STUDIES ON THE LOCAL (PARACRINE) ACTIONS OF GLUCAGON, SOMATOSTATIN AND INSULIN IN ISOLATED ISLETS OF RAT PANCREAS

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1. Introduction

In isolated pancreatic islets, we found that insulin biosynthesis and release were stimulated by addition of glucagon [1] whereas insulin release could be inhibited by exogenous somatostatin [2,3] but not by insulin itself [4]. On the other hand, in attempts to bind endogenous islet hormones by incubating islets with corresponding antisera, conflicting results concerning insulin release were obtained with anti-somatostatin serum [5,6]. No such data were available about the influence of endogenous islet hormones on insulin biosynthesis or somatostatin release. Therefore, we decided to study insulin biosynthesis and release as well as somatostatin release from isolated rat islets during incubation with antisera against glucagon, somatostatin and insulin.

2. Material and methods

Islets were isolated by collagenase from the pancreata of male rats (strain FW 49 Lemgo-Kirchb.-Bib.) fed ad libitum. Batches of 20 islets were incubated in 1 ml gassed, supplemented Krebs-Ringer-bicarbonate buffer (pH 7.4) at 50 or 300 mg/100 ml glucose together with [^{3}H]leucine (50 μCi) and the hormone antisera or normal control sera (see below). Hormone release was determined in aliquots of the incubation media by radioimmunoassay. (Pro-)Insulin biosynthesis was estimated from incorporation data for [^{3}H]leucine into the proinsulin and insulin fraction of the islet proteins after extraction and fractionation on a Sephadex G-50 column. All these procedures were performed as in [1].

Rabbit antiglucagon serum (K30) was purchased from Serono, Freiburg (lot no. 2903-1). The 3.5 μl of this antiserum which were added to 1 ml incubation medium exhibited a binding capacity of 2.5 ng glucagon. Anti-somatostatin serum from the rabbit was a gift of Professor Arnold, Göttingen. The 5 μl of this antiserum added to 1 ml medium bound 10 ng somatostatin. Guinea pig antiserum to bovine insulin was raised by Dr Bretzel from our group. The 5 μl antiserum used in 1 ml medium bound 5 mU (~200 ng) insulin.

Statistical evaluations were carried out by the paired or unpaired Student's *t*-test.

3. Results

Presence of anti-glucagon serum resulted in a significant reduction of both glucose-stimulated insulin release and biosynthesis. At the sub-stimulatory glucose level of 50 mg/100 ml, no changes were observed (fig.1). Glucose-stimulated somatostatin release was significantly depressed in the presence of anti-glucagon serum (table 1).

With anti-somatostatin serum, an enhancement of insulin release provoked by 300 mg glucose/100 ml was found whereas insulin release at 50 mg glucose/100 ml and proinsulin–insulin biosynthesis at both glucose levels did not change (fig.2).

As was expected and can be seen from fig.3, no insulin was measurable by immunoassay in the media in which anti-insulin serum was present during the incubations. Glucose (300 mg/100 ml)-stimulated proinsulin–insulin biosynthesis was found to be unchanged. There was also no significant difference in glucose-stimulated somatostatin release: It amounted to 107 ± 10 and 172 ± 20 pg/20 islets after

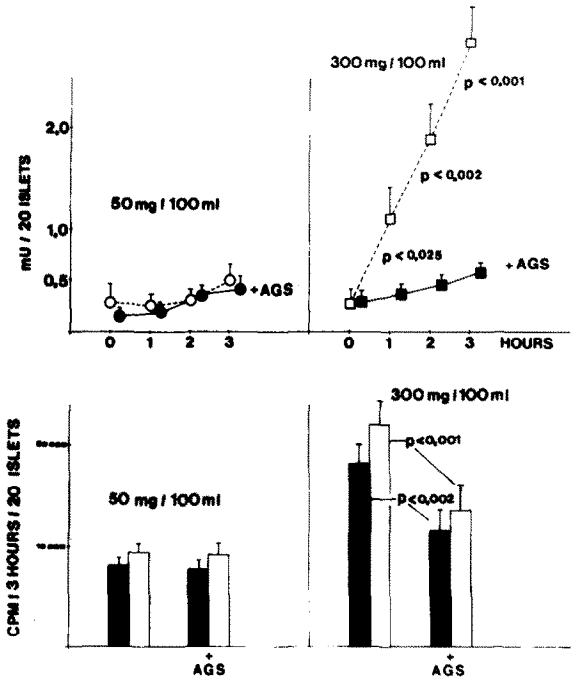

Fig.1. Influence of anti-glucagon serum on insulin release (upper part) and proinsulin–insulin biosynthesis (black and open bars, respectively; lower part). Isolated rat islets were incubated for 3 h at 50 or 300 mg glucose/100 ml together with anti-glucagon serum from the rabbit (+AGS) or normal rabbit serum. Mean ± SEM, $n = 7$.

