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Research Article

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Virus-Induced Alterations in Insulin Release in Hamster Islets of Langerhans

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A B S T R A C T   After the inoculation of Golden Syrian hamsters with the TC-83 vaccine strain of Venezuelan encephalitis (VE) virus, a sustained diminution in glucose-stimulated insulin release and glucose intolerance of shorter duration develops. To understand better the mechanism of this defect in insulin release, we examined insulin secretion in response to several test agents in isolated perifused islets from control and 24-d post-VE virus-infected hamsters. 50 islets were used in all perifusion experiments, and data were expressed as total insulin released as well as peak response for each test agent during a 30-min perifusion period from control and VE-infected islets. After perifusion with 20 mM glucose, a 45% diminution of insulin release was noted in VE-infected islets in comparison with control islets, which in turn was similar to in vivo findings. However, following 1-mM tolbutamide stimulation, insulin release was similar in control and VE-infected islets. In separate studies, 1 mM tolbutamide, 10 mM theophilline, 1 mM dibutyryl cyclic (c)AMP, and 1 mM 8-bromo-cAMP resulted in statistically similar insulin-release curves in control and VE-infected islets. Additional experiments assessing [5-^{3}H]glucose use in control and infected islets after 20 min of perifusion with 20 mM glucose revealed virtually identical values (239±30-control; and 222 ±27-VE-infected islets). Morphological and morphometric evaluation of VE-infected islets (21 d following virus inoculation) showed no changes in islet volume density, beta cell density, and beta cell granulation. Thus, VE virus induces a defect in glucose-stimulated insulin release from hamster beta cells that can be corrected by cAMP analogues and does not alter islet glucose use.

INTRODUCTION

A diabetes mellitus-like syndrome has been reported in genetically susceptible mice inoculated with either the M variant of encephalomyocarditis (EMC)[1] virus (1, 2) or a diabetogenic variant of coxsackie B_4 virus (3). In both of these systems, a rapid lysis of beta cells occurs with the severity of hyperglycemia closely correlating with the degree of virus-induced beta cell destruction (3, 4). Another example of virus-induced alterations of carbohydrate metabolism occurs after inoculation of Golden Syrian hamsters with the TC-83 vaccine strain of Venezuelan encephalitis (VE) virus (5). After TC-83 VE infection, there is a 20% mortality rate with surviving animals exhibiting a 24-d period of glucose intolerance and a severe diminution of glucose-stimulated insulin release for the 3-mo duration of study (5). This model differs from the EMC and Coxsackie B_4 systems in that VE is an enveloped virus in which viral proteins become incorporated in membranes of host tissue (6, 7). Although some cell lysis occurs following VE infection, long-term pathologic changes in the pancreas do not develop (5). To understand the mechanism for this virus-induced inhibition of glucose-stimulated insulin release at a more basic level, we performed a series of experiments using isolated perifused hamster islets obtained from virus-infected and control animals. The results suggest that there is a virus-induced defect, possibly at the level of the beta cell membrane, but that events distal to the cyclic (c)AMP system of the beta cell are intact.

METHODS

Animals and virus preparation.   Golden Syrian hamsters (Charles River Breeding Laboratories, Inc., Wilmington, Mass.), weighing between 110 and 130 g, were used in all experiments. The animals had free access to hamster chow

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[1] *Abbreviations used in this paper*: EMC, M-variant of encephalomyocarditis; VE, Venezuelan encephalitis.

and water until the time of study. Before glucose tolerance testing or isolation of islets, hamsters were anesthetized with 10 mg of sodium pentobarbital (Abbott Diagnostics, Diagnostic Products, North Chicago, Ill.). Groups of hamsters were inoculated with either 100,000 plaque-forming units of TC-83 VE virus in 0.2 ml of 1% bovine albumin (Miles Laboratories, Inc., Elkart, Ind.) in Hanks' solution (Gibco, Grand Island Biological Laboratories, Grand Island, N. Y.), adjusted to pH 8.0 or sham inoculated with 0.2 ml i.p. of diluent. The TC-83 VE vaccine strain (lot 3-2-L6) was produced at the United States Army Medical Research Institute of Infectious Diseases, Frederick, Md. After reconstitution from the lyophilized state, the virus was passed one additional time in chicken embryonic cell cultures. Viruses were assayed by counting plaque-forming units on chicken embryonic cell cultures monolayers under agar (8). Preinoculation and 3-wk postinoculation acetone-extracted sera were measured for the presence of antiviral antibody by a microtiter hemagglutination-inhibition test (9). Neutralizing antibodies to VE were measured in several animals 1 yr after inoculation with VE (3).

