Defective Early Phase Insulin Release in Perfused Isolated Pancreatic Islets of Spiny Mice (*Acomys Cahirinus*)*

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Summary. In order to characterize pancreatic beta cell function in Geneva bred spiny mice (*acomys cahirinus*), the dynamics of immunoreactive insulin release were examined during perfusion of pancreatic islets isolated from normoglycemic *acomys*. The initial insulin response of *acomys* was slow: no clear-cut early (1 to 10 min) peak of insulin release was observed when glucose in the perfusion medium was abruptly raised from 2.8 mM to concentrations as high as 56 mM. This was true for islets of either young, or older more obese *acomys*. However, after 20 to 30 min of perfusion at the high glucose concentrations, the rate of insulin release from *acomys* islets became similar to that from islets of rats or mice. By contrast, glucose-induced insulin release responses observed with islets of Wistar-derived rats, Swiss albino mice, and inbred C57 BL/6J lean or obese (*ob/ob*) mice, were clearly biphasic. Tolbutamide 1.5 mM, arginine 16 mM, and theophylline 10 mM were ineffective in stimulating insulin release from *acomys* islets in the presence of a

substimulatory glucose concentration (2.8 mM), whereas these agents were effective in rat islets at the same substimulatory concentration of glucose. On the other hand, when these agents, as well as cyclic AMP 10 mM or cytochalasin B 10 µg/ml were applied in the presence of a stimulating concentration of glucose (16.8 mM), the glucose-stimulated insulin release from *acomys* islets was increased to the same or to a greater extent than from rat islets. It is suggested that the failure of all the agents tested to stimulate an early rapid phase of insulin release from *acomys* islets may be secondary to the observed initial insensitivity to glucose, which insensitivity may in turn reflect a selective impairment in the recognition of glucose as an insulinogenic signal in this species.

Key words: Insulin secretion, perfused islets, spiny mouse (*acomys cahirinus*), obese (*ob/ob*) C57 BL/6J mice, glucose, tolbutamide, arginine, theophylline, cyclic AMP, cytochalasin B.

Previous studies of the spiny mice (*acomys cahirinus*) in our colony have established that the *acomys* species is one of several among small rodents that may develop a diabetes-like syndrome of spontaneous inappropriate hyperglycemia and obesity [1, 2, 3], at least under certain conditions and when bred in the laboratory. Furthermore, low insulin secretory responses *in vivo* (by comparison with mice and rats) have been demonstrated even in normoglycemic *acomys* [4, 5], in spite of the considerable hyperplasia of the pancreatic islets of Langerhans and the increased insulin stores characteristic of this species [1, 2]. Our aim in the present experiments was to characterize the insulin secretory response of *acomys* more directly, using isolated pancreatic islets *in vitro*, the comparison being again with rats and mice. Since a

number of studies have indicated that the dynamics of insulin release are modified in the diabetic syndrome in man [6–11], and in other species [12], an experimental procedure that allowed for analysis of insulin release kinetics, i.e. islet perfusion, was selected.

Material and Methods

Animals

The characteristics of the *acomys cahirinus*, rats, and mice used in this study are shown in Table 1. *Acomys*, of both sexes, were obtained from the colony bred in our laboratory, as were the Swiss albino mice, and Wistar-derived rats. Male obese (*ob/ob*) C57 BL/6J mice and their appropriate lean controls were purchased from the Jackson Laboratories, Bar Harbour, Maine, at the age of 6 weeks, and used at 20 weeks of age.