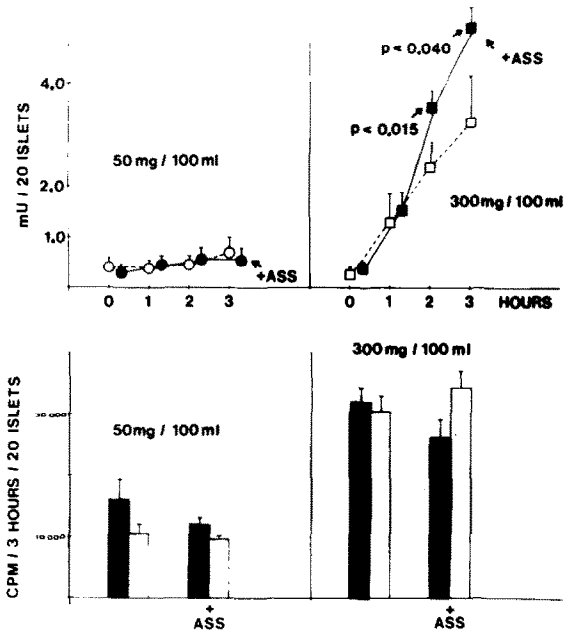

Fig.2. Influence of anti-somatostatin serum on insulin release (upper part) and proinsulin–insulin biosynthesis (black and open bars, respectively; lower part). Isolated rat islets were incubated for 3 h at 50 or 300 mg glucose/100 ml together with anti-somatostatin serum from the rabbit (+ASS) or normal rabbit serum. Mean ± SEM, $n = 7$.

1 and 3 h in the controls and to 89 ± 15 and 225 ± 42 pg/20 islets after 1 and 3 h in the presence of anti-insulin serum, respectively (mean ± SEM, $n = 12$).

4. Discussion

It thus appears that endogenous glucagon is indeed involved by local (paracrine) action in regulation of insulin release as well as (pro-)insulin biosynthesis (fig.1) and also somatostatin release (table 1). Such local effects have long been assumed to exist from the results of studies on the influence of added exogeneous glucagon on the regulation of both the B-cell (cf. [1]) and D-cell function [7–9], and also from morphologic findings [10]. A decrease in insulin release in the pres-

Table 1
Somatostatin (pg) secreted into the incubation media of 20 isolated rat islets incubated for 3 h in the presence of rabbit anti-glucagon serum or normal rabbit serum (controls)

Glucose (mg/100 ml)	Mean ± SEM, $n = 12$		
	Controls		Anti-glucagon serum
50	175 ± 29		207 ± 30
	$p < 0.01$		
300	290 ± 28	$p < 0.01$	184 ± 23

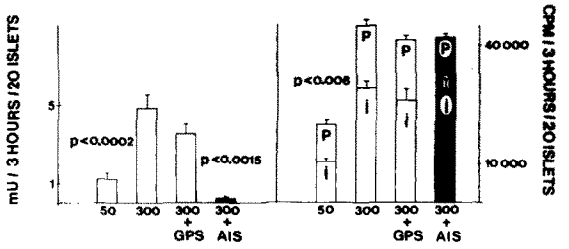

Fig.3. Influence of anti-insulin serum on (pro-)insulin biosynthesis. Left, insulin measured by radioimmunoassay in the media; right, proinsulin (P)–insulin (i) biosynthesis. Isolated rat islets were incubated for 3 h at 50 or 300 mg glucose/100 ml alone or together with guinea pig anti-insulin serum (+AIS) or normal guinea pig serum (+GPS). Mean ± SEM, $n = 11–16$.

ence of anti-glucagon serum has also been reported in abstract form, however, no effect at high glucose concentration was found [11].

Our result that glucose stimulates somatostatin release (table 1) confirms the findings in [12,13], however, it is at variance with those of in [6,14]. The increase of glucose-stimulated insulin release but not of biosynthesis observed in the presence of anti-somatostatin serum (fig.2) agrees with current views on the involvement of somatostatin in regulation of B-cell function; whereas insulin release is obviously controlled by the hormone of the D-cells (cf. [15]), this appears not to be the case for (pro-)insulin biosynthesis (unpublished, [16–18], but cf. [19]). In [5,20] an increase in insulin release with antisomatostatin serum was also observed but only at a sub-stimulatory or intermediate but not at high glucose concentration. However, the experimental design in [5,20] differed from ours; they preincubated the islets with the antiserum and tested insulin release thereafter without antiserum in the presence of glucose or/and leucine only. Work with precultured rat islets [6] revealed no influence of anti-somatostatin serum on insulin output. But in [6], addition of somatostatin also did not change insulin release from the islets.

To our experience, antisera against somatostatin should always be tested for cross-reactivity against glucagon. In contrast to the anti-somatostatin serum used here, a commercially available anti-somatostatin serum (which we had taken for a first series of experiments) bound, to our surprise, large amounts of glucagon, although synthetic somatostatin had been used for immunisation according to the information given by the producer.

The presence of insulin antibodies and the binding of all secreted insulin by anti-insulin serum did not influence (pro-)insulin biosynthesis (fig.3), arguing against an immediate feedback inhibition of insulin secretion on its own synthesis [4,21], an action which has been claimed, however for proinsulin [22]. Indirect evidence for the absence of such a direct, negative feedback emerges also from studies of the effects of addition of insulin antibodies to the culture media of islets [23]. The fact that there was no significant change in somatostatin release in the presence of anti-insulin serum might be taken as an argument against a local (paracrine) influence of insulin on somatostatin release. Such a notion would agree with numerous negative results obtained with exogenous insulin concerning somatostatin secretion ([7,9,15] but cf. [24]).

It can be concluded that, by local action:

1. Endogenous glucagon stimulates insulin biosynthesis and release as well as somatostatin release;
2. Somatostatin reduces release but not biosynthesis of insulin; and
3. Insulin has no direct action on its own biosynthesis. No influence of insulin on somatostatin release is apparent.

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