Alterations in Calcium Channel Currents Underlie Defective Insulin Secretion in a Transgenic Mouse*

(Received for publication, March 4, 1996)

Chung-Ren J an†§¶, Thomas J. Ribar‡¶, Anthony R. Means** and George J. Augustine§

From the Departments of †Pharmacology and §Neurobiology, Duke University Medical Center, Durham, North Carolina 27710

A transgenic mouse overexpressing a mutant form of calmodulin (CaM-8) that is selectively targeted to pancreatic beta-cells has an impaired ability to secrete insulin in response to elevated blood glucose. Fluorescence measurements of cytosolic Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) showed that intracellular Ca\(^{2+}\) rises produced by glucose were smaller than normal in beta-cells of CaM-8 mice. Glucose utilization rates were not different between the CaM-8 and control beta-cells, suggesting that glucose metabolism was unperturbed by CaM-8. Ion channel defects were implicated in the phenotype of CaM-8 beta-cells because treatment of these cells with tolbutamide, a blocker of ATP-sensitive K\(^+\) channels, produced smaller than normal amounts of insulin secretion and Ca\(^{2+}\) rises. Depolarization of elevated extracellular K\(^+\) also produced smaller Ca\(^{2+}\) rises in beta-cells from CaM-8 mice. Whole-cell patch-clamp recordings revealed that Ca\(^{2+}\) channel currents of beta-cells from CaM-8 mice were half as large as Ca\(^{2+}\) currents in control cells, while the currents carried by delayed rectifier and ATP-sensitive K\(^+\) channels were similar in magnitude in both cell types. We conclude that expression of the CaM-8 form of calmodulin causes a down-regulation of Ca\(^{2+}\) channel currents, which reduces Ca\(^{2+}\) entry and accumulation when glucose stimulates closure of the ATP-sensitive K\(^+\) channels. The reduction in intracellular Ca\(^{2+}\) accumulation then prevents an adequate amount of insulin from being secreted from beta-cells of CaM-8 mice.

The exocytotic secretion of insulin from pancreatic beta-cells requires the transduction of numerous internal and external signals, beginning with the sensing of elevated glucose levels (1, 2). The transduction of the glucose signal through metabolic pathways results in the generation of ATP which closes ATP-dependent K\(^+\) channels in the plasma membrane (3, 4). This depolarizes the membrane and opens voltage-gated Ca\(^{2+}\) channels, elevating the intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) and triggering exocytotic insulin secretion (5-7). The molecular basis of the action of Ca\(^{2+}\) in insulin secretion is not clear, although it has been proposed that the intracellular Ca\(^{2+}\) - receptor protein, calmodulin (CaM), may play a key role in this process (8-10).

As part of an effort to elucidate the functional role of calmodulin in pancreatic beta-cells, transgenic mouse lines have been established that specifically overexpress CaM in their beta-cells (11). These mice have defective insulin secretion at an early age, due to an impairment in the metabolism of glucose and the subsequent generation of ATP (12, 13). Another mouse line, referred to as CaM-8, expresses in its beta-cells a mutant form of CaM that lacks 8 amino acids in its central helix and does not activate calmodulin-dependent proteins in vitro even though this protein binds Ca\(^{2+}\) with normal affinity (14). These mice also demonstrate a reduced insulin secretion in response to fuel secretagogues such as glucose (15), which is surprising because overexpression of this mutant form of CaM in cardiac atrial cells produces no phenotypic abnormalities (16).

Subtle differences in pancreatic beta-cell responses of 6-8-day-old postnatal CaM-8 and CaM mice suggest that the secretory defects may be different in the two lines of mice. For example, the onset of diabetes begins later in CaM-8 mice than in CaM mice and the kinetics of the glucose-induced secretion response is different in these two mice at this age (15). In addition, depolarization of the beta-cell membrane potential increases insulin secretion much more in CaM mice than in CaM-8 mice (reviewed in Ref. 15). Here we examine several key signaling events that underlie insulin secretion (1, 2, 17) in pancreatic beta-cells of CaM-8 mice. We find that the primary defect in beta-cells from CaM-8 mice is a remarkable reduction in the amount of Ca\(^{2+}\) flowing through voltage-gated Ca\(^{2+}\) channels, which results in abnormal small rises in [Ca\(^{2+}\)]\(_i\), during glucose stimulation and presumably leads to or directly accounts for the diabetic phenotype of these animals. These results confirm that the nature and origin of the insulin secretory defect exhibited in CaM-8 mice is quite different from that of CaM mice (13) and provide a useful experimental system for the study of intracellular Ca\(^{2+}\) signaling and Ca\(^{2+}\) channel regulation.