Isolation of islets and general perifusion techniques. 3.5 wk after inoculation of hamsters with TC-83 VE or diluent, pancreatic islets were isolated from two infected and two control animals by the collagenase (Worthington Biochemical Corp., Freehold, N. J.) digestion method of Lacy and Kostianovsky (10), followed by the use of a Ficoll (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.) gradient for separation. 50 islets from two infected or two control hamsters were used for each preparation with the n given in each figure designating the corresponding number of preparations. Islets were perifused according to the method of Tomita and Lacy (11) with a control and experimental chamber run in parallel in each experiment. The basal perifusion medium consisted of 115 mM NaCl, 5 mM KCl, 25 mM NaHCO$_3$, 1 mM MgCl$_2$, 2.2 mM CaCl$_2$, and 0.5% bovine serum albumin that was gassed continuously with a mixture of 95% O$_2$ and 5% CO$_2$. The flow rate was 1 ml/min in all perifusion studies. Perifusion experiments were performed as described by Zawalich et al. (12). At each sampling time, a 0.5-ml aliquot of the perifusate was collected and stored at −20°C until assayed for glucose and insulin. Because of the desirability of normalizing secretion in relation to islet cell mass, insulin release rates are expressed in terms of microunits per minute per microgram of islet DNA. Insulin was measured by a standard double antibody radioimmunoassay (13) with human insulin (Eli Lilly & Co., Indianapolis, Ind.) as standard. Glucose was measured by a glucose oxidase method (14) (Eskalab glucose reagent kit, SmithKlein Instruments, Inc., Div. SmithKline Corp., Sunnyvale, Calif.) in select samples to validate the accuracy of perifusion buffers. The following test agents were used in the various perifusion protocols: sodium tolbutamide (Upjohn Co., Kalamazoo, Mich.), theophilline, dibutyryl cAMP, butyrate, and 8-bromo-cAMP (Sigma Chemical Co., St. Louis, Mo.).

Metabolic studies. Isolation of islets was performed as above, and metabolic studies carried out using the method of Zawalich et al. (12). The formula of Ashcroft et al. (15) was used to calculate the glucose use rate. Glucose use is expressed as picomoles glucose used per microgram islet DNA.

Islet DNA analysis. After each perifusion experiment, the DNA content of 50 islets in each chamber was determined by the diaminobenzoic acid (DABA, Aldrich Chemical Co., Inc., Milwaukee, Wis.) method (16). The hormones were extracted first with acid-ethanol, and then the insoluble material was further extracted with ethanol and ether before DNA assay.

Morphology and morphometry. To evaluate pathologic correlation of the perifusion experiments performed in these studies, infected hamsters and age-matched controls were killed 3 and 21 d after infection. The pancreas was removed, dissected into small segments, and fixed in Bouin's solution. Several segments from each pancreas were dehydrated and embedded together in paraffin. The blocks were serially sectioned and two adjacent serial sections at intervals of 200 μm were mounted on separate slides. Insulin- and glucagon-positive cells were then localized immunocytochemically on consecutive slides (adjacent sections) by a modification (17) of the unlabeled antibody-enzyme method of Sternberger et al. (18). Primary antisera against insulin and pancreatic glucagon for these studies were raised in rabbits. Sheep anti-rabbit gammaglobulin was obtained from Antibodies, Inc., Davis, Calif., peroxidase-antiperoxidase antiserum was purchased from N. L. Capell Laboratories, Inc., Cochranville, Pa., and diaminobenzidine-tetraHCl was obtained from Aldrich Chemical Co. The islet volume density within the pancreas was determined by a linear scanning method (19). At least 1,000 mm of total scan was made over each pancreas. The volume densities of beta and alpha cells within the islet were estimated by the point count method (20). The points over beta and alpha cells in adjacent sections of at least 50 islets were determined in each pancreas.

Analysis of perifusion data. Data were analyzed statistically by measuring both the area under the curve for a test agent by trapezoidal rule and peak insulin responses using Student's t test for paired variates.

RESULTS

Serology. Presence of TC-83 VE in inoculated hamsters was verified by comparing sera from infected and uninfected hamsters using two methods. In one group of hamsters, before infection with TC-83 VE, all hamsters showed hemagglutination inhibition titers of <10. By 21–24 d after viral inoculation, titers increased to 40–160. In another group of hamsters, endpoint-neutralizing antibody titers increased from <4 in uninfected animals to 64–256 in animals inoculated with virus 21–24 d previously. At 12 mo after infection, anti-VE-neutralizing antibody was still detectable in four of five hamsters tested (titers ranged from 8 to 32). In addition, using the double-label fluorescent antibody technique (21), viral antigen was localized in islets in fresh frozen sections of pancreas isolated from hamsters 3–5 d after viral infection (data not shown).