Islets were obtained from all animals in the fed state, between 9 and 10 am. Insulin responses from islets of young *acomys* (8–14 weeks) were compared to those from rat and albino mouse islets, whose insulin contents were similar to that of the young

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Table 1. *Characteristics of *acomys cahirinus*, rats and mice used in this study*

Species and strain	Age	Body weight	Plasma Glucose ^a	Islet insulin content ^b
	(wks)	(g)	(mg/100 ml)	(ng/islet)
<i>Acomys cahirinus</i>	8–14	25– 40	145 ± 7 (12)	19.8 ± 2.5 (23)
<i>Acomys cahirinus</i>	22–36	60– 90	181 ± 12 (9)	36.3 ± 4.8 (7)
Wistar-derived rats	6–12	150–200	129 ± 8 (6)	21.3 ± 2.0 (21)
Swiss albino mice	8–14	25– 35	157 ± 11 (8)	18.8 ± 2.8 (15)
Lean +/+ C57BL/6J mice	20	24– 27	158 ± 11 (4)	19.5 ± 3.5 (7)
Obese <i>ob/ob</i> C57BL/6J mice	20	42– 46	224 ± 14 (4)	92.8 ± 8.0 (6)

^a Mean (± SEM) values in the fed state, at the time of islet isolation; numbers of animals shown in brackets.

^b Mean (± SEM) values for 40 islets (not perfused); numbers of estimations shown in brackets.

acomys. Also, the mean (± SEM) plasma glucose concentration of the young *acomys* (145 ± 7 mg/100 ml) was intermediate between that of the rats and albino mice, and not significantly different from either. The older *acomys* (22–36 weeks) were more obese (60–90 g), and exhibited a significantly elevated mean (± SEM) plasma glucose concentration (181 ± 12 mg/100 ml, $p < 0.02$), and approximately double the islet insulin content compared to young *acomys*. Insulin responses from islets of these older, more obese *acomys* were compared to those from islets of these obese (*ob/ob*) C57 BL/6J mice, who exhibited still greater elevations of plasma glucose and islet insulin content.

Experimental Procedures

Pancreatic islets of Langerhans were isolated from the different rodents by a modification of the collagenase (Worthington Chemical Corp., Freehold, N. J.) method of Lacy and Kostianovsky [13]. Pancreatic digestion (at 37° C) and subsequent isolation of the islets (at 4° C) was carried out in Hank's solution containing glucose 2.8 mM and gassed with 95% O₂:5% CO₂ (pH 7.4). After digestion (4 to 6 min, with 7 mg collagenase per ml), the pancreatic sediment was centrifuged and washed four times, again with centrifugation, then transferred to a blackbottomed Petri dish. By visualization through a binocular dissecting microscope, and using a 10 µl micropipette, the islets were then further separated from any surrounding exocrine tissue by three successive isolations and transfers into Petri dishes containing fresh Hank's solution. For each perfusion experiment, the islets used could be obtained from a single *acomys* pancreas, or from pancreases pooled from 2 rats, 4 albino mice, 4 C57 BL/6J lean mice, or 1 *ob/ob* mouse. In each instance, 40 islets were transferred into each of six individual perfusion chambers.

The perfusion system as applied to fragments of whole pancreas in this laboratory, has been described in considerable detail [14]. The *basic medium* was Krebs-Ringer bicarbonate buffer (pH 7.4), continuously gassed with 95% O₂:5%CO₂, and containing bovine albumin 5 mg/ml (Fraction V, Behringwerke, Marburg, Germany) as well as glucose 2.8 mM. The buffer reservoirs and islet chambers were maintained at 37° C. Perfusion of islets, at a constant flow rate of 2.5 to 3.0 ml/min was begun between 40 and 60 min after sacrifice of the animals.

For all perfusions, an initial 20 min equilibration period with glucose 2.8 mM preceded the stimulation period, which lasted 30 min; this was followed by return to glucose 2.8 mM for 10 min. In the first set of experiments, the glucose dose-response studies were performed using glucose 2.8, 5.6, 8.4, 16.8, or 56 mM in the stimulation buffer. In the second set, tolbutamide Na 1.5 mM (Farbwerke Hoechst A.G., Frankfurt/Main-Hoechst, Germany), arginine hydrochloride 16 mM, theophylline 10 mM, cyclic AMP 10 mM (Sigma Chemical Co., St. Louis, Mo.), or cytochalasin B 10 µg/ml (Imperial Chem. Industries, Macclesfield, U.K.) was added to the stimulation buffer containing glucose at one of 3 concentrations: 2.8, 8.4 or 16.8 mM. Control perfusions in the absence of non-glucose stimulating agents, but at each corresponding glucose concentration, were always performed on islets obtained from the same collagenase isolation batch. For the experiments with cytochalasin B, the agent was also present during the 20 min pre-stimulation period with glucose 2.8 mM, and control experiments with glucose 16.8 mM alone were performed in the presence of dimethylsulfoxide 10 µl/ml (Fluka A.G., Buchs, Switzerland), the vehicle used to dissolve cytochalasin B.

