and of addition of growth hormone to the culture medium on rat islets of Langerhans

Parameter	Normal rat islets		Hypophysectomised rat islets		Statistical significance *(p)*			
	− GH	+ GH	− GH	+ GH	B vs A	D vs C	C vs A	D vs B
Insulin release (μU/islet/h)	70 ± 5 (12)	151 ± 13 (12)	46 ± 3 (12)	74 ± 6 (12)	⩽0.001	⩽0.001	⩽0.001	⩽0.001
Insulin plus proinsulin biosynthesis (dpm/islet per 90 min)	3755 ± 153 (18)	4506 ± 63 (18)	1944 ± 47 (11)	2203 ± 67 (11)	⩽0.001	⩽0.001	⩽0.001	⩽0.001
Total protein biosynthesis (dpm/islet per 90 min)	19540 ± 186 (18)	22087 ± 378 (18)	16367 ± 494 (11)	18014 ± 488 (11)	⩽0.001	⩽0.05	⩽0.001	⩽0.001
Glucose oxidation (pmol/islet per h)	28.6 ± 3.7 (7)	49.5 ± 3.4 (5)	26.2 ± 2.2 (6)	36.2 ± 2.3 (6)	⩽0.001	⩽0.05	NS	⩽0.01
Cyclic AMP content (fmol/islet)	18.7 ± 1.3 (11)	22.6 ± 1.0 (11)	16.9 ± 2.9 (9)	13.8 ± 1.2 (10)	⩽0.05	NS	NS	⩽0.001
Calmodulin content (ng/islet)	1.6 ± 0.31 (9)	2.17 ± 0.47 (9)	0.54 ± 0.16 (11)	0.72 ± 0.16 (11)	NS	NS	⩽0.01	⩽0.05

Islets were prepared by collagenase digestion from normal or hypophysectomised rats and cultured for 4 days in RPMI 1640 [8] in the absence (− GH) or presence (+ GH) of rat growth hormone 1 μg/ml. After culture the indicated parameters were measured as described in the text. The rates of insulin release given are those determined when the medium contained glucose 20 mmol/l: the basal insulin release found with glucose 2 mmol/l was 12.7 ± 3.4 μU/islet per h ($n = 8$) for islets from normal rats cultured without growth hormone and was not significantly different from this value in the other three groups. Rates of incorporation of {^{3}H}-leucine into insulin plus proinsulin and total islet protein and of glucose oxidation are those found in the presence of glucose 20 mmol/l. The cyclic AMP content of similar islets incubated for 12.5 min at glucose 2 mmol/l was not significantly different for any of the groups from the values given here for islets incubated for 10 min at 2 mmol/l followed by 2.5 min at glucose 20 mmol/l. Results are expressed as mean ± SEM for the number of separate batches of islets given in parentheses. Significance of differences was assessed by the two-tailed Student's 't'test. NS = not significant.

albumin (2 mg/ml) and D-{U-^{14}C} glucose (20 mmol/l; 3 mCi/mmol). The $^{14}CO_2$ was absorbed in hyamine and counted by liquid scintillation spectrometry as described by Ashcroft et al. [13]. For measurements of islet content of cyclic AMP, batches of 20 islets were first incubated in 10 μl bicarbonate-medium containing 2 mmol/l of glucose for 10 min at 37 °C and then incubated for a further 2.5 min following addition of 5 μl medium containing glucose 2 or 50 mmol/l. Metabolism was arrested and islets were extracted by addition of 100 μl perchloric acid (5% by volume) followed by sonication at 4 °C. The cyclic AMP was measured by a protein-binding assay [14] using a commercial kit (Radiochemical Centre, Amersham, UK). Appropriate blanks and standards were similarly treated with perchloric acid. For the measurement of islet calmodulin content, batches of 20 islets were extracted in 50 μl Tris-HCl (10 mmol/l; pH 8) by heating for 5 min on a boiling water bath. Aliquots of the extracts were tested for calmodulin by their ability to activate calmodulin-deficient brain phosphodiesterase and quantitated by comparison with standard curves constructed using purified calmodulin as previously described in detail [15].