Effects of 20 mM glucose on insulin secretion. In Fig. 1, the effect of 20 mM glucose, a near maximal stimulatory dose for insulin release (22), is depicted in relation to insulin secretion from islets obtained from hamsters 24 d after inoculation with TC-83 VE or diluent. Under basal conditions (3 mM glucose), insulin secretion was similar to both control and TC-83 VE-infected islets. In contrast, in response to 20 mM glucose, insulin release in the infected islets was only 55% that of control islets ($P < 0.001$). Although a biphasic insulin response typically is present during stimulation with 16.7–20 mM glucose in rat islets in perifusion experiments, such a response frequently does not occur in Syrian hamster islets (23). Other
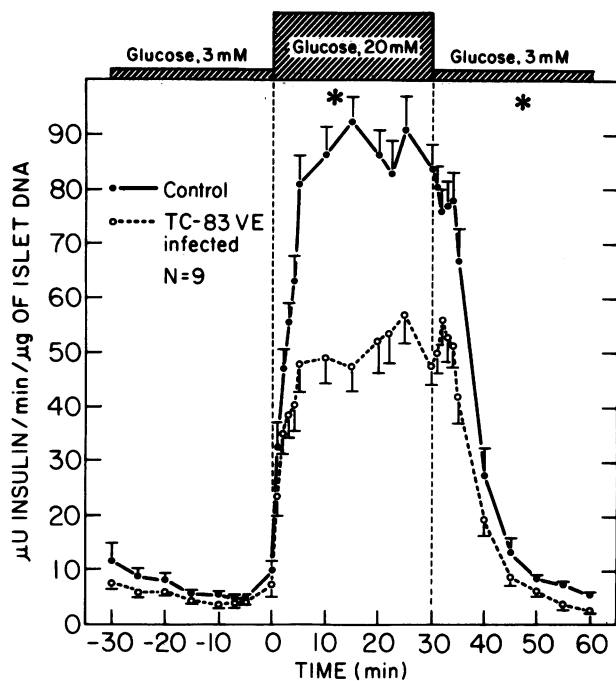
FIGURE 1 Effect of basal (3 mM) and 20-mM glucose concentration on insulin release from perifused control and infected (24-d post TC-83 VE inoculation) hamster islets. The perifusion periods are indicated in each of the panels. n represents the number of observations. An asterisk indicates a P value of <0.05 measuring the area under the curve by trapezoidal rule using Student's t test for paired variates.

FIGURE 2 Effect of basal (3 mM) and 20-mM glucose concentration on insulin release from perifused control and infected (8-mo post TC-83 VE inoculation) hamster islets. n represents the number of observations. An asterisk indicates a P value of <0.05.

animals were maintained for 8 mo after inoculation with TC-83 VE or diluent in order to assess whether the decrease in insulin secretion would persist for this duration. Fig. 2 demonstrates that following this period, a sustained statistically significant decrease in insulin secretion remains in response to 20 mM glucose. The insulin profile is very similar to that noted in Fig. 1. Despite the prolonged period of limited insulin reserve, the plasma glucose levels of these hamsters were within the normal range (data not shown).

Effects of tolbutamide on insulin secretion. Tolbutamide was used as a second stimulus for insulin secretion. It is known to stimulate adenylate cyclase in islet homogenates (24), to inhibit cAMP phosphodiesterase (25, 26), and to increase intraislet cAMP content in the rat (2, 3). 1 min after 1-mM tolbutamide administration, there was a prompt rise in insulin response with a peak response occurring at 3 min in both control and TC-83 VE-infected islets with virtually identical secretory profiles in both groups (Fig. 3). After 30 min of reequilibration in 3 mM glucose, both groups of islets were presented with the combination of 1 mM tolbutamide and 20 mM glucose. No significant difference was observed in insulin secretion with respect to areas under the curve or
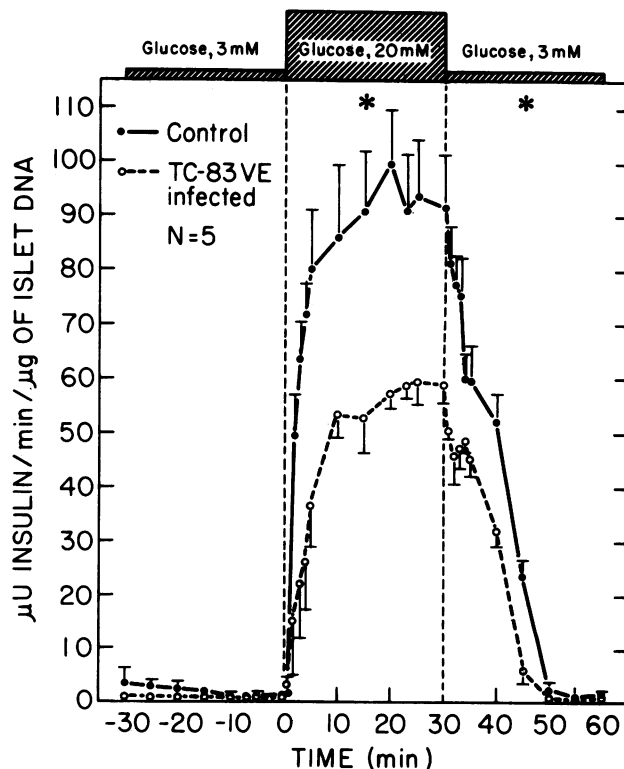
peak insulin responses in control or virus-infected islets.

