Evidence for Decreased Sensitivity to Glucose of Isolated Islets from Spiny Mice* (*Acomys cahirinus*)

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Summary. Immunoreactive insulin (IRI) release from collagenase isolated pancreatic islets of spiny mice (*Acomys cahirinus*) was examined in 15 min and 2 h incubations at glucose concentrations between 2.8 and 56 mM. The resultant glucose-insulin dose-response curves for *Acomys* islets were compared to those for similarly incubated rat islets. After 15 min incubations, IRI release from *Acomys* islets was significantly lower than that from rat islets at all stimulating glucose concentrations. After 2 h incubations, however, maximal responses were similar for *Acomys* and rat islets, whereas the sensitivity of *Acomys* islets to glucose was significantly less. Cyclic AMP 10 mM, theophylline 5 mM, arginine 10 mM, and cytochalasin B 10 µg/ml, all enhanced IRI release in the

presence of glucose 16.7 mM to a relatively greater extent from *Acomys* than from rat islets. Vincristine 10^{-5} M reduced IRI release from both *Acomys* and rat islets, and this to a similar extent. The results suggest that defective IRI release from *Acomys* islets may be the expression of decreased B-cell sensitivity to glucose, even though IRI release from *Acomys* islets may improve in time with continued exposure to elevated glucose concentrations. The ability of B-cells of *Acomys* to recognize other agents that enhance the action of glucose appeared unaltered.

Key words: Spiny mice (*Acomys cahirinus*), isolated islets, glucose, theophylline, arginine, cytochalasin B, vincristine, insulin release *in vitro*, sensitivity to glucose.

Introduction

The pancreas of the rodent *Acomys cahirinus* of the Geneva colony is characterized histologically by marked hyperplasia of the islets of Langerhans. The immunoreactive insulin (IRI) content of the pancreas is correspondingly elevated [10, 13, 23]. Nevertheless, *in vivo* studies in the *Acomys* have demonstrated that IRI response to insulinotropic agents is slower and smaller [2, 3, 11] than in other rodents. The purpose of the experiments with collagenase isolated islets reported here was to assess whether the low IRI responses observed *in vivo* could also be demonstrated *in vitro*.

Materials and Methods

Animals

Thirty-one 2–3 month old male *Acomys* were used. Bred in our laboratory, they were fed *ad libitum* and weighed 28 to 44 g; at decapitation their plasma glucose was 152 ± 4 mg/100 ml. Comparison was with

male albino Wistar rats, weighing 150–200 g. Their plasma glucose averaged 126 ± 6 mg/100 ml.

Experimental Procedures

Islets were isolated using collagenase according to Lacy *et al.* [15] either from a single *Acomys* pancreas, or from pancreata pooled from two rats. 10 islets were placed into each incubation flask, containing 1 ml Krebs-Ringer bicarbonate buffer with bovine serum albumin 5 mg/ml and Trasylol 500 units/ml, kindly provided by Dr. H. Ruef, Bayer Pharma, A.G., Zurich. The flasks were incubated for 15 min or for 2 h in a Dubnoff shaking incubator at 37°C, and the atmosphere above the open flasks was continuously gassed with O₂/CO₂:93/7. Each different condition or agent was tested in at least 5 flasks in a given experiment.

Measurements

Insulin (IRI) released into the medium was determined by the charcoal separation radioimmunoassay method of Herbert *et al.* [12]. Islet IRI content was also measured on groups of 10 islets (not incubated) using acid-ethanol extraction according to Scott and Fisher [21]. IRI of *Acomys* was assayed using anti-beef insulin antibody and sheep insulin standard [2] while rat IRI was measured using anti-pork insulin antibody and rat insulin standard. The antibodies were kindly donated by Dr. Peter Wright, Indianapolis, Indiana, and the insulin standards by Dr. J. Schlichtkrull, Novo Industri, Copenhagen, Denmark. Islet ATP content was assayed by the luciferin-luciferase method of Kilbert *et al.* [14]. For statistical evaluation Student's unpaired t-test was used.

