Insulin Secretion in the Spiny Mouse (Acomys Cahirinus)

Dose and Time Kinetic Studies with Glucose in Vivo and in Vitro

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SUMMARY

Plasma insulin responses to the intravenous injection of glucose in the doses of 0.5, 1.5, and 3.0 gm./kg. were compared in spiny mice (Acomys cahirinus) and weight-matched Swiss albino mice. The mean early (two-minute) plasma insulin response was significantly lower in Acomys at all doses of glucose injected; whereas, at later times (5, 15, and 30 minutes), differences in plasma insulin concentrations in the two species of mice were smaller or nonexistent. Plasma glucose clearance was significantly less in the Acomys. In terms of glucose dose kinetics, there was a decreased capacity of the mean plasma insulin response in Acomys compared with albino mice at two minutes; whereas, at later times, the mean dose response curve for Acomys was shifted to the right of that for albino mice, indicating a decreased sensitivity to glucose in the Acomys. There was, however, a large variation between the plasma insulin responses of the eight individual Acomys mice tested. There was a significant correlation in individual Acomys between the plasma insulin response, expressed as an insulinogenic index, integrated over the thirty minutes after injection of glucose 3.0 gm./kg. in vivo, and the insulin released from pancreatic islets obtained from the corresponding Acomys and perifused for thirty minutes with glucose 1,000 mg./100 ml. in vitro ($r = 0.77$, $p < 0.05$). It is concluded that the rate and magnitude of the insulin response to glucose in an individual Acomys reflects mainly the degree of sensitivity to glucose of the pancreatic beta cells in that animal.

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A colony of spiny mice, Acomys cahirinus, bred in our laboratory is one among several strains of rodents that has been shown to develop a diabetes-like syndrome of spontaneous, inappropriate hyperglycemia and obesity. Furthermore, the Acomys pancreas has been characterized by considerable hyperplasia of the islets of Langerhans.[1-3] Cameron and co-workers[4] demonstrated that plasma insulin responses to the intraperitoneal administration of glucose and other secretory stimuli were impaired in otherwise normoglycemic Acomys in comparison with Swiss albino mice. Recent studies employing intravenous glucose injection confirmed the lesser plasma insulin responses as well as impaired glucose tolerance in this species.[5] The aim of the present experiments was to further characterize the nature of defective insulin secretion in Acomys. The dose dependency as well as the time kinetics of glucose-induced insulin release were examined, since these parameters have been shown to be deranged in the various stages of the diabetic syndrome in man[6-11] and other species.[3] Dynamic insulin release was examined in vivo by serial blood sampling after intravenous injections of glucose in different doses and in vitro by perifusion of pancreatic islets obtained from the same Acomys that had been tested in vivo.

This work was presented in part at the Third Brook Lodge Workshop on Spontaneous Diabetes in Laboratory Animals, Augusta, Michigan, May, 1974, and at the Meeting of the Israel Societies of Diabetes and Endocrinology, Jerusalem, September, 1974.

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MATERIALS AND METHODS

Animals

The eight Acomys cahirinus studied were males and females, aged eight to sixteen weeks and weighing 30 to 48 gm. The Swiss albino mice were of both sexes and similar in age and body weight to the Acomys. All animals were bred in our laboratory and were fed a commercial laboratory chow ad libitum (protein 21 per cent, fat 8 per cent, carbohydrate 71 per cent of total calories). The Acomys received an additional daily ration of a seed mixture (protein 19 per cent, fat 16 per cent, carbohydrate 65 per cent), since this supplement was found necessary to maintain reproductive activity although it did not measurably affect beta cell function or ultrastructure.[1,2] Some of the

albino mice used in the present study were also maintained on the seed mixture, and their insulin responses were similar to those of mice not receiving the seed supplement.