Effects of theophilline on insulin secretion. Theophilline was used to assess its ability to correct the decrease in glucose-stimulated insulin release observed with tolbutamide because both agents are phosphodiesterase inhibitors and elevate islet cAMP (23, 27–30). Fig. 4 shows that at the outset of this protocol, the TC-83 VE-infected islets demonstrated the expected significantly decreased insulin response to 20 mM glucose and that the combination of 10 mM theophilline and 20 mM glucose completely corrected the insulin response to that noted in control islets.

Effects of dibutyryl cAMP on insulin secretion. Because the phosphodiesterase inhibitors (tolbutamide and theophilline) corrected the decreased insulin secretion in combination with 20 mM glucose, a cAMP analogue was next used in an effort to augment insulin secretion (Fig. 5). 2 mM butyric acid was used as a control to show that the cAMP moiety and not the butyrate moiety results in correction in the diminished insulin release observed with 20 mM glucose alone (31). Butyrate is also a fatty acid that has been reported to stimulate insulin secretion in vitro (32, 33).
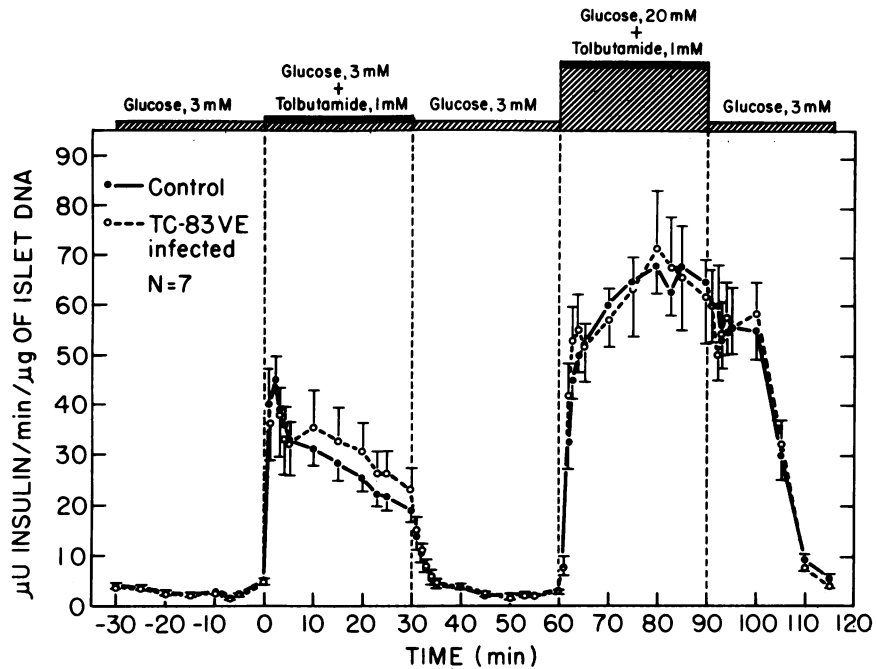
FIGURE 3 Effect of 1 mM tolbutamide + basal (3 mM) glucose and 1 mM tolbutamide + 20 mM glucose on insulin release from perifused control and infected (24-d post TC-83 VE inoculation) hamster islets. The perifusion periods are indicated in each of the panels. n represents the number of observations.
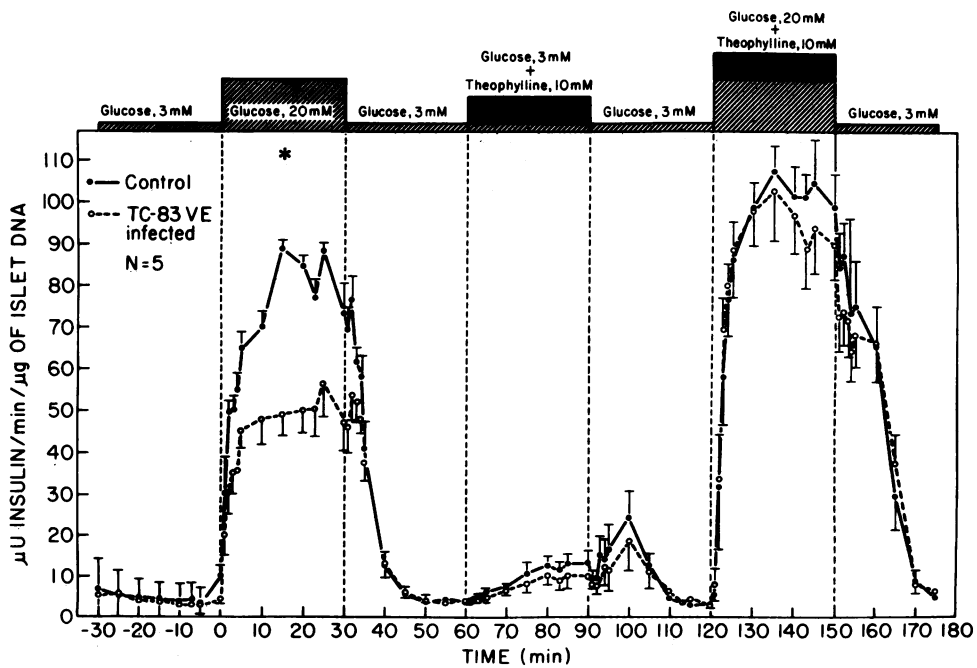