MATERIALS AND METHODS

Chemical Reagents—C-SOH was from Mallinkrodt Chemical Co., West Germany. All other chemical reagents were from Sigma, unless otherwise indicated.

Isolation of Islets and Beta-cells—6-8-day-old postnatal mice were obtained from active breeding colonies of transgenic CaM-8 and control mice. All animals were cared for in accordance with the rules and regulations set forth by the United States National Institutes of Health. Isolated islets were prepared from normal and CaM-8 mice by collagenase digestion and cultured in fully supplemented RPMI 1640 (Life Technologies, Inc.) at 37°C in an atmosphere of 95% O\(_2\), 5% CO\(_2\) as described previously (12, 15). Single beta-cells used for patch-clamp analysis were prepared from freshly isolated islets using EDTA and trypsin treatment as described by Ribar et al. (13). Isolated beta-cells were then suspended in Dulbecco's modified Eagle's medium (Life Technologies, Inc.) containing 10% fetal bovine serum, 11.1 mM glucose, 100 units/ml penicillin, and 100 \mu g/ml streptomycin, plated into 24-well
Calculus Channel Defects in Transgenic Mice

RESULTS

Reduced [Ca\(^{2+}\)], Signaling in Beta-cells from CaM-8 Mice—
Given the essential role of intracellular calcium in glucose-induced insulin secretion (23, 24), we examined the ability of glucose to elevate cytosolic [Ca\(^{2+}\)], in beta-cells of CaM-8 and normal mice. For these experiments, isolated pancreatic islets were loaded with the membrane-permeant, fluorescent indicator dye, fura-2-AM (18), to monitor [Ca\(^{2+}\)]. Fig. 1 shows examples of experiments illustrating that perfusion of control and CaM-8 islets with Krebs-Ringer bicarbonate saline (KRB) containing a low concentration of glucose (2.8 mM) provided resting [Ca\(^{2+}\)], levels that were similar for both types of islets. Resting [Ca\(^{2+}\)], measured in KRB containing 2.8 mM glucose, was approximately 100 nM for both control and CaM-8 islets (Fig. 2A) and did not differ significantly between the two groups (p > 0.60). Elevation of the extracellular glucose concentration to 16.8 mM produced robust rises in [Ca\(^{2+}\)], from control cells (Fig. 1, top), as reported in earlier work (24). In contrast, glucose-induced [Ca\(^{2+}\)], rises were much smaller in islets isolated from CaM-8 mice (Fig. 1, bottom). While [Ca\(^{2+}\)], in normal islets increased by approximately 200 nM above the resting level upon exposure to KRB containing 16.8 mM glucose, this rise in [Ca\(^{2+}\)], was only about one-third as large as in islets from CaM-8 mice (Fig. 2B). The difference between the mean rises in [Ca\(^{2+}\)], induced by glucose in the two types of islets was significantly different (p < 0.05). Thus, the ability of glucose to elevate [Ca\(^{2+}\)], is impaired in islets from CaM-8 mice.

Because it has been suggested that dissociated beta-cells can produce glucose-induced [Ca\(^{2+}\)], responses which differ from those of intact islets (25), we also measured [Ca\(^{2+}\)], in dissociated beta-cells loaded with indo-1-AM, another membrane-permeant indicator dye. We found that glucose-induced [Ca\(^{2+}\)], transients also were smaller in beta-cells from CaM-8 mice than in beta-cells from normal mice (data not shown). Thus, in our case the [Ca\(^{2+}\)], responses of isolated beta-cells paralleled those of intact islets, with both indicating that beta-cells from CaM-8 mice produced smaller glucose-induced rises in [Ca\(^{2+}\)], than cells from control mice.