Procedures in Vivo

The animals were tested in the fed state (between 9 and 10 a.m.), and after anesthesia with sodium pentobarbital 50 mg./kg. intraperitoneally and 25 mg./kg. subcutaneously. Because of the absence of injectable tail veins in the Acomys, a femoral vein was exposed in the thigh, and after a thirty-minute stabilization period glucose was injected over forty-five to ninety seconds, as a 5-20 per cent solution in water, in one of three doses: 0.5, 1.5, and 3.0 gm./kg. Each Acomys was tested with all three doses of glucose, administered in random order on separate occasions two weeks apart. The injection procedure was facilitated by use of a butterfly infusion set with a 25-gauge short needle (Abbott Laboratories, North Chicago, Ill.) and by visualization through a binocular dissecting microscope. Sequential blood sampling was performed by puncture of the retrorbital venous plexus[12] with heparinized hematocrit capillary tubes five minutes before and at 2, 5, 15, and 30 minutes after termination of the glucose injection. A total of 500 to 700 μl. of blood was withdrawn from each animal, and the Acomys tolerated the procedure well and recovered for later testing as described. The blood samples were immediately transferred to heparinized microcentrifuge tubes kept at 4° C., and within thirty minutes plasma was separated by centrifugation for three minutes in a microcentrifuge. A portion of each plasma sample was kept at 4° C., and the glucose concentration was measured within one hour. The remainder was stored at −20° C., and the immunoreactive insulin (IRI) concentration was estimated within one week. The insulin content of the pancreas of seven other comparable Acomys and nine albino mice was also estimated on acid-ethanol extracts of pancreas.[13]

Procedures in Vitro

Islets were isolated by a modification of the collagenase digestion method of Lacy et al.[14] from the pancreases of the same eight Acomys tested in vivo two weeks after the last (of three) glucose injections. Pancreatic digestion (at 37° C.) and subsequent isolation of islets (at 4° C.) were carried out in Hank's solution gassed with 95 per cent O_2:5 per cent CO_2 and containing glucose 50 mg./100 ml. After digestion, the pancreatic sediment was washed four times, with centrifugation, and transferred to a black-bottomed Petri dish. Islets were individually separated from any surrounding exocrine tissue by three successive transfers (by use of a 10-μl. micropipette, and under microscopic control) into Petri dishes containing fresh Hank's solution. In each experiment, the islets isolated from a single Acomys pancreas or from pancreases pooled from four albino mice were distributed into each of three perifusion chambers (forty islets per chamber). Two other groups of forty islets were set aside for estimation of insulin content after acid-ethanol extraction.[13]

The perifusion system, as applied to fragments of whole pancreas in this laboratory, has been described in detail.[15] The basic medium was Krebs-Ringer bicarbonate buffer, continuously gassed with 95 per cent O_2:5 per cent CO_2, and containing bovine albumin 5 mg./ml. and glucose 50 mg./100 ml. Perifusion of islets, at a constant flow rate of 2.5 to 3.0 ml./min., was begun between forty and sixty minutes after sacrifice of the animals. For all perifusions, an initial twenty-minute equilibration period with glucose 50 mg./100 ml. preceded the thirty-minute stimulation with glucose 1,000 mg./100 ml., and this was followed by ten minutes with glucose 50 mg./100 ml. Effluent from each islet chamber was collected in one-minute periods by fraction collectors, and the insulin output rate was calculated as the product of the hormone concentration measured in the effluent and the perifusion flow rate.

Assay Procedures

Insulin was measured by a charcoal separation method of radioimmunoassay.[16] For the experiments in vivo, a micromodification of the assay[5] permitted estimation of insulin in 10-μl. plasma samples, assayed in duplicate. Acomys samples were assayed with human insulin used as standard, since no purified Acomys insulin was available, and since serial dilution of plasma, pancreas, islet, and perifusate samples from Acomys reacted parallel to human (or porcine) but not to rat or mouse insulin standards. Samples from albino mice were assayed with mouse insulin used as standard. The labeled insulin was monocomponent porcine insulin iodinated with Na-^{125}I by the chloramine-T method of Hunter and Greenwood[17] and purified on G50 fine Sephadex.