FIGURE 4 Effect of 10 mM theophylline + basal (3 mM) glucose and 10 mM theophylline + 20 mM glucose on insulin release from perifused control and infected (24-d post TC-83 VE inoculation) hamster islets. The perifusion periods are indicated in each of the panels with 3 mM glucose followed by 20 mM glucose as the initial control. n represents the number of observations. An asterisk indicates a P value of <0.05.
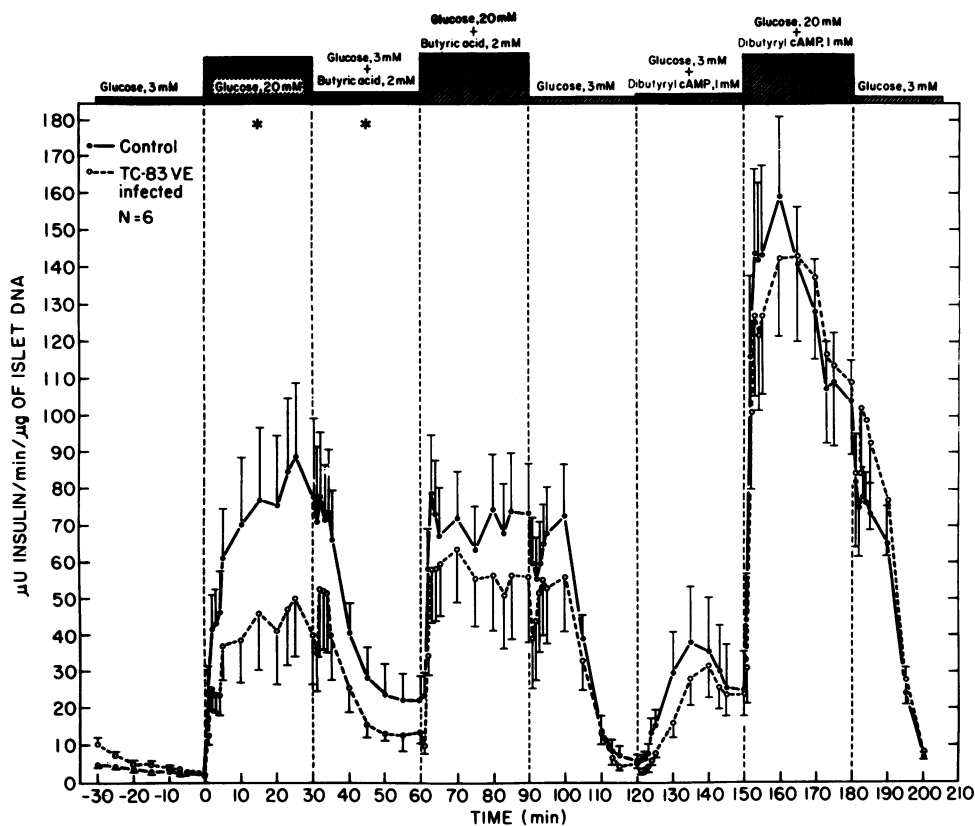
FIGURE 5   Effect of 1 mM dibutyryl cAMP + basal (3 mM) glucose and 1 mM dibutyryl cAMP + 20 mM glucose on insulin release from perifused control and infected (24-d post TC-83 VE inoculation) hamster islets. The perifusion periods are indicated in each of the panels with 3 mM glucose, 20 mM glucose, and 2 mM butyric acid as controls. n represents the number of observations. An asterisk indicates a P value of <0.05.

In these experiments, control islets released a similar amount of insulin when perifused with 20 mM glucose alone or the combination of 20 mM glucose and 2 mM butyric acid. Likewise, the amount of insulin released by VE-infected islets when perifused with 20 mM glucose alone or the combination of 20 mM glucose and 2 mM butyric acid was not significantly different. Insulin secretion was comparable in control and VE-infected islets that were perifused with 20 mM glucose and 2 mM butyric acid. This observation suggests that the regulation of insulin secretion by short-chain fatty acids in the presence of high glucose is intact in VE-infected islets. The combination of 20 mM glucose and 1 mM dibutyryl cAMP on insulin secretion resulted in a two- to threefold increase of insulin values in control and VE-infected islets when compared with respective insulin release areas in the presence of 20 mM glucose and 2 mM butyric acid. Therefore, the cAMP moiety of the cyclic nucleotide analogue clearly corrects the beta cell defect in virus-infected islets.

Effect of 8-bromo-cAMP on insulin secretion.

The effect of another cAMP analogue, 8-bromo-cAMP, was assessed in control and virus-infected islets. As is noted in Fig. 6, the combination of 1 mM 8-bromo-cAMP and 20 mM glucose completely corrected the reduced insulin response that occurred with 20 mM glucose alone.