In summary, our measurements of [Ca\(^{2+}\)], reveal significant deficiencies in the glucose-induced calcium signaling pathway of CaM-8 beta-cells. Because of the essential role of this pathway for glucose-dependent insulin secretion, it is likely that these deficiencies account for the defective insulin secretion characteristic of the CaM-8 mouse. We next determined the mechanisms underlying this lesion in the calcium signaling pathway.

Glucose Metabolism Is Normal in CaM-8 Mice—The glucose-induced [Ca\(^{2+}\)], signaling pathway begins with extracellular glucose being transported into \(\beta\)-cells and converted into ATP. To determine whether these early steps in the pathway were altered in CaM-8 mice, we measured the rate of glucose utilization of CaM-8 islets. Fig. 3 shows that under basal metabolic conditions, CaM-8 islets (n = 23) utilized glucose at a rate that was not significantly different (p > 0.10) from that of control islets (n = 32). Elevating the glucose concentration to 16.8 mM resulted in an approximately 4-fold increase in glucose utilization by both CaM-8 and control islets (p > 0.70). These measurements show that glucose metabolism is normal in CaM-8 mice and indicate that the defect in glucose-induced Ca\(^{2+}\) signaling must be downstream of this early step in the signaling cascade. Further, because glucose utilization can be used to predict whether sufficient ATP will be produced to drive ATP-sensitive K\(^{+}\) channels (21), our results suggest that these channels should experience normal ATP levels in CaM-8 cells.

Ion Channel Abnormalities in CaM-8 Beta-cells—The re-
duced glucose-induced \([\text{Ca}^{2+}]_{i}\) signaling of CaM-8 beta-cells could result from a defect in any (or all) of the ion channels that regulate the membrane potential of beta-cells. We next performed experiments designed to identify the ion channel abnormalities that underlie this defect in the glucose-induced calcium signaling pathway.

Glucose metabolism in beta-cells induces insulin secretion by closing ATP-sensitive \(K^+\) channels in the plasma membrane (4, 26). As an initial test of the function of these channels and their downstream effectors, we examined the sensitivity of CaM-8 islets to tolbutamide, a sulfonylurea drug that blocks ATP-sensitive \(K^+\) channels and induces insulin secretion by depolarizing the beta-cell membrane potential (4, 27). Fig. 4 shows that, under basal conditions (2.8 mM glucose), CaM-8 islets and control islets secreted similar amounts of insulin. Addition of tolbutamide (1 mM) induced a 4-fold increase in insulin secretion in the presence of 2.8 mM glucose in control islets. However, while exposure of CaM-8 islets to this concentration of tolbutamide produced an initial burst of insulin secretion, subsequent secretion was only about twice the basal rate \((p < 0.05)\). Furthermore, whereas addition of a high concentration (16.8 mM) of glucose to the tolbutamide-containing saline caused control islets to produce an additional 3-fold increase in insulin secretion, CaM-8 islets (Fig. 4) failed to respond further \((p < 0.001)\). Thus, tolbutamide was less capable of evoking insulin secretion from islets from CaM-8 mice.

The differential effects of tolbutamide on insulin secretion in control and CaM-8 islets were paralleled by the actions of this drug upon \([\text{Ca}^{2+}]_{i}\). For both control and CaM-8 islets, treatment with 1 mM tolbutamide (in the presence of 2.8 mM glucose) resulted in sustained and reversible increases in \([\text{Ca}^{2+}]_{i}\), as measured with fura-2 (Fig. 5A). However, the mean rise in \([\text{Ca}^{2+}]_{i}\), induced by tolbutamide was approximately half as large in CaM-8 islets as in islets from normal mice (Fig. 5B); this difference was statistically significant \((p < 0.05)\). Taken together, these results indicate that the secretory defect in CaM-8 cells could reside at the level of the ATP-sensitive \(K^+\) channels or at some subsequent step in the pathway that normally leads to a rise in \([\text{Ca}^{2+}]_{i}\), and resultant secretion of insulin.