Plasma glucose was measured in 5-μl samples (in duplicate) by a glucose-oxidase method.[18]

Reagents and Chemicals

Collagenase was purchased from Worthington Chemical Corporation, New Jersey; bovine albumin from Behringwerke, Marburg, Germany; Na-^{125}I from Eidg. Institut für Reaktorforschung, Würenlingen, Switzerland; and G50 fine Sephadex from Pharmacia Fine Chemicals, Uppsala, Sweden.

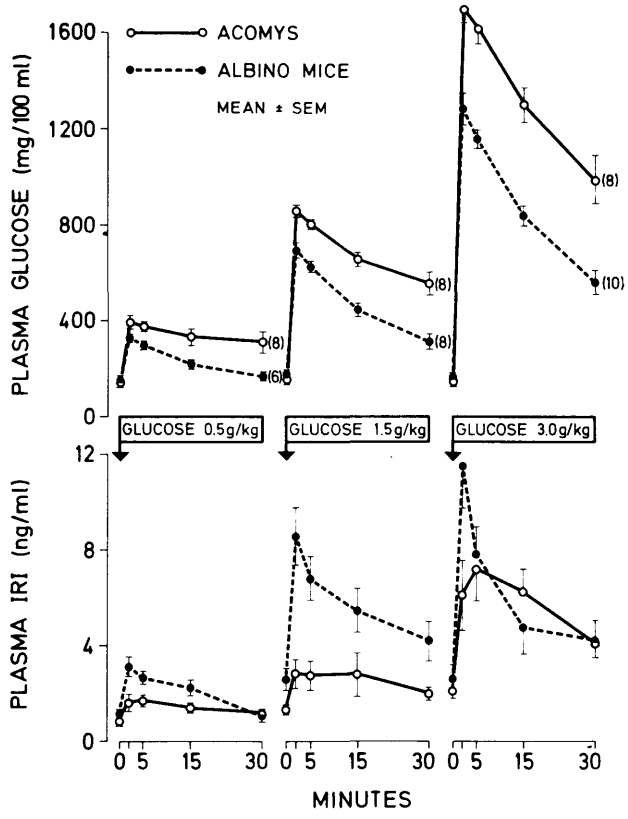

FIG. 1. Mean (± S.E.M.) plasma glucose (above) and insulin (IRI, below) concentrations in Acomys cahirinus and weight-matched Swiss albino mice injected intravenously at zero minutes with glucose 0.5, 1.5, and 3.0 gm./kg. Each dose of glucose was injected in the same eight Acomys on different occasions two weeks apart; glucose 0.5 gm./kg. was injected into six albino mice, glucose 1.5 gm./kg. in eight different albino mice, and glucose 3.0 gm./kg. in ten others.

The guinea-pig antiporcine insulin antiserum and the purified crystalline insulins used as standards were generous gifts of Dr. P. H. Wright (Indiana University) and Dr. J. Schlichtkrull (Novo Research Institute, Bagsvaerd, Denmark), respectively. Glucose-oxidase kits were kindly donated by Dr. F. Schmidt (Boehringer-Mannheim GmbH, Germany).

RESULTS

The over-all mean (± S.E.M.) preinfusion plasma insulin concentration was significantly ($p < 0.05$) lower in the Acomys (1.46 ± 0.18 ng./ml., n = 23) than in the albino mice (2.25 ± 0.32 ng./ml., n = 24); however, the corresponding plasma glucose concentration was slightly but not significantly lower in the Acomys (151 ± 5 mg./100 ml.) than in the albino mice (166 ± 6 mg./100 ml.). Figure 1 shows that the mean plasma insulin responses to injections of glucose 0.5, 1.5, and 3.0 gm./kg. were generally less in the Acomys than in the albino mice, in spite of significantly greater elevations of plasma glucose concentrations in the Acomys. Mean plasma insulin concentrations were significantly lower in the Acomys than in the albino mice at two minutes ($p < 0.02$), five minutes ($p < 0.02$), and fifteen minutes ($p < 0.05$) after injection of glucose 0.5 gm./kg.; at two minutes ($p < 0.001$), five minutes ($p < 0.005$), and thirty minutes ($p < 0.05$) after injection of glucose 1.5 gm./kg.; and only at two minutes ($p < 0.05$) after injection of glucose 3.0 gm./kg.