The effluent from each islet chamber was collected in 1 min periods using fraction collectors; the insulin output rate was calculated as the product of the

hormone concentration measured in the effluent and the perfusion flow rate. In about one-third of all perfusions, the insulin content of 40 islets of each species, set aside before perfusion, was measured after acid-ethanol extraction [15].

Assay Procedures

Insulin was measured by a charcoal separation method of radioimmunoassay [16]. Samples of perfusate and islet extracts from *acomys* were assayed using human insulin as standard, since no purified *acomys* insulin was available, and since serial dilutions of *acomys* samples reacted parallel to human (or porcine) but not to rat or mouse insulin standards. Samples from rats and the different mice tested were assayed using rat insulin as standard, since rat and mouse insulin provided identical standard curves. The immunoassay antibody used was guinea-pig anti-porcine insulin antiserum (kindly provided by Dr. P. H. Wright, Indiana University, Indianapolis, Ind.). Monocomponent porcine insulin was iodinated with [125 I] (Eidg. Institut für Reaktorforschung, Würenlingen, Switzerland) by the chloramine-T method of Hunter and Greenwood [17] and purified on G 50 fine Sephadex (Pharmacia Fine Chemicals, Uppsala, Sweden). Purified crystalline insulins of the different species were kindly supplied by Dr. J. Schlichtkrull, Novo Research Institute, Bagsvaerd, Denmark.

Perfusate glucose was measured by a glucose-oxidase method [18], using reagents kindly donated by Dr. F. Schmidt, Boehringer-Mannheim GmbH, Germany.

Results

Dose-Response Studies with Glucose

As seen in Fig. 1, basal insulin release rates at the end of the initial 20 min perfusion with glucose 2.8 mM (i.e. 0 min on the abscissa) were low, stable and approximately the same for the islets from young *acomys*, albino mice, and rats. At 0 min the source of the perfusion medium was switched from the basic buffer reservoir containing glucose 2.8 mM to that containing glucose 16.8 mM. The concentration of glucose in the effluent to the fraction collector started to increase at 1 min, was 13 mM at 2 min, and reached 16.8 mM at 3 min. The rate of insulin release was first significantly increased at 2 min for rat islets ($P < 0.01$), at 3 min for albino mouse islets ($P < 0.01$), and at 5 min for *acomys* islets ($P < 0.05$), i.e. not until 2

Fig. 1. Mean insulin release rates of isolated islets from rat ($n = 15$ expts), albino mouse ($n = 8$ expts), and *acomys* ($n = 14$ expts) related to mean glucose concentration (the solid line) in the effluent from the islet chambers during the first 10 min after changing the perfusion buffer from glucose 2.8 to 16.8 mM. For 20 min prior to 0 time all islets were perfused with glucose 2.8 mM. The encircled value on each profile indicates the first rate of insulin release that is significantly increased above basal for the species concerned

Fig. 2. Mean insulin release rates of islets from rat, albino mouse, and *acomys* perfused with glucose 2.8 mM for 20 min (only last 5 min shown) followed by an increase in the perfusion medium glucose concentration to 5.6, 8.4, 16.8 or 56 mM for 30 min, finally a return of medium glucose to 2.8 mM for 10 min. The numbers of experiments are indicated in brackets

min after the glucose concentration had reached 16.8 mM.