The results are expressed as mean ± SEM for the number of separate batches of islets given in parentheses. The statistical significance of differences was assessed by the two-tailed Student's 't'-test.

Results and Discussion

Table 1 summarises the effects of hypophysectomy and supplementation of the culture medium with growth hormone on rates of insulin release, insulin plus proinsulin and total protein biosynthesis and glucose oxidation, and on the islet content of cyclic AMP and calmodulin.

The basal rate of insulin release seen with glucose 2 mmol/l was unaffected by hypophysectomy or by addition of growth hormone to the culture medium. However insulin release stimulated by glucose (20 mmol/l) was 34% lower in islets from hypophysectomised rats than in paired control rat islets. For both groups, culture with rat growth hormone augmented the secretory response to glucose by 116% in control islets and by 61% in islets from hypophysectomised rats. The rate of release of insulin from islets from hypophysectomised rats following culture with growth hormone was similar to that from normal rat islets cultured in the absence of growth hormone, but remained 51% lower than that from normal rat islets cultured in the presence of growth hormone. This observation may suggest that the lack of pituitary factors beside growth hormone contributes directly or indirectly to the diminished secretory response of islets from hypophysectomised rats. Earlier studies have shown that glucose-stimulated insulin release is impaired in islets from hypophysectomised rats [2–5] and the impairment is counteracted by injection of growth hormone [2, 4]. The enhancing effect of growth hormone added to the culture medium on the insulin secretory responses to glucose of islets from

both normal and hypophysectomised rats was previously noted [2]: in the present study, the use of rat growth hormone permitted the demonstration of these effects of growth hormone at a lower concentration than that of the bovine growth hormone used earlier [2]. We have now investigated possible mechanisms.

The rate of incorporation of {^{3}H}-leucine into insulin plus proinsulin in the presence of glucose 20 mmol/l was lower by 48% in islets from hypophysectomised rats compared with control rats; for both groups, incorporation was slightly (13–20%) but significantly increased by the presence of growth hormone during the culture period (Table 1). Similar changes were seen at 2 mmol/l glucose although the extent of incorporation was about eightfold lower (results not shown). Incorporation of {^{3}H}-leucine into total islet protein was also influenced in a qualitatively similar manner although the percentage changes were less than for insulin plus proinsulin. Thus the ratio of incorporation of {^{3}H}-leucine into insulin plus proinsulin to that into total islet protein was decreased from 0.195 ± 0.007 in control islets to 0.120 ± 0.002 in islets from hypophysectomized rats ($p < 0.001$). In neither group was this ratio significantly affected by the presence of growth hormone in the culture medium. The combination of decreased insulin biosynthesis with decreased insulin secretion may underlie the reported lack of effect of hypophysectomy on the islet content of insulin [2]. In freshly prepared rat islets, insulin biosynthesis was decreased by hypophysectomy of the donor rats but the incorporation of {^{3}H}-uridine into islet total RNA was unaffected [16]. As for insulin release, the present findings imply that the enhancing effect of growth hormone on insulin biosynthesis was not simply a reversal of the effect of hypophysectomy since the biosynthesis rate, although increased slightly by growth hormone, remained well below that of control islets.

Since rates of release and biosynthesis of insulin in response to glucose are both critically dependent on rates of islet glucose metabolism [17], it was important to assess the effects of hypophysectomy and growth hormone on islet glucose metabolism. No significant difference was found between rates of glucose oxidation by islets from control and hypophysectomised rats after culture in the absence of growth hormone (Table 1). Hence decreased glucose metabolism does not underlie the reduced rates of insulin release and biosynthesis in islets from hypophysectomised rats in agreement with studies [4] on freshly prepared islets. However the presence of growth hormone during the culture period increased the observed rates of glucose oxidation for both groups; the increase was most marked for the islets from normal rats.