In order to relate the plasma insulin responses, in each species, to the concentration of plasma glucose achieved after injection of each dose of glucose, the results shown in figure 1 are illustrated in the form of glucose dose-response curves in figure 2. At two minutes after injection of glucose, the capacity of the mean plasma insulin response in Acomys was clearly less (about 50 per cent) than in albino mice; whereas at five, fifteen, and thirty minutes, the mean plasma insulin responses in Acomys injected with the highest dose of glucose (3.0 gm./kg.) were similar to the mean maximal plasma insulin responses in albino mice (achieved with glucose 1.5 gm./kg.).

Table 1 indicates the large variation between the plasma insulin responses to glucose of the eight individual Acomys tested. The over-all magnitude of the plasma insulin response in each animal was calculated as the increases of plasma insulin concentration above

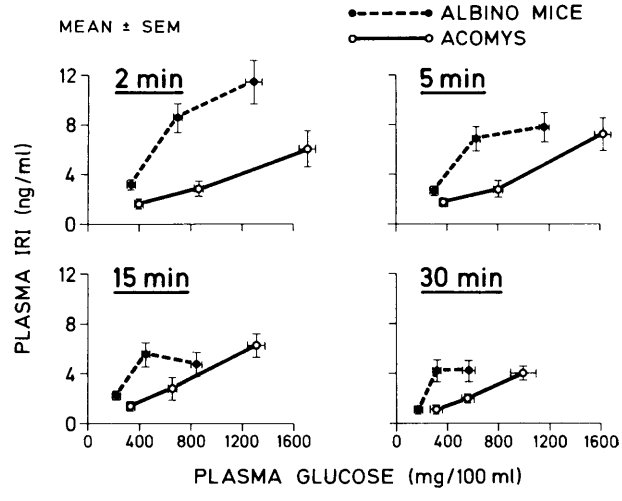

FIG. 2. Glucose dose-response curves in albino mice and Acomys obtained by plotting (on the ordinate) the mean (± S.E.M.) plasma insulin (IRI) concentration after injection of glucose 0.5, 1.5, and 3.0 gm./kg., and (on the abscissa) the corresponding mean (± S.E.M.) plasma glucose concentration at 2, 5, 15, and 30 minutes after the glucose injections. Experimental details are given in figure 1.

TABLE 1

Plasma glucose and insulin at different times after injection of glucose 0.5, 1.5, and 3.0 gm./kg. in eight individual Acomys cahirinus