Metabolic studies of ^3H_2O formation from [5-^3H]-glucose.   Under certain circumstances, e.g., with the absence of Ca^{2+} in the perifusate, the insulin-releasing function of glucose can be dissociated from the ability of islets to utilize glucose (34). Accordingly, the relationship between insulin release from TC-83 VE-infected islets in response to 20 mM glucose (20-min time point) and the sequential assessment of glucose use in the same islets was determined (Table I). There was no significant difference in the ability of control and virus-infected islets to metabolize glucose to CO_2 and ^3H_2O.

Morphologic and morphometric studies.   Fig. 7 depicts adjacent and serial sections from control and TC-83 VE-infected hamster pancreas stained immunocytochemically for insulin and glucagon. There were
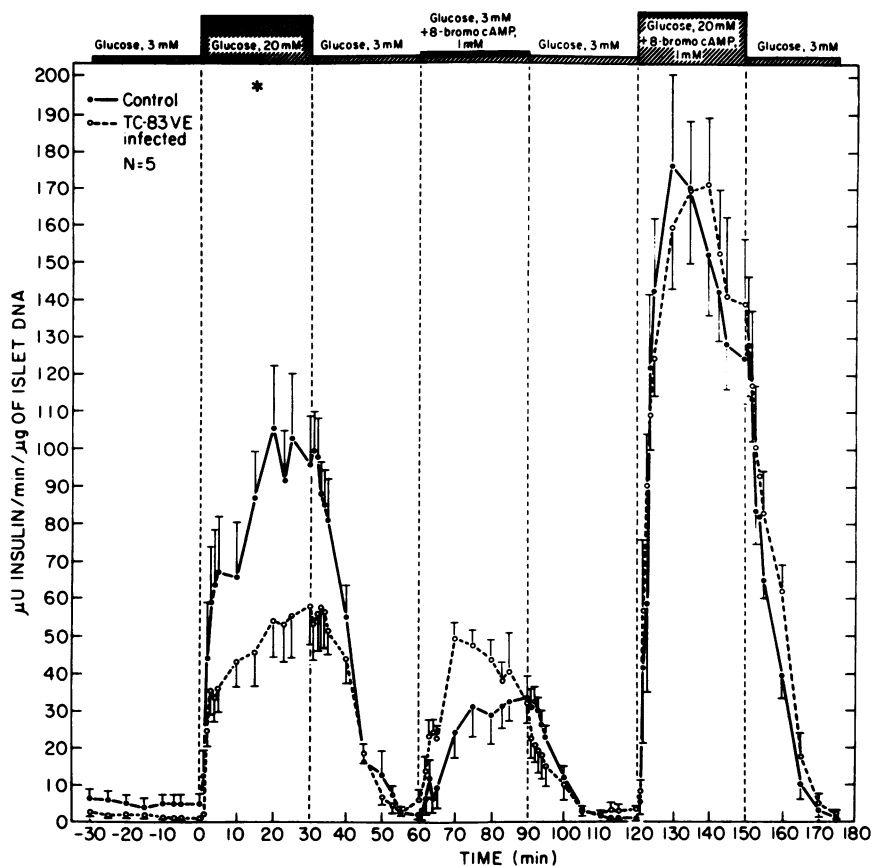
FIGURE 6 Effect of 1 mM 8-bromo-cAMP + basal (3 mM) glucose and 1 mM 8-bromo-cAMP + 20 mM glucose on insulin release from perifused control and infected (24-d post TC-83 VE inoculation) hamster islets. The perifusion periods are indicated in each of the panels with 3 mM glucose and 20 mM glucose as controls. *n* represents the number of observations. An asterisk represents a *P* value of <0.05.

no significant morphological differences between the control and virus-infected islets 3 wk after TC-83 VE inoculation. Assessment of beta cell granulation showed degranulation 3 d after VE infection. In contrast, 21 d postvirus inoculation, beta cell granulation was normal (Cf. Fig. 7a, b). Morphometric analysis of control and virus-infected pancreas 3 and 21 d postinfection confirmed the subjective impression. Furthermore, the DNA content of 50 control and infected islets was virtually identical (data not shown). Table II shows that the percentage of islets of the various subpopulations, i.e., beta, alpha, delta, and pancreatic polypeptide, did not change significantly in response to acute infection with TC-83 VE 3 d or 21 d postinfection, the latter corresponding to the time of the perifusion protocols.

DISCUSSION

Several animal models of virus-induced diabetes mellitus have been developed over the past decade

(1–5, 35). In the case of infection with both Coxsackie B₄ virus and the M variant of EMC virus in genetically susceptible mice, an acute diabeteslike syndrome develops within 7 d. The severity of hyperglycemia correlates closely pathologically with the degree of pancreatic damage (4). This acute model of virus-induced diabetes is not applicable to all cases of human, juvenile-onset diabetes, some of which may take months to years to develop after infection. In fact, a 10–20-yr period may elapse between congenital rubella infection and the onset of diabetes mellitus (36). In addition, multiple viral infections may be involved in the clinical expression of disease (35). For these reasons, the finding that the TC-83 vaccine strain of VE virus infection in Golden Syrian hamsters causes a transient glucose intolerance with sustained diminution of glucose-stimulated insulin release and no long-term morphologic abnormalities (5) provided an attractive additional model of virus-islet cell interaction.