In view of the importance of cyclic AMP as a modulator of insulin secretion, and of reports of long-term changes in the adenyl cyclase system in situations such as starvation [18], we measured islet cyclic AMP content. Islets after culture were incubated either for 12.5 min with 2 mmol/l glucose or for 10 min with 2 mmol/l glucose followed by a further 2.5 min with 20 mmol/l glucose. In agreement with other reports on cultured islets [19, 20], glucose did not elevate the islet content of cyclic AMP. As shown in Table 1, islet cyclic AMP content was not significantly different in islets from hypophysectomised rats compared with control islets. When growth hormone was present during the culture period the islets from normal rats had a greater content of cyclic AMP than islets from hypophysectomised rats. However it is unlikely that such an effect can explain the enhancing effect of growth hormone on insulin secretion since in the hypophysectomised group the effect of growth hormone addition was actually to lower somewhat the cyclic AMP content. It has previously been reported that basal adenyl cyclase activity in rat islets is unaffected by culture for 16 h with growth hormone although total adenyl cyclase was increased [21].

Evidence has been presented [15, 22] that calmodulin may play a key role in stimulus-secretion coupling in the B-cell. We therefore measured the concentration of calmodulin in the cultured islets. It was found (Table 1) that the islets from hypophysectomised rats had a significantly decreased content of calmodulin compared with control islets. The presence of growth hormone during the culture period did not significantly affect the calmodulin content of islets from either control or hypophysectomised rats.

The expression of results of this study on a per islet basis requires justification, since any change in islet size or B-cell content following hypophysectomy would be reflected in apparent changes in the parameters studied. In order to exclude this possiblility, in all experiments islets from hypophysectomised rats were carefully matched for size with control islets using an eye-piece micrometer on the dissecting microscope. It was thus possible to obtain a mean islet diameter that was constant in all experimental groups. Moreover, the islet insulin content was also not significantly different in any of the experimental groups. Others have shown [23] that the mean DNA per islet is not significantly different in islets from hypophysectomised rats compared with control islets. Thus the impaired secretion response of islets from hypophysectomised rats is not attributable to change in islet size or insulin content.

From the present and earlier observations [2, 4] the following conclusions seem warranted. Hypophysectomy produces a long-lasting impairment of the ability of B-cells to synthesize and secrete insulin in re-

sponse to glucose. These changes are not explicable by changes in islet glucose or cyclic AMP metabolism. The decreased secretory response may be ascribed, at least in part, to a decrease in islet calmodulin content. Since calmodulin does not appear to play a role in glucose-stimulated insulin biosynthesis [22], the decreased rate of insulin biosynthesis in islets from hypophysectomised rats may occur in parallel with rather than consequential to the diminished calmodulin content. As a minimal hypothesis, therefore, we may speculate that hypophysectomy impairs rather specifically the synthesis of proteins involved in secretion, including calmodulin and insulin itself; the diminished secretory response may result from these changes. Although growth hormone clearly has a direct effect on islet function our findings suggest that this is not simply a reversal of the effects of hypophysectomy. Thus, although both biosynthesis and secretion of insulin by islets from hypophysectomised rats are enhanced by culture with growth hormone, the rates do not attain those seen in normal islets cultured with growth hormone. Moreover the islet content of calmodulin remained low in the islets from hypophysectomised rats after culture with growth hormone. The observed changes in insulin biosynthesis and secretion were associated with, and could plausibly result from, the greatly increased rate of glucose oxidation in islets cultured with growth hormone; we suggest therefore that growth hormone in vitro may elicit increased synthesis of enzyme(s) catalysing a rate-limiting step for islet glucose-metabolism, in particular the high Km glucose-phosphorylating activity [24]. Such a hypothesis is open to experimental testing.

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