Acomys No.	Glucose dose gm./kg.	Plasma glucose mg./ml.					Δ plasma glucose area* mg./min./ml.	Plasma insulin ng./ml.					Δ plasma insulin area* ng./min./ml.
		0 min.	2 min.	5 min.	15 min.	30 min.	0-30 min.	0 min.	2 min.	5 min.	15 min.	30 min.	0-30 min.
1	0.5	152	348	363	220	185	29	0.65	2.21	1.52	1.00	1.10	17.3
	1.5	168	767	730	523	396	113	2.72	4.99	7.00	8.90	2.75	111.0
	3.0	194	1,816	1,659	1,384	1,088	351	2.70	13.40	13.60	10.00	4.96	205.8
2	0.5	—	—	—	—	—	—	—	—	—	—	—	—
	1.5	158	879	818	666	523	151	0.52	0.89	2.24	1.68	2.08	38.3
	3.0	176	1,874	1,815	1,538	1,196	395	2.28	3.36	4.05	5.17	3.52	59.6
3	0.5	129	311	310	228	188	33	0.75	1.15	1.21	2.10	1.50	26.5
	1.5	128	832	787	607	503	148	1.17	1.37	2.50	1.45	2.26	20.8
	3.0	143	1,628	1,432	1,060	608	270	2.61	8.70	5.90	5.60	4.80	90.4
4	0.5	143	344	329	267	245	40	0.23	0.70	1.60	1.22	1.33	30.7
	1.5	169	839	837	651	530	147	1.12	3.20	2.45	2.50	2.40	40.7
	3.0	151	1,768	1,693	1,454	1,409	397	1.61	9.52	10.85	8.15	4.96	186.7
5	0.5	152	497	448	437	437	84	1.15	1.80	2.70	1.58	1.09	16.6
	1.5	134	819	800	814	811	196	1.06	2.20	1.89	0.97	0.86	5.6
	3.0	150	1,608	1,736	1,450	992	365	2.43	3.40	6.14	6.30	5.20	95.7
6	0.5	140	475	417	449	415	85	1.75	3.50	2.45	2.00	1.25	8.3
	1.5	186	1,006	840	633	488	144	1.20	5.80	2.60	3.50	3.05	63.2
	3.0	119	1,539	1,419	948	571	257	1.13	3.97	7.49	6.30	3.25	129.0
7	0.5	161	384	378	318	275	47	0.59	1.03	1.28	0.84	0.90	11.0
	1.5	203	869	830	724	715	160	1.08	1.40	1.40	1.08	1.08	2.9
	3.0	157	1,911	1,816	1,303	868	348	3.42	5.25	7.93	7.40	4.78	92.5
8	0.5	162	399	396	390	433	70	0.82	0.92	1.18	1.04	0.91	6.0
	1.5	105	870	773	646	496	159	1.70	2.90	2.10	2.30	1.45	11.2
	3.0	100	1,481	1,411	1,301	1,201	352	0.79	1.05	1.54	1.05	0.76	8.6

*Increases (Δ) of plasma glucose and insulin, above the corresponding preinfusion value (zero minutes), integrated over the thirty minutes after injection of each dose of glucose.

the preinfusion value, integrated over the thirty minutes after injection of each dose of glucose, and these insulin responses are shown in the form of glucose dose-response curves in figure 3. Only two of the eight Acomys tested (animals 1 and 6) clearly exhibited increased plasma insulin responses when the glucose dose was increased from 0.5 to 1.5 gm./kg.; four other Acomys (animals 4, 3, 7, and 5) exhibited substantial increases in their plasma insulin responses with glucose 3.0 gm./kg., and two (animals 2 and 8) did not respond well, even with glucose 3.0 gm./kg. Thus, animals 1 and 6 were the most *sensitive* to glucose, both in terms of thirty-minute integrated incremental plasma insulin responses to glucose 1.5 gm./kg. (figure 3) and plasma insulin concentrations at two minutes after injection of glucose 1.5 gm./kg. (table 1). Conversely, animals 2 and 8 were the least sensitive to glucose, again in terms of thirty-minute integrated plasma insulin responses as well as the re-

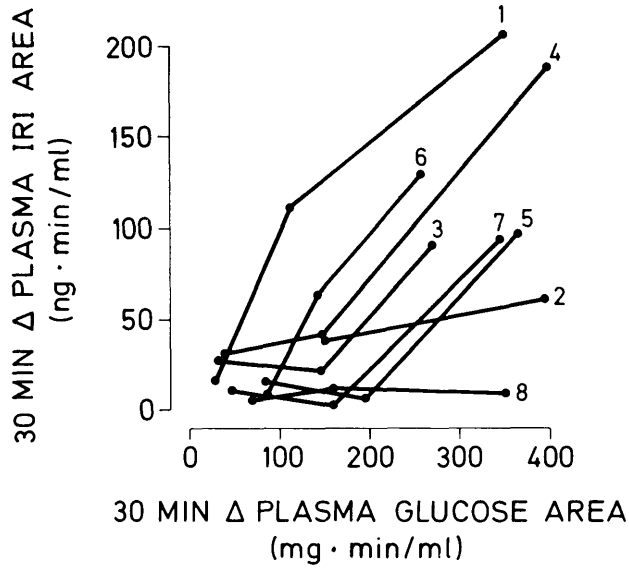

FIG. 3. Individual glucose dose-response curves for eight Acomys (numbered 1 to 8) obtained by plotting (on the ordinate) the increases (Δ) of plasma insulin (IRI) concentration, above the preinfusion value, integrated over the thirty minutes after injection of glucose 0.5, 1.5, and 3.0 gm./kg. and (on the abscissa) the corresponding Δ plasma glucose area. The values are taken from table 1.

TABLE 2

Plasma and islet insulin responses to glucose and insulin content of islets of eight Acomys cahirinus

Acomys* No.	Plasma Δ IRI / glucose area† $\times 10^{-5}$	Islet Δ IRI release area‡ mg./30 min./40 islets	Islet IRI content§ ng./40 islets
1	59	121	512
6	50	92	656
4	47	106	504
3	33	94	592
7	27	89	608
5	26	120	492
2	15	67	536
8	2	52	428

*Acomys cahirinus are ranked in descending order of plasma insulinogenic indices.

†Plasma insulinogenic index; calculated as increases (Δ) of plasma insulin concentration, above the preinfusion value, integrated over the thirty minutes after injection of glucose 3.0 gm./kg., divided by the corresponding Δ plasma glucose area. Values taken from table 1.

‡Increases of insulin release rates (above the mean value during the last five minutes of perifusion with glucose 50 mg./100 ml.) integrated over the thirty minutes of perifusion of the islets with glucose 1,000 mg./100 ml.; mean value for three perifusions.

§Mean value for two groups of forty islets, not perifused.

sponses at two minutes. The Acomys were ranked according to a plasma insulinogenic index, as shown in table 2.

In order to determine whether these differences in the plasma insulin responses of individual Acomys could be accounted for by differences in sensitivity of their pancreatic beta cells to glucose, islets obtained from each of the eight Acomys were stimulated with glucose, as shown in figure 4. As in the findings in vivo, insulin release from Acomys islets was decreased more at early than at later times after glucose stimulation, in comparison with albino mice. Again, as in vivo, there was a large variation between the rates of insulin release from islets of the different Acomys. Islets of animal 1 responded the best and those of animal 8 the poorest, both early and later during stimulation with glucose 1,000 mg./100 ml. Furthermore, there was a significant correlation between the plasma insulinogenic index and the insulin response to glucose of islets obtained from the corresponding Acomys (figure 5). Differences between the amounts of insulin released from islets of each Acomys could not be accounted for by differences in islet insulin content, as shown by the lack of correlation between release and content of insulin in these islets (figure 6).

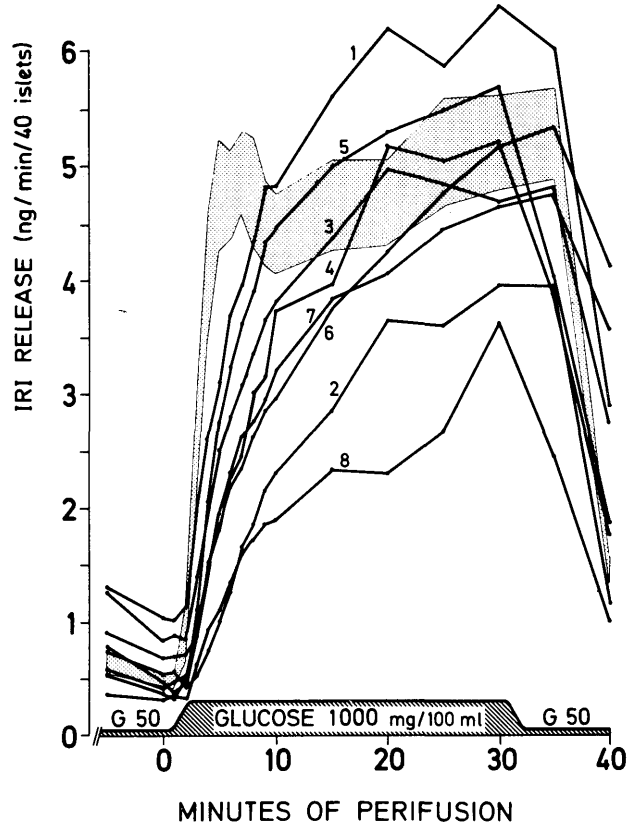

FIG. 4. Individual profiles of insulin (IRI) release rates with perifused islets obtained from the same eight Acomys tested in vivo (figure 3) and mean (± S.E.M.) IRI release rates for fifteen perifusions with albino mouse islets (shaded area). In each experiment forty islets were perifused for twenty minutes with glucose 50 mg./100 ml. (G50), then for thirty minutes with glucose 1,000 mg./100 ml. followed by ten minutes with glucose 50 mg./100 ml. Each profile of insulin release for Acomys islets represents the mean of three experiments.

DISCUSSION

In the present study, the time as well as the dose kinetics of glucose-induced insulin secretion were compared in normoglycemic spiny mice (Acomys cahirinus) and Swiss albino mice. The latter served as controls only insofar as they represent rodents similar

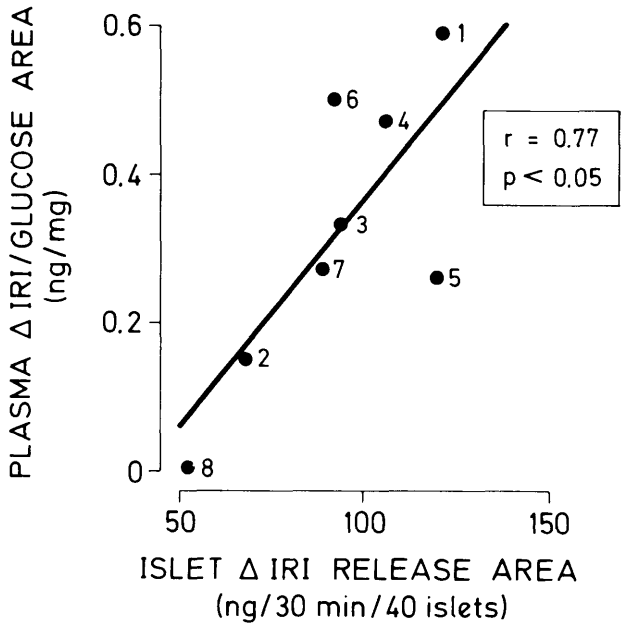

FIG. 5. Correlation (r = 0.77, p < 0.05) between the plasma insulinogenic index after injection of glucose 3.0 gm./kg. in eight Acomys (on the ordinate) and the insulin response from islets obtained from the corresponding Acomys and perifused with glucose 1,000 mg./100 ml. (on the abscissa). The numbers identify the Acomys, and the values are taken from table 2.

to Acomys in age and body weight and different in not developing spontaneous hyperglycemia and ketosis. The early or first phase of glucose-induced insulin release was reduced in Acomys both in vivo and in vitro. Injection of glucose, in a dose (3.0 gm./kg.) sufficient to elevate the plasma glucose concentration above 1,000 mg./100 ml., produced increases of plasma insulin significantly less in Acomys than in albino mice at two minutes but not at later times (figures 1 and 2). Similarly, perifusion of Acomys islets with glucose 1,000 mg./100 ml. in vitro failed to stimulate a rapid early phase of insulin release, whereas a typical biphasic insulin response to glucose was observed with islets of albino mice (figure 4). This decreased capacity of the early insulin response to glucose in Acomys is even more striking when considered in terms of the greater pancreatic insulin content (mean ± S.E.M.) in Acomys (139 ± 20 ng./mg. pancreas, n = 7) than in albino mice (51 ± 5 ng./mg. pancreas, n = 9; $p < 0.001$).

Although the maximal early (two-minute) plasma insulin response in Acomys was decreased below that of albino mice, at later times (five, fifteen, and thirty minutes) the mean glucose dose-response curves for Acomys were shifted to the right of those for albino mice, thereby indicating a decreased sensitivity rather than a decreased capacity of the insulin response in Acomys. There was, however, a large variation between the plasma insulin responses of the eight individual Acomys tested (figure 3). While most Acomys showed a more-or-less pronounced displacement of their glucose dose-response curve toward the right, two of the eight animals (nos. 1 and 6) exhibited similar sensitivities to glucose as the mean observed in albino mice. If confirmed in a larger Acomys population, the presence of animals with a "normal" insulin response may solve the problem of the adequacy of using other species as controls.

The wide range of the plasma insulin responses to glucose in the different Acomys tested appears to reflect the secretory behavior of the pancreatic islets of individual animals, as indicated by the significant correlation between the response to glucose of the whole animal and of its pancreatic islets in vitro. This suggests that the sensitivity to glucose demonstrated in vivo reflects mainly the characteristics of the beta cells of the individual animal and that humoral and neural modulation of insulin secretion may have a lesser importance in this respect.

The present findings that insulin responses in most Acomys equal or even exceed responses in albino mice in vivo and in vitro or those in rat islets,[19] provided stimulation with glucose is maintained both in time and in dose, suggest to us that there may be a selec-

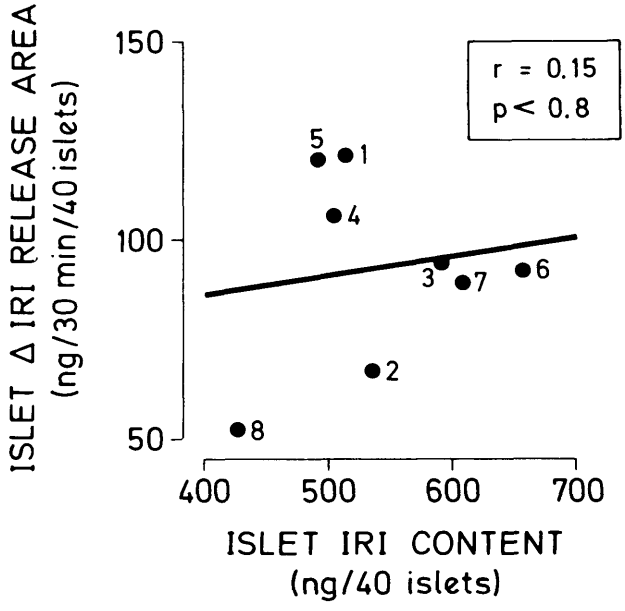

FIG. 6. Lack of significant correlation (r = 0.15, p < 0.8) between the insulin response from islets of eight Acomys perifused with glucose 1,000 mg./100 ml. (on the ordinate) and the insulin content of islets of the corresponding Acomys (on the abscissa). The numbers identify the Acomys, and the values are taken from table 2.

tive impairment in the initial recognition of glucose by the Acomys beta cell. However, since mechanisms normally regulating either early- or late-phase glucose-induced insulin release are not yet well defined, it may not be surprising that the presumably defective insulin secretory mechanism in Acomys has not been precisely located in the present study. Malaisse-Lagae et al.[20] have recently reported a deficiency of vincristine-induced paracrystalline deposits in Acomys beta cells, thereby suggesting a defect in the microtubular-microfilamentous system. However, this finding does not appear to explain the apparently selective deficiency of early-phase insulin release in Acomys, since a symmetric and only partial reduction of both phases of glucose-induced insulin release has been reported in rat islets after extensive disruption of the microtubular apparatus by vincristine.[21]

Finally, since the dose and time kinetics of glucose-induced insulin secretion in normoglycemic spiny mice, Acomys cahirinus, resemble those in prediabetic and diabetic human subjects,[22] the Acomys appears to be a useful model for further investigation of insulin secretory mechanisms associated with, if not ultimately responsible for, the diabetic state.

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