TABLE I

Comparison of Peak Insulin Secretion following 20 min of 20-mM Glucose and Sequentially Measured Glucose Use in Control and TC-83 VE-Infected Islets*

Experi-ment	Peak insulin‡		Glucose use§	
	Control	TC-83 VE-infected	Control	TC-83 VE-infected
1	125	100	87	126
2	105	50	180	168
3	170	40	250	178
4	175	90	381	264
5	135	60	328	139
6	160	85	102	150
7	150	70	230	179
8	110	60	418	307
9	150	75	106	216
10	175	135	140	163
11	150	105	182	433
12	180	115	321	95
13	170	120	396	387
14	175	115	228	305
Mean	150	105	239	222
SEM	±5	±10‖	±30	±27

* In each experiment for both control and TC-83 VE-infected islets, the peak insulin response used was taken at the 20-min point.
‡ Microunits insulin per minute per microgram of islet DNA.
§ [5-³H]glucose used in picomoles per hour per microgram islet DNA.
‖ $P < 0.001$.

In the present investigations, we used isolated hamster islets in a perifusion system with micro-chambers to study the mechanism of the decrease in glucose-stimulated insulin release after VE infection. This in vitro system provided an accurate assessment of insulin secretion without the influence of neural and hormonal factors that are present in vivo. The elimination of neural factors is particularly relevant because the TC-83 vaccine strain of VE is a pantropic virus that localizes not only in hamster pancreas (5), but also in brain [Purkinje cells, vascular, and olfactory systems (37, 38)]. It should also be noted that the morphologic results showed that beta cell granulation, total islet volume density, beta cell volume density, and DNA per islet were not significantly different in control and VE-infected islets 21 d after virus inoculation. However, the insulin secretion data were calculated with respect to DNA content of islets as an added precaution to correct for possible virus-related difference in size that might cause spuriously low secretion profiles in 21–24-d post infection islets.

Interestingly, similar to the in vivo hamster data (5), there was a 45% decrease in insulin secretion in response to 20 mM glucose in the perifusion system; yet these animals did not exhibit nonfasting hyperglycemia in vivo. Furthermore, the profound decrease in insulin seretion during glucose administration persisted in islets obtained 8 mo after VE inoculation, and these hamsters were also not hyperglycemic. The presence of hypoinsulinemia in response to glucose in the absence of elevated plasma glucose levels would be compatible with increased peripheral sensitivity to insulin in vivo. Certainly, diphenylhydantoin and other agents are known to decrease insulin levels after glucose administration without impairing glucose tolerance in individuals not genetically predisposed to diabetes (39). This latter observation supports the notion that a larger amount of insulin is released in response to secretagogues than is really necessary for biologic effect.

One possible mechanism by which VE virus could lead to abnormalities in insulin release after glucose, without causing morphologic abnormalities, would be a virus-related defect at the level of the beta cell membrane. VE belongs to a group of enveloped viruses that have a lipoglycoprotein coat with lipid derived totally from host and proteins although glycosylated by host

TABLE II

Total Islet Volume Density and Volume Density of Individual Islet Subtypes 3 and 21 d after Inoculation with TC-83 VE Virus

	n*	Total islet	Insulin	Glucagon	SRIF	PP
		%‡	%§	%§	%§	%§
3-d control	3	1.12±0.19‖	65.1±3.0	20.8±1.9	13.1±1.7	0.9±0.9
TC-83 VE-infected	4	1.09±0.12	70.6±1.7	18.5±2.1	10.2±2.5	0.6±0.4
21-d TC-83 VE-infected	4	1.06±0.17	67.9±3.9	17.9±2.6	13.3±1.9	0.8±0.7

* n = number of pancreases studied.
‡ % of total pancreas.
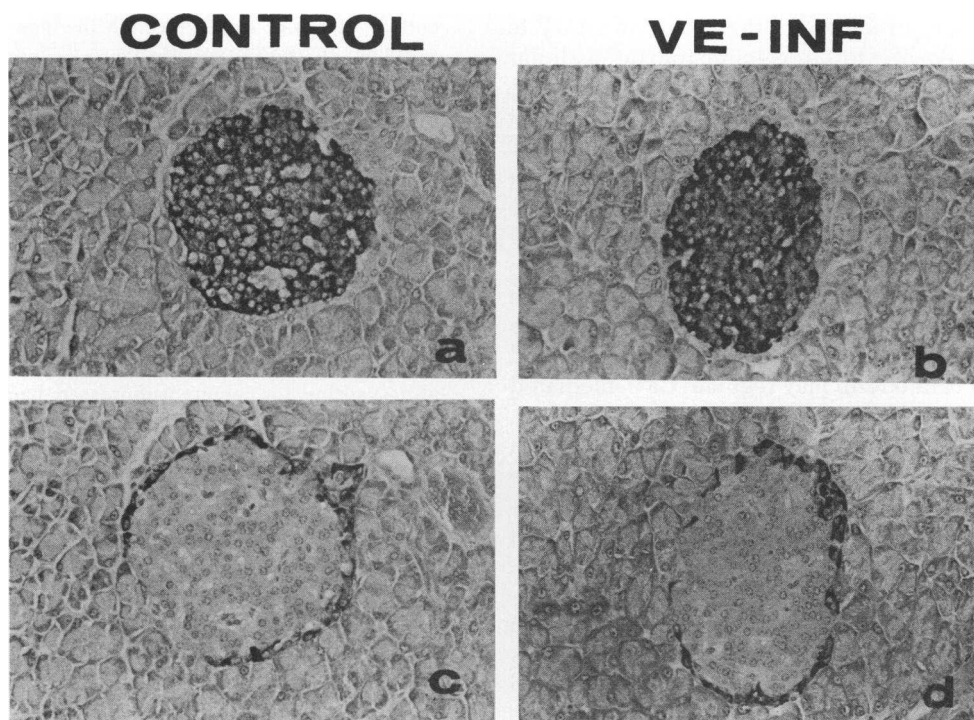§ % of islet
‖ Mean±SEM.

FIGURE 7 Adjacent serial sections from control (left) and infected (21-d post TC-83 VE inoculation) (right) hamster pancreas stained immunocytochemically for insulin (A and B) and glucagon (C and D). Hematoxylin counterstain ×195.

enzymes specified totally by the virus (6, 7). During the replication process, viral proteins become incorporated into the host membrane potentially leading to alterations in membrane function and immunogenicity. It is known that in vitro both glucose and tolbutamide-stimulated insulin release result in elevated islet cAMP levels. Because tolbutamide-triggered insulin release was intact while glucose-stimulated insulin release was impaired in the infected islets, it is possible that viral modification has led to alteration in the beta cell membrane and its ability to respond to increased glucose. Changes in membrane receptors have been described after infection with other viruses (43). Both herpes simplex virus and vesicular stomatitis virus have been shown to decrease the number of insulin receptors in human amnion (WISH) cells between 4 and 12 h after addition of the virus, a time when viral antigens can be found in the plasma membranes of cells infected with these viruses (40–43).

Zawalich et al. (12) have developed a method for sequentially assessing insulin release and the metabolism of [5-³H]glucose (to ³H$_2$O production) from isolated rat islets. This technique has demonstrated that agents such as iodoacetate and mannoheptulose that block glucose-induced insulin release interfere concomitantly with glucose metabolism. By contrast, in the present study, there is an interesting dissociation in which the insulin release in VE-infected islets is markedly decreased following 20 min of 20 mM glucose without any change in the ability of the islets to utilize glucose. There is a precedent for this dissociation. The elimination of calcium from the perifusion medium of mouse pancreatic islets does not alter the rate of glucose oxidation, but decreases glucose-stimulated insulin release (34).

Further efforts to elucidate the changes in insulin secretion in vitro in perifused hamster islets following VE-infection focused on the cAMP system of the islets. Tolbutamide and theophylline, both of which inhibit phosphodiesterase, were able to correct the decrease in insulin release when the agent was added to 20 mM glucose in the perifusion system. The derivatives, dibutyryl cAMP and 8-bromo-cAMP, were also able to correct the diminished insulin response to glucose when added in combination with 20 mM glucose to the perifusion buffer. Therefore, the data are consistent with normal beta cell function distal to the cAMP system.

Because glucose-induced insulin release is decreased but can be normalized by certain cAMP analogues in VE-infected islets, it is reasonable to believe that a virus-induced alteration exists between the beta cell membrane and cAMP itself. It is thought that the relationship of glucose and cAMP to insulin

release is in part a function of the ability of cAMP to inhibit the uptake of cytosolic calcium into intracellular organelles (44, 45). Cytosolic calcium is therefore raised and stimulates insulin release when the rate of calcium efflux across the cell membrane is reduced by glucose (44, 45). In this regard, it is noteworthy that Schubart et al. (46) recently demonstrated the presence of Ca^{++}-calmodulin-dependent protein kinase activity in a hamster insulinoma cell line. In this line, similar to the VE-infected islet model, insulin is not released appropriately in response to an elevation in glucose concentration (47). These workers also presented evidence that calmodulin may regulate Ca^{++}-mediated insulin release in these insulinoma cells (46). Thus, following TC-83 VE infection, it is possible that an abnormality exists in beta cell membrane adenylate cyclase, calmodulin, and/or islet cAMP generation— any of which could contribute to the abnormalities in insulin release observed in this system.

In conclusion, our studies show the feasibility of using a perifusion system to assess subtle alterations in beta cell function after infection with VE virus. This virus induces a defect in glucose-stimulated insulin release from hamster beta cells despite no abnormalities in glucose oxidation. Insulin response to phosphodiesterase inhibitors and cAMP analogues in the high glucose experiments are consistent with normal beta cell function distal to the cAMP system. Further studies will be required to determine the precise locus of the lesion.

ACKNOWLEDGMENTS

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