We next used whole-cell patch-clamp methods (22) to examine directly the properties of ATP-sensitive \(K^+\) channels in CaM-8 beta-cells. Ionic currents flowing through ATP-sensitive \(K^+\) channels were measured using the paradigm illustrated in Fig. 6A. These currents were activated by progressively lowering the cytosolic concentration of ATP by dialyzing the cell interior with a patch pipette solution containing a low concentration (0.3 mM) of ATP (5, 13). The amplitude of these currents reached a plateau and then slowly declined after about 10 min (not shown), presumably because the ATP-sensitive \(K^+\) channels exhibit "wash-out" during prolonged intracellular dialysis (5). These dialysis-induced currents were unequivocally identified as ATP-sensitive \(K^+\) currents because they were rapidly blocked by tolbutamide (0.1 mM; Fig. 6A). While the ATP-sensitive \(K^+\) channels were opening during removal of intracellular ATP, the beta-cell membrane potential was alternated between -60, -70, and -80 mV to study the voltage-dependence of the induced currents. These currents were inward at -80 mV and outward at -60 mV; since the equilibrium potential for \(K^+\) is approximately -76 mV under our experimental conditions, this behavior is as expected for a current carried by \(K^+\) ions. The slope of the relationship between the magnitude of the ATP-sensitive \(K^+\) currents and the membrane potential gives the ATP-sensitive \(K^+\) conductance; this value was normalized by dividing by the membrane capacitance measured for each cell, to take into account variations in cell membrane.

![Graph](https://example.com/graph.png)
When measured in this way, as shown in Fig. 6B, the average ATP-sensitive K⁺ conductance of CaM-8 beta-cells (n = 19) was found to be significantly larger (p < 0.05) than that of control beta-cells (n = 27). This indicates that beta-cells of CaM-8 mice have a relatively high density of ATP-sensitive K⁺ channels that are able to function normally. Thus, the defects in glucose-induced and tolbutamide-induced insulin secretion and [Ca²⁺]ᵢ signaling must have a source other than loss of ATP-sensitive K⁺ channels.

The delayed rectifier K⁺ channel is another type of ion channel that regulates the Ca²⁺ entry that triggers insulin secretion (5, 17). Currents flowing through this type of K⁺ channel were measured in isolation by including the Ca²⁺ buffer, EGTA, in the pipette solution to prevent the rise in [Ca²⁺] that would normally activate Ca²⁺-dependent K⁺ currents (28), as well as ATP to close the ATP-sensitive K⁺ channels. Fig. 7A illustrates the ionic currents produced under these conditions when beta-cells were held at a membrane potential of −70 mV and briefly depolarized to a variety of more positive potentials. The resultant currents were outward in polarity, activated rapidly and did not inactivate during 200 ms long depolarizations. Further, they were eliminated by extracellular application of tetraethylammonium ions (data not shown), a blocker of delayed rectifier channels in these cells (28). These properties indicate that the currents measured were K⁺ currents flowing through delayed rectifier channels.

The time course and magnitude of delayed rectifier currents were very similar in beta-cells from control and CaM-8 mice. Membrane current density was quantified for each individual cell by dividing current amplitude by the membrane capacitance. The voltage-dependence of K⁺ current density was very similar for the two types of beta-cells (Fig. 7B). In both types of cells, delayed rectifier K⁺ currents activated at membrane potentials of −60 mV and increased in magnitude at more positive potentials. The peak delayed rectifier K⁺ conductance, measured at +30 mV, was 0.92 ± 0.14 nS/pF in CaM-8 cells (n = 12) and 1.23 ± 0.25 nS/pF in control beta-cells (n = 27).
in control cells \((n = 25)\) at \(+30\,\text{mV}\). These two means are not significantly different from each other \((p > 0.20)\), indicating that the delayed rectifier K\(^+\) currents in CaM-8 cells are indistinguishable from those of controls. Thus, delayed rectifier K\(^+\) channels are not the cause of the low \([\text{Ca}^{2+}]_i\) transients that lead to deficient insulin secretion in the CaM-8 mice.