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Reagents and Chemicals

Theophylline and arginine hydrochloride were purchased from Merck and Co., Darmstadt, Germany; adenosine 3',5'-cyclic monophosphate (cyclic AMP), dimethylsulfoxide (DMSO) and perchloric acid from Fluka A.G., Buchs, Switzerland; cytochalasin B from Imperial Chem. Industries, Macclesfield, England; crude collagenase from Worthington Chem., New Jersey; firefly lantern extract from Sigma Chemical Corporation, St. Louis, Missouri; ^{125}I -insulin from Cambridge Nuclear, Massachusetts; bovine serum albumin

cantly different from that of rat islets (289 ± 17 ng/10 islets, $n = 34$), although the number of islets isolated from any one *Acomys* pancreas was approximately double that isolated from any one rat pancreas. The similarity of islet IRI content permitted the use of equal numbers of islets from each species (10 per vial) in the secretion studies described below.

Glucose Dose-Response Studies

In order to characterize the dose relationships for the early and late IRI responses, islets were incubated

Fig. 1. Glucose-insulin (IRI) dose-response curves from rat [○—○] and *Acomys* [●—●] islets incubated for 15 min. The p-values between the two curves indicate the degree of statistical difference between IRI release for the two species at corresponding glucose concentrations. IRI release values significantly ($p < 0.05$) higher than those at glucose 2.8 mM are indicated as ○ or as ●. Numbers in parentheses denote the number of experiments

Fig. 2. Glucose-insulin (IRI) dose-response curves from islets incubated for 2 h. Data are presented as in Fig. 1. Note the smaller scale for IRI release

(fraction V), from Behringwerke, Marburg, Germany. Vincristine sulfate, lot NDC 2-N55-A (Oncovin, Eli Lilly), was a gift from Schweiz. Serum- und Impfstoffinstitut, Bern, Switzerland.

Results

Insulin Content of the Islets

The acid-ethanol extractable IRI content of *Acomys* islets (299 ± 10 ng/10 islets, $n = 88$) was not signifi-

in the presence of different concentrations of glucose, either for 15 min (reflecting mainly the early response) or for 2 h (mainly the late response). Fig. 1 shows the glucose-IRI dose-response curves during the 15 min incubations for each species. The lowest glucose concentration which resulted in significant stimulation of IRI release above basal levels was 11.2 mM for *Acomys* islets ($p < 0.05$ vs. basal release), and 8.4 mM for rat islets ($p < 0.01$). For both species the plateau of maximal release was reached at 16.7 mM (the higher levels noted at 28.0 mM glucose for rat islets were not signifi-

Table 1. Effect of cyclic AMP, theophylline, arginine, and cytochalasin B on IRI release in the presence of glucose (G) 2.8 and 16.7 mM

Agent (A)	Rat				Acomys			
	G2.8 mM	G2.8 mM + A	p	G16.7 mM + A	G2.8 mM	G2.8 mM + A	p	G16.7 mM + A
Cyclic AMP 10 mM	6.0 ± 1.3 (6)	5.9 ± 0.6 (5)	N.S.	123.1 ± 5.8 (7)	5.4 ± 1.2 (5)	7.2 ± 0.5 (5)	N.S.	60.0 ± 10.7 (6)
Theophylline 5 mM	4.6 ± 0.4 (5)	5.7 ± 0.6 (6)	N.S.	174.3 ± 10.8 (6)	3.1 ± 1.0 (6)	4.3 ± 1.2 (5)	N.S.	50.5 ± 2.5 (6)
Arginine 10 mM	3.4 ± 0.3 (6)	16.1 ± 3.4 (6)	< 0.001	112.0 ± 9.0 (8)	3.6 ± 0.7 (12)	15.3 ± 3.4 (12)	< 0.001	103.8 ± 6.4 (12)
Cytochalasin B 10 µg/ml	4.8 ± 1.1 (6)	3.4 ± 0.4 (6)	N.S.	134.5 ± 12.6 (14)	2.9 ± 0.5 (6)	2.1 ± 0.2 (5)	N.S.	85.8 ± 8.1 (5)
								155.8 ± 5.8 (6)
								< 0.001

cantly different from the values at 16.7 or 56.0 mM). Also, the glucose concentration inducing half-maximal insulin release was similar in both species (around 10 mM). Thus, during a short incubation (15 min), *Acomys* islets exhibited a higher threshold for glucose and significantly lower rates of insulin release at all stimulating glucose levels by comparison with rat islets ($p < 0.01$).

The insulin release responses observed during 2 h of incubation are shown in Fig. 2. Again, the threshold glucose concentration eliciting IRI release above basal levels was higher for *Acomys* islets (8.4 mM, $p < 0.05$ vs. basal release) than for rat islets (5.6 mM, $p < 0.01$). The maximal IRI response of *Acomys* islets to glucose, however, was similar to that of rat islets, although this maximal response was observed at 28 mM glucose for *Acomys*, as compared to 16.7 mM for rat islets. Half-maximal response was seen at about 12.0 mM glucose for *Acomys* islets and 9.5 mM for rat islets. Thus, over 2 h of incubation, the glucose-IRI dose, response curve for *Acomys* islets was shifted to the right of that for rat islets, still indicating a decreased sensitivity of the *Acomys* islets to glucose, even though the maximal response reached did not differ from that of rat islets.

Enhancement of the Late Insulin Response by Agents Other than Glucose

The effect of some agents known to enhance the insulin response to glucose was evaluated in islets incubated for 2 h in the presence of either low (2.8 mM) or high (16.7 mM) glucose (Table 1). The islets used for comparison between different experimental conditions were obtained from the same collagenase isolation batch. Cyclic AMP (10 mM) did not modify the response to glucose 2.8 mM in either species, nor did it significantly increase the IRI response to glucose 16.7 mM in rat islets (+14%, NS). However, it augmented significantly the IRI release at high glucose concentration in *Acomys* islets (+90%, $p < 0.01$). Theophylline 5 mM, an agent that raises the intracellular concentration of cyclic AMP [1], had no effect on IRI release at glucose 2.8 mM, but enhanced the IRI secretion at glucose 16.7 mM in both species, the effect being greater in *Acomys* islets (+68%, $p < 0.01$) than in rat islets (+31%, $p < 0.05$). Arginine 10 mM had similar pronounced effects on IRI release at glucose 2.8 mM in both species (+300%, $p < 0.001$). In the presence of glucose 16.7 mM it stimulated *Acomys* islets (+39%, $p < 0.001$) to a greater extent than rat islets (+18%, NS). Cytochalasin B, an agent known to affect the microfilamentous system and to enhance glucose-induced IRI release [17, 24] had no effect on basal IRI release, but augmented the response to high glucose in both species. Again, the effect was greater in *Acomys* islets (+82%, $p < 0.001$) than in rat islets (+40%, $p < 0.01$).

Effects of Vincristine

(Fig. 3). All incubations were carried out at glucose 16.7 mM for 2 h. Vincristine, at the concentration of

10^{-5} M, slightly but significantly reduced the stimulating effect of glucose 16.7 mM on IRI release by islets of both species (-25 to -30% , $p < 0.05$). Increasing the concentration to 10^{-4} M did not reduce IRI release any further, but significantly decreased islet ATP levels, again in both species (-25 to -30% , $p < 0.01$).

Fig. 3. IRI release (upper panel) and intracellular ATP levels (lower panel) from rat and acomys islets incubated for 2 hrs in the presence of glucose 16.7 mM. The Vincristine (Vc) concentrations varied between 0 and 10^{-4} M. Values above bars indicate significant differences from control (absence of Vincristine, set as 100%) for both species

Discussion

The results presented in this report show clearly that the low insulin responses observed *in vivo* in *Acomys* [2, 3, 11] are also to be seen *in vitro*. This decreased responsiveness is thus not likely to be due to modifications in the metabolism of the hormone but may well be a characteristic of the pancreatic B-cells of *Acomys*. Whether the choice of the rat as the control animal was appropriate or not is, of course, open to question. However, since the islets of these two species contain similar amounts of insulin, in spite of the marked differences in body size of their hosts, this comparison appears justified.

The striking finding in this comparison is the difference between the two species in their respective glucose-insulin dose response relationship. Both regarding the early (15 min) and prolonged (2 h) insulin responses, *sensitivity* of the *Acomys* islet was less, as evidenced by the higher glucose "threshold" values needed to induce stimulation of insulin release. By contrast, whereas the maximal *Acomys* response at 15 min was much smaller than that of rat islets, after 2 h the islets of both species reached comparable

maximal rates of release (albeit at different glucose levels). Thus, in the present *in vitro* system, while both the early and the late phase insulin responses of *Acomys* islets exhibited decreased sensitivity to glucose (as compared to the sensitivity of rat islets), decreased *capacity* (i.e. a low maximal response) was observed only for the early insulin release phase.

Observations in man [8] may suggest an explanation for the augmentation in maximal response seen during prolonged incubation of *Acomys* islets with glucose. It was shown that glucose, with time, enhances markedly its own effect on insulin release. Applying these concepts to the insulin response of *Acomys*, the data available support the hypothesis of impaired ability to recognize glucose as an initiating signal, while recognition of the time-related enhancing action of the sugar may be intact. Support for this interpretation derives from the observation that all agents which have been tested because of their known enhancing action on glucose-induced IRI release were effective in islets of *Acomys*, indeed more effective than was true for islets from rats, when considering the prolonged (two hours) incubations. The agents tested were cyclic AMP, theophylline, arginine and cytochalasin B. The point to be emphasized here is the presence of at least normal enhancement by agents other than glucose, not the still tentative demonstration of a greater than normal one. Before accepting the latter observation the comparison with rat islets will have to be carried out under a range of different experimental conditions, since the present one may not be optimal for the demonstration of enhancement in islets from rats. It may nevertheless be concluded that *Acomys* islets do not show impairment in the recognition of agents which modulate the effects of glucose.

The cause of the lower insulin release from the B-cells of *Acomys* in response to glucose remains obscure. Several possibilities have been discussed previously [20] one of them being that suggested by Malaisse-Lagae *et al.* [18] who demonstrated that the number of Vincristine-induced crystals was much less in *Acomys* B-cells than in those of other rodents. A possible interpretation of that observation is that a deficiency of the microtubular system might be involved in producing the characteristic insulin release pattern of *Acomys*. The results reported here with vincristine neither support nor invalidate this view, as sensitivity of insulin release to vincristine was similar in *Acomys* and rat islets; toxic effects of the drugs, in terms of decrease in ATP, also occurred at equal concentrations in both species, thereby suggesting that the penetration of vincristine across the B-cell membrane was not altered.

In closing, a brief discussion of purely speculative nature may be in order. The insulin release pattern observed in *Acomys* appears to resemble that observed at various stages of the diabetic syndrome in man [4, 19, 22]. Decreased insulin response to glucose administration, much more marked for the initial response phase, is frequently found in subjects with latent or

chemical diabetes as well as in patients with maturity-onset diabetes. The same pattern, and again it is the pattern seen in *Acomys* as well, has been described in a number of subjects with entirely normal glucose tolerance in whom, however, the probability to develop diabetes at a later stage is greater than for the general population [7]. In the studies referred to in man, decreased sensitivity to glucose in terms of insulin release, as in the present study, was demonstrated [6]. Furthermore, the enhancing effect of agents such as theophylline [5] or arginine [9] was either intact or augmented in the subjects exhibiting a decreased insulin response to glucose alone. Despite all the qualifications which must be kept in mind before information obtained in one species may be transferred to another, the results described in this report clearly encourage us in continued attempts to analyze *Acomys* as completely as possible, as one possible model for some aspects of diabetes-related endocrine function.

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