Fig. 2 illustrates differences in the patterns of insulin release from islets of the three species perfused over a range of glucose concentrations extending from 5.6 to 56 mM. With the exception of 5.6 mM glucose applied to albino mice islets, glucose-induced insulin responses from islets of rats and albino mice exhibited a clear biphasic profile; the early (rapid) peak in the rate of release was usually seen at 4 min for rat islets and at 5 min for albino mouse islets, and was followed by a transient decrease with a nadir at about 7 min in both these control species; the secondary increase in insulin release then occurred but tended to flatten toward a plateau between 15 and 30 min after first increasing glucose in the perfusion medium. In clear contrast, *acomys* (8 to 14 weeks of age) islets appeared to lack the early phase of rapid insulin release almost completely, whereas the late phase was successively augmented with higher glucose concentrations, reaching levels not too different from those of the islets of mice or rats. When perfusion glucose was returned to 2.8 mM from 30 min on, however, insulin release rates decreased equally promptly for the islets of all three species. It therefore appears unlikely that the early delay of insulin response to glucose in *acomys* islets had resulted from slowed outward diffusion of insulin released into the extracellular space within islets. Slight delays in the decrease of insulin release, upon returning from very high glucose concentration to glucose 2.8 mM, were noted for the islets of all three species, and have been reported elsewhere to result from abrupt decrease in medium osmolarity [19].

Fig. 3 characterizes the glucose dose-response relation for insulin release rates achieved either early (4 min) or late (30 min) after raising the concentration of glucose. The "threshold" for the early insulin response was glucose 5.6 mM for rat islets ($P < 0.01$ vs. basal release), and glucose 8.4 mM for albino mouse islets ($P < 0.01$), whereas, even with glucose 56 mM, there was no significant early insulin response from *acomys* islets. By contrast to the flat dose-response curve for *acomys* islets at 4 min, the sensitivity of *acomys* islets to glucose closely approximated that of albino mouse islets, at 30 min. On the other hand, both *acomys* and albino mouse islets remained somewhat less sensitive to glucose than rat islets after 30 min, half-maximal insulin response after 30 min occurring at glucose levels of approximately 8 mM for rat islets, and 12 mM for *acomys* and albino mouse islets. Similar maximal rates of insulin release were observed for all three rodents at 30 min.

Fig. 3. Dose-response relation between glucose concentration (abscissa, logarithmic) and insulin release response (ordinate) utilizing the data obtained in the experiments described in Fig. 2. The relation is shown at both the 4th and the 30th min after switching to the stimulating glucose concentration

Effects of Ageing and Obesity in Acomys

The curves at the left of Fig. 4 show that the insulin response to glucose 16.8 mM was biphasic for islets of both lean and obese (*ob/ob*) C57 BL/6J mice, whereas no early phase of rapid insulin release was observed either with islets of young *acomys* (8 to 14 weeks, 25 to 40 g) — these summarizing data being pooled from those in Figs. 1 and 2 — or with islets of older, more obese *acomys* (22 to 36 weeks, 60 to 90 g). The magnitude of the insulin response over the entire 30 min stimulation period with glucose 16.8 mM appeared roughly proportional to the insulin content of the corresponding islets, represented by the columns to the right of Fig. 4. Thus, a low early phase of insulin

Fig. 4. Mean ($\pm$ SEM) insulin release rates (left panels); and islet insulin contents (on the right) for lean, and obese (ob/ob) C57 BL/6J mice (upper panels); and of young (8–14 weeks), and older (24–42 weeks) and more obese *acomys* (lower panels). The numbers of experiments or estimations are indicated in brackets

release was a constant feature in the *acomys* population, present also in older and relatively obese animals.

Effects of Other Insulin Secretagogues

Table 2 compares, in islets from *acomys* (8 to 14 weeks) and rats, the effects of theophylline, cyclic AMP, arginine, tolbutamide and cytochalasin B on total insulin release during 30 min of perfusion with these agents, at three different glucose concentrations. With glucose 2.8 mM, a substimulatory glucose concentration in both species, theophylline 10 mM resulted in a 91% increase in insulin release from rat islets ($P < 0.02$); arginine 16 mM in a 138% increase ($P < 0.005$); and tolbutamide 1.5 mM in a 169% increase ($P < 0.005$). At the same low glucose concentrations, however, all three agents exerted no significant effects on insulin release from *acomys* islets, either total release (Table 2) or any part of the insulin release profile (Fig. 5). By contrast, in the presence of stimulatory concentrations of glucose (8.4 and 16.8 mM), all agents tested increased insulin release to the same or even to a greater extent in *acomys* than in rat islets (Table 2 and Fig. 6); however, none of these agents elicited an early peak in the rate of insulin release from *acomys* islets.

Table 2. Effects of agents on total insulin release from islets of *acomys cahirinus* and of rats perfused over 30 min at different glucose concentrations

Additions	2.8 mM Glucose		8.4 mM Glucose		16.8 mM Glucose	
	<i>Acomys</i>	Rat	<i>Acomys</i>	Rat	<i>Acomys</i>	Rat
Glucose (G) alone	11.4 $\pm$ 1.2 ^a	8.5 $\pm$ 1.5	23.1 $\pm$ 2.7	83.7 $\pm$ 9.1	64.0 $\pm$ 5.1	99.6 $\pm$ 8.1
G + theophylline 10 mM	12.9 $\pm$ 1.0	16.2 $\pm$ 0.8	53.5 $\pm$ 5.1	116.7 $\pm$ 6.0	100.0 $\pm$ 8.7	125.3 $\pm$ 18.6
	P ^b NS (6) ^c	< 0.02 (6)	< 0.01 (6)	< 0.02 (6)	< 0.01 (8)	NS (8)
G alone	5.8 $\pm$ 1.1	11.5 $\pm$ 3.5			58.8 $\pm$ 5.0	113.0 $\pm$ 9.4
G + cyclic AMP 10 mM	4.3 $\pm$ 0.8	10.1 $\pm$ 2.8			111.9 $\pm$ 4.0	118.5 $\pm$ 5.0
	P NS (4)	NS (4)			< 0.005 (6)	NS (6)
G alone	7.8 $\pm$ 1.1	14.0 $\pm$ 2.8	26.6 $\pm$ 1.1	103.2 $\pm$ 9.1	60.3 $\pm$ 6.8	91.3 $\pm$ 11.8
G + arginine 16 mM	8.7 $\pm$ 2.1	33.3 $\pm$ 4.5	47.2 $\pm$ 5.0	155.6 $\pm$ 19.5	98.5 $\pm$ 10.4	147.7 $\pm$ 11.8
	P NS (6)	< 0.005 (8)	< 0.01 (6)	< 0.05 (6)	< 0.01 (6)	< 0.01 (6)
G alone	9.0 $\pm$ 1.3	11.7 $\pm$ 1.6	20.5 $\pm$ 6.4	57.9 $\pm$ 5.5		
G + tolbutamide 1.5 mM	12.2 $\pm$ 1.1	31.5 $\pm$ 3.4	22.1 $\pm$ 4.5	55.3 $\pm$ 4.6		
	P NS (6)	< 0.005 (6)	NS (6)	NS (6)		
G alone					63.5 $\pm$ 4.6	132.0 $\pm$ 9.1
G + cytochalasin B 10 μ g/ml					112.6 $\pm$ 5.1	256.1 $\pm$ 15.7
	P				< 0.005 (4)	< 0.005 (4)

^a mean ($\pm$ SEM) insulin release (ng/40 islets) integrated over 30 min.

^b p values compare insulin release in the presence and in the absence of each agent by Student's paired t test. NS = not significant.

^c numbers of paired experiments.

Fig. 5. Mean insulin release rates from islets of *acomys* (left panels) and rat (right panels) perfused (during shaded periods) with glucose 2.8 mM plus theophylline 10 mM, arginine 16 mM, or tolbutamide 1.5 mM (●—●) and in paired control experiments with glucose 2.8 mM alone (○—○). Numbers of paired experiments are shown in brackets

Discussion

A sharp and early peak in the rate of insulin release followed by a transient decrease, then a slower rise, is a characteristic feature of the insulin response pattern to abrupt and sustained elevation of the glucose concentration, in man and in several animal species [20–22]. In the present study, similar biphasic insulin release responses to glucose have been observed in perfused islets isolated from normoglycemic rats, albino mice and C57 BL/6J, lean mice, as well as from a mouse mutant, *ob/ob* C57/6J characterized by the tendency to develop obesity and hyperglycemia. By contrast, glucose-induced insulin release from islets of spiny mice, *acomys cahirinus*, was relatively delayed (Fig. 1), and no early peak insulin response was apparent, even when very high glucose concentrations were used (Fig. 2). This was true, whether the islets were obtained from young (8–14 weeks) or older and obese *acomys* (Fig. 4). The difference between the early response of *acomys* islets, and that of rat or mouse islets, could not be accounted for by differences in the insulin content of the islets of the three species, in the basal insulin release rates, or in the ease of

Fig. 6. Mean insulin release rates from islets of *acomys* (left panels) and rat (right panels) perfused (during shaded periods) with glucose 16.8 mM plus theophylline 10 mM, arginine 16 mM, or cytochalasin B 10 µg/ml, (●—●) and in paired control experiments with glucose 16.8 mM alone (○—○). For the experiments with cytochalasin, the agent was also present during the 20 min pre-stimulation period with glucose 2.8 mM, and control experiments with glucose 16.8 mM alone were performed in the presence of dimethylsulfoxide 10 µL/mL, the vehicle used to dissolve cytochalasin B. For SEMs and significance estimates of effectiveness of the agents used, see Table 2

diffusion of already released insulin from the extracellular space to the incubation medium.

Although studies *in vivo* [5] have demonstrated increases of plasma insulin as early as 2 min after intravenous injection of glucose in *acomys*, nevertheless, this early response was significantly less than that observed in control albino mice. Thus, the already small early response of the *acomys* B-cell *in vivo* may disappear entirely *in vitro*, perhaps as a result of further loss in sensitivity of *acomys* islets during isolation. Nevertheless, both *in vivo* [5] and in the present dynamic experiments *in vitro*, the differ-

ences in insulin release between *acomys* and control rodents were greatest during the first few minutes of glucose stimulation, becoming progressively less with continued exposure to higher glucose concentrations. Indeed, after 15 to 30 min of constant glucose stimulation, the rate of insulin release from *acomys* islets had become similar to that from albino mouse islets, and approached that from rat islets. This time related difference is best seen when comparing the early and late dose response patterns shown in Fig. 3: whereas *acomys* islets were initially (4 min) insensitive to glucose (in concentrations up to 56 mM), their sensitivity after 30 min exposure to stimulatory concentrations of glucose was identical to that of albino mouse islets, although still less than that of rat islets, at all but the highest concentration of glucose (56 mM). Thus, the insulin response of *acomys* islets could be clearly distinguished from that of both rat and albino mouse islets, by its *early* insensitivity to glucose. Fig. 3 also demonstrates a slightly lower sensitivity and higher threshold for glucose-induced insulin release for both *acomys* and albino mouse islets, when compared to rat islets. These findings may account for the higher plasma glucose levels observed in the *acomys* (145 ± 7 mg/100 ml) and albino mice (157 ± 16 mg/100 ml) than in the rats (129 ± 8 mg/100 ml) used in these experiments.

In order to determine whether the insensitivity of *acomys* islets was exclusive for glucose, several other insulin secretagogues were tested. Tolbutamide, arginine, theophylline, cyclic AMP and cytochalasin B all failed to elicit an early spikelike insulin secretory response from *acomys* islets, whereas they did from rat islets, at least when associated with 16.8 mM glucose. These findings *in vitro* are in agreement with those of Cameron *et al.* [4] *in vivo*, who reported that the intraperitoneal administration of glucose, arginine, glucagon, isoproterenol, aminophylline, and dibutyryl cyclic AMP caused either no early increases of plasma insulin in normoglycemic *acomys*, or increases very much smaller than in albino mice, or several mouse mutants. Thus the early secretory response for insulin would appear to be considerably less in *acomys* than in rats or mice, even when using a variety of agents presumably stimulating insulin secretion by different mechanisms, and whether stimulation is carried out *in vivo* or *in vitro*. This could be interpreted as suggesting a defect in the transport of insulin from a labile compartment, proposed as the source of initial insulin responses to a variety of agents [23].

Indeed, the recent demonstration by Malaisse-Lagae *et al.* [24] of a significant decrease in vincristine-induced paracrystalline deposits in

acomys B-cells, by comparison to several other rodents, may suggest that the defect in *acomys* B-cells could be in the microtubular-microfilamentous system, which is involved in insulin secretion [25]. However, a symmetrical and only partial reduction of both phases of glucose-induced insulin release has been reported in rat islets after extensive disruption of the micro tubular apparatus by Vincristine [26]. Similarly, the apparently intact later phase of insulin release to glucose, as well as the intact potentiating action of other insulin secretagogues demonstrated in *acomys* islets in the present experiments, is difficult to explain on the basis of the apparently decreased microtubular content demonstrated in islets of this species.

Alternatively, the failure of theophylline, arginine and tolbutamide to stimulate insulin release from *acomys* islets in the presence of glucose 2.8 mM, by contrast to their effects on rat islets at the same glucose concentration (Fig. 5), could be the result of the higher threshold for glucose-induced insulin release in *acomys* islets (between 5.6 and 8.4 mM, Fig. 3) than in rat islets (between 2.8 and 5.6 mM, Fig. 3). Indeed, at glucose concentrations which were stimulatory in *acomys* islets (8.4 and 16.8 mM), all agents tested were as effective, occasionally more effective amplifiers of the glucose effect in *acomys* than in rat islets (Fig. 6 and Table 2). It therefore appears to us that the impaired early responses of *acomys* islets, to a variety of insulinogenic agents, may be based upon a more fundamental insensitivity to glucose.

The present experiments, however, do not permit further precise localization of the lesser sensitivity to glucose observed in *acomys* islets. This is hardly surprising since the mechanisms normally regulating biphasic insulin release are still far from understood [27]. Evidence for a common glucose receptor [28] or glucose mediated signal for both phases of insulin release is derived from the observations that in perfused rat pancreas [23], and in normal man [29], both phases of insulin release exhibit similar sensitivities to glucose. Also, both phases of insulin release have recently been reported to be impaired, and to a similar extent, in diabetic Chinese hamsters [30], although insulin responses were assessed at only a single glucose concentration. In the present study, sensitivities to glucose were also similar for the early (4 min) and late (30 min) insulin responses in rat islets (half maximal stimulation occurring at about 8 mM glucose, Fig. 3), while in albino mouse islets the corresponding concentration was 12 mM for both phases. In *acomys* islets, however, sensitivity to glucose was considerably less in the early than in the

late phase of insulin release, an observation which has also been reported in "prediabetic" human subjects [29]. Thus, regulation of early and late phases of insulin secretion may not necessarily be identical, at least not in all cases.

Although comparisons between *acomys* (or any animal) and man must remain guarded, nevertheless some understanding of the derangement of insulin secretion in diabetic man may be provided by examination of insulin secretion in the *acomys*, a species with the liability to develop spontaneous hyperglycemia and ketosis. Similar to our observations in the *acomys*, the early insulin response to glucose in prediabetic and diabetic human subjects has been shown by several investigators [6–11, 29] to be relatively more impaired than the late response. It has also been observed in man, that glucose, with time, may enhance markedly its own effect on insulin release, and that this "potentiating" action of glucose may be fully operative in subjects with low insulin responses [31]. It may be that such an action of glucose may account for the normal rates of insulin release observed in *acomys* islets after stimulation with elevated glucose concentrations for 30 min. Furthermore, the enhancing effects of arginine [32, 33] and theophylline [34] on glucose-induced insulin release have been reported to be intact in subjects with decreased insulin responses, as demonstrated for *acomys* in the present experiments. Finally, it is proposed that the present findings in islets obtained from normoglycemic *acomys* may support the hypothesis that a basic defect in the diabetic islet, possibly preceding the occurrence of glucose intolerance, may involve some impairment in the recognition of glucose as an insulinogenic signal [29].

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