The reduced glucose-induced \([\text{Ca}^{2+}]_i\) signaling of CaM-8 beta-cells could result from a defect in voltage-gated Ca\(^{2+}\) channels. We initially tested this possibility by using fura-2 to measure the rises in \([\text{Ca}^{2+}]_i\), produced when these channels were opened by depolarizing the beta-cell membrane potential by treatment with saline containing 50 \text{mM} K\(^+\). As has been reported previously \((7, 13)\), this treatment produced a prompt elevation of \([\text{Ca}^{2+}]_i\) in islets from normal mice \(\text{(right side of Fig. 1A)}\). These responses were substantially reduced in islets from CaM-8 mice \(\text{(Fig. 1B)}\). The peak amplitude of the \([\text{Ca}^{2+}]_i\)-change produced by depolarization of control islets was approximately 400 \text{nM} above resting \([\text{Ca}^{2+}]_i\), but was approximately half this value in CaM-8 mice \(\text{(Fig. 2C)}\). Although this difference was not statistically significant \((p > 0.30)\), these data suggest possible changes in the Ca\(^{2+}\) channels of CaM-8 beta-cells. Experiments on dissociated beta-cells loaded with indo-1 also indicated similar reductions in the K\(^+\) induced \([\text{Ca}^{2+}]_i\) rise in CaM-8 cells \(\text{(data not shown)}\).

To examine Ca\(^{2+}\) channel properties more directly, we used patch-clamp methods to measure currents flowing through these channels. Ca\(^{2+}\) channel currents were examined in isolation by including cesium ions in the intracellular pipette solution to block K\(^+\) channels \((29)\). Families of Ca\(^{2+}\) currents recorded from control and CaM-8 cells under such conditions are shown in Fig. 8A. In both cases, the membrane potential initially was held at \(-70\,\text{mV}\) and then briefly stepped to more depolarized voltages to open voltage-gated Ca\(^{2+}\) channels. While the kinetics of these currents were similar in the two
populations of cells, Ca\textsuperscript{2+} current amplitude appeared to be smaller in the CaM-8 cells. This was quantified by calculating Ca\textsuperscript{2+} current density, using the procedure already described for K\textsuperscript{+} currents, and determining this parameter over a range of membrane potentials (Fig. 8B). There were no obvious differences in the voltage dependence of Ca\textsuperscript{2+} currents in beta-cells from the two types of mice; in both cases, Ca\textsuperscript{2+} currents were activated at \(-250\) mV, increased in magnitude as voltage was increased up to 0 mV, and decreased at more positive potentials. For both cell types, the currents reversed polarity at \(-50\) mV, though efflux of Cs\textsuperscript{+} makes this value an underestimate of the true reversal potential for Ca\textsuperscript{2+} currents (30). However, the peak magnitude of Ca\textsuperscript{2+} currents recorded from CaM-8 cells was about 50% smaller than that of control cells at all membrane potentials (Fig. 8B). For example, at 0 mV the mean Ca\textsuperscript{2+} current density was 8.9 ± 0.9 pA/pico farad (n = 34) in CaM-8 cells, slightly less than half that measured in control cells (18.1 ± 1.3 pA/pico farad; n = 23). The difference between these two means is significant (p < 0.05), indicating much smaller amounts of Ca\textsuperscript{2+} current in CaM-8 cells. This reduction in Ca\textsuperscript{2+} current is qualitatively sufficient to account for the defects in glucose-induced Ca\textsuperscript{2+} signaling and insulin secretion that lead to the diabetic phenotype of CaM-8 mice.

**DISCUSSION**

In this study we have identified the physiological defect that leads to the nonimmune diabetic condition of the CaM-8 mouse. We have found that the glucose-regulated Ca\textsuperscript{2+} signaling pathway is deficient in pancreatic beta-cells of this animal. Specifically, depolarization of CaM-8 beta-cells, in response to extracellular glucose, tolbutamide, or potassium, resulted in smaller rises in [Ca\textsuperscript{2+}], than in beta-cells from normal mice. These same treatments also consistently produced less insulin secretion from CaM-8 beta-cells than from normal beta-cells. Given that insulin secretion requires elevation of [Ca\textsuperscript{2+}], (23, 31, 32), it is likely that this defect in Ca\textsuperscript{2+} signaling accounts for the mild hyperglycemic phenotype demonstrated by the CaM-8 mice at the age we have examined (6–8 days old). Prolonged elevation of blood glucose levels in these young mice acts as a catalyst to further desensitize the beta-cells to external stimuli, so that the diabetic condition will become more severe as the animals get older (33).

The underlying cause of the Ca\textsuperscript{2+} signaling defect of CaM-8 beta-cells appears to be a defect in the voltage-gated Ca\textsuperscript{2+} channels of their plasma membrane. Patch-clamp measurements revealed a significant decline in the peak amplitude of voltage-gated Ca\textsuperscript{2+} channel currents while the voltage dependence and kinetics of these currents were similar to normal. Though multiple types of Ca\textsuperscript{2+} channels have been found in beta-cells and may contribute to [Ca\textsuperscript{2+}], elevation and insulin secretion (34, 35), the Ca\textsuperscript{2+} channels affected by the CaM-8 mutation are the L-type Ca\textsuperscript{2+} channel currents because dihydropyridines blocked these currents.\textsuperscript{2} This defect in voltage-gated Ca\textsuperscript{2+} channels should yield reduced elevation of [Ca\textsuperscript{2+}],

\textsuperscript{2} C.-R. Jan, T. J. Ribar, A. R. Means, and G. J. Augustine, unpublished results.
and reduced insulin secretion when CaM-8 beta-cells are depolarized. The fact that insulin secretion evoked by tolbutamide (Fig. 4) or elevated external K+ (15) (both treatments that trigger insulin secretion by opening voltage-gated Ca2+ channels) was also reduced in CaM-8 mice provides independent evidence for this interpretation.

Currents flowing through the other types of ion channels that regulate insulin secretion were largely normal in CaM-8 cells. Delayed rectifier K+ channels, which aid in repolarization of the membrane potential during each action potential (5, 17), were unaffected by expression of the CaM-8 protein. However, ATP-sensitive K+ channels, which initiate the electrical component of the secretory response by depolarizing the beta-cell (4, 26), carried larger than normal currents in the CaM-8 cells. This increase in ATP-sensitive K+ current might somehow contribute to the CaM-8 phenotype by altering the beta-cell membrane potential. While the cause of this increase in ATP-sensitive K+ channel current is not known, it may represent some sort of compensatory mechanism. For example, Yan et al. (36) have demonstrated that changes in the level of expression of one β-cell protein can result in a compensatory change in other associated proteins. An up-regulation of the number of ATP-sensitive K+ channels would make the resting potential more negative and the glucose-induced depolarization larger, which might compensate for the CaM-8 phenotype by allowing glucose to open a larger fraction of available voltage-gated Ca2+ channels. Currents carried by the Ca2+-activated K+ channels, which hyperpolarize the beta-cell membrane potential between bursts of action potentials (17), were not examined in our experiments.

The decreased magnitude of voltage-gated Ca2+ channel currents in CaM-8 cells could arise from a number of causes. For example, a reduction in Ca2+ channel currents could result from a decrease in the rate of Ca2+ channel synthesis and/or membrane insertion, an increased rate of Ca2+ channel degradation, post-translational changes in the channel, or changes in the regulation of the channel. We do not yet know which of these processes are involved in the CaM-8 mice.

The CaM-8 gene was originally designed to produce a protein that would serve as a control for overexpression of CaM (12, 15). The rationale was that the CaM-8 protein should mimic the ability of CaM to bind Ca2+, yet not activate effector proteins such as protein kinases and phosphatases (14–16). However, our data indicate that the CaM-8 protein must possess some intrinsic biological activity because its presence in beta-cells results in decreased Ca2+ channel currents. Overexpression of another E-F hand Ca2+ binding protein, calbindin-D28k, in GH3 pituitary cells also results in reduced Ca2+ channel currents through an unknown mechanism (37). Like CaM-8, calbindin-D28k, has not been shown to bind to other proteins, but has been suggested to regulate [Ca2+]i of the cells (37). Thus, it is possible that the Ca2+ binding properties of both calbindin-D28k and CaM-8 are sufficient in themselves to reduce Ca2+ channel currents through an unknown pathway. If so, this must not be an acute effect of Ca2+ binding because dialysis of the Ca2+- chelator EGTA during patch-clamp experiments does not inhibit Ca2+ currents in normal cells.

It is possible that truncating the central helix of CaM may yield an unusual conformation that allows the CaM-8 protein to bind to or otherwise block the voltage-gated Ca2+ channels. In Paramecium, changes in single amino acids in the C-terminal half of CaM prevents regulation of Ca2+-activated K+ channels (38). Additionally, changes in single amino acids in the N-terminal half of CaM lead to down-regulation of Ca2+-activated Na+ channels (38). Such studies suggest that CaM may modulate ion channels through a direct binding mechanism (39). If this were true for the voltage-dependent Ca2+ channels of beta-cells, then CaM-8 may interfere with the binding of Ca2+/CaM to these channels. However, currently there is no evidence that either CaM-8 or CaM participate in modulating this channel through a direct binding mechanism (10, 40).

Although there is some controversy (41), Ca2+/calmodulin-independent kinase, type II has been reported to modulate the activity of beta-cell Ca2+ channels through phosphorylation (40). While the CaM-8 protein conceivably could compete with native CaM for binding to this kinase in the CaM-8 cells, the fact that CaM-8 protein is about 100 times less potent in binding to CaM-binding proteins (15) makes this possibility unlikely. Alternatively, it is conceivable that CaM-8 could bind to other proteins involved in regulation of the voltage-gated Ca2+ channels.

There are also similarities between the reduction of the Ca2+ channel currents in the CaM-8 beta-cells and the reductions in Ca2+ currents seen in beta-cells depleted of protein kinase C (42). In both instances Ca2+ currents are substantially reduced in the beta-cells while the voltage dependence and kinetics of Ca2+ channel gating do not appear to be altered (Fig. 8) (42). In normal beta-cells activation of protein kinase C results from a Ca2+ dependent hydrolysis of phosphoinositides by a phosphoinositide-specific phospholipase C (43). This hydrolysis generates inositol 1,4,5-triphosphate, which rapidly releases internal Ca2+ stores, and diacylglycerol which activates protein kinase C (43). It has been suggested that one of the consequences of activating protein kinase C in beta-cells is to shift the voltage dependence of the Ca2+ channel so that it activates at more negative potentials, which could enhance and sustain long term glucose induced insulin secretion (42, 43). It is possible that the action of CaM-8 protein is inhibiting some component of this protein kinase C-mediated pathway. Consistent with the possibility that the reduction in Ca2+ currents seen in CaM-8 mice may be mediated via the protein kinase C pathway comes from the fact that stimulating islets with a muscarinic agonist (carbachol) that activates protein kinase C can restore the secretory response in islets from CaM-8 mice (15).

One of the original aims in developing mouse lines which overexpress CaM and CaM-8 was to explore the role of CaM in cell growth and differentiated cellular function (10, 44, 45) in the pancreatic beta-cell. Initial analysis of these transgenic mice suggested that CaM might be part of the glucose-regulated signaling pathway that triggers the exocytotic secretion of insulin (11, 12, 15). However, detailed analysis of the lesions produced by these genetic manipulations indicates that this approach is not as straightforward as intended. The results presented here show that the presence of CaM-8 yields a lesion in Ca2+ channel currents which not only is upstream of the reactions directly responsible for insulin secretion but also is completely unanticipated based on the known properties of the CaM-8 protein and the voltage-gated Ca2+ channel. Likewise, overexpression of CaM yields unpredicted upstream effects on ATP utilization in the beta-cell (13). Taken together, these results indicate that considerable caution must be exercised in interpreting the experimental consequences of long term manipulation of gene expression and cast some uncertainty on the utility of this approach for sorting out the molecular constituents of the secretory pathway (46). However, given that the nature of the signaling defect that now has been identified in the CaM-8 mouse, this mouse line could be useful for studying Ca2+ signaling pathways and the long term regulation of Ca2+ channels. Further, having diabetic mice with defined physiological lesions should be valuable for understanding the mechanisms involved in diabetes.

Downloaded from http://www.jbc.org/ by guest on July 21, 2018
ACKNOWLEDGMENTS—We thank Hui Qiu for writing the Axobasic Ca²⁺ measurement software used in these experiments, Lin Coyle for network assistance on the computer, and Bruno Alicke for participating in several experiments.

REFERENCES
1. Holz, G. G., and Habener, J. F. (1992) Trends Biochem. Sci. 17, 388–393
2. German, M. S. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 1781–1785
3. Rorsman, P., and Overbeek, P. A., and Means, A. R. (1985) Pflugers Arch. 405, 305–309
4. Ashcroft, S. J., and Ashcroft, F. M. (1990) Cdl. Signaling 2, 197–214
5. Rorsman, P., and Trube, G. (1986) J. Physiol. 374, 531–550
6. Smith, P. A., Rorsman, P., and Ashcroft, F. M. (1989) Nature 342, 550–553
7. Herchuelz, A., Pochet, R., Pastiels, C., and VanPraet, A. (1991) Cell Calcium 12, 577–586
8. Sugden, M. C., Christie, M. R., and Ashcroft, S. T. (1979) FEBS Lett. 105, 95–100
9. Henquin, J. C. (1981) Biochem. J. 196, 771–780
10. Valverde, I., and Malaisse, W. J. (1984) Experientia 40, 1061–1068
11. Epstein, P. N., Overbeek, P. A., and Means, A. R. (1989) Cell 58, 1067–1073
12. Epstein, P. N., Ribar, T. J., Decker, G. L., Yaney, G., and Means, A. R. (1992) Endocrinology 130, 1387–1393
13. Ribar, T. J., Jan, C. R., Augustine, G. J., and Means, A. R. (1995) J. Biol. Chem. 270, 28688–28695
14. VanBerkum, M. F. A., George, S. E., and Means, A. R. (1990) J. Biol. Chem. 265, 3750–3756
15. Ribar, T. J., Epstein, P. N., Overbeek, P. A., and Means, A. R. (1995) Endocrinology 136, 106–115
16. Gruver, C. L., DeMayo, F., Goldstein, M. A., and Means, A. R. (1993) Endocrinology 133, 376–388
17. Rajan, A. S., Aguilar-Bryan, L., Nelson, D. A., Yaney, G. C., Hsu, W. H., Kunze, D. L., and Boyd, A. E., III (1990) Diabetes Care 13, 340–363
18. Grynkiewicz, G., Poenie, M., and Tsien, R. Y. (1985) J. Biol. Chem. 260, 3440–3450
19. Neher, E (1989) in Sellin, L. C. Libelius, R., and Theileff, S. (eds) Neuronal Nucleolar J. uncton, pp. 65–76, Elsevier, Amsterdam
20. Augustine, G. J., and Neher, E. (1992) J. Physiol. 450, 247–271
21. Ashcroft, S. J., Weerasinge, L. C. C., Bassett, J. M., and Randle, P. J. (1972) Biochem. J. 126, 525–532
22. Hamill, O. P., Marty, A., Neher, E., Sakmann, B., and Sigworth, F. T. (1981) Pflugers Archiv. 391, 85–100
23. Grodsky, G. M., and Bennett, L. L. (1966) Diabetes 15, 910–912
24. Wang, J., Baimbridge, K. G., and Brown, J. C. (1992) Endocrinology 131, 46–52
25. Smolen, P., Rinzell, J., and Sherman, A. (1993) Biophys. J. 64, 1668–1680
26. Ashcroft, F. M. (1988) Annu. Rev. Neurosci. 11, 97–118
27. Trube, G., Rorsman, P., and Ohno Shosaku, T. (1986) Pflugers Arch. 407, 493–499
28. Bokvist, K., Rorsman, P., and Smith, P. A. (1990) J. Physiol. 423, 327–342
29. Smith, P. A., Ashcroft, F. M., and Fewtrell, C. M. S. (1993) J. Gen. Physiol. 101, 767–779
30. Hess, P., and Tsien, R. W. (1984) Nature 309, 453–456
31. Gillis, K. D., and Misler, S. (1991) Pflugers Arch. 420, 121–123
32. Penner, R., and Neher, E. (1988) J. Exp. Biol. 139, 329–345
33. Bokvist, K., Rorsman, P., Yan, H. M., Bright, D., and Grodsky, G. M. (1991) Endocrinology 129, 2131–2138
34. Leech, C. A., Holz, G. G., IV, and Habener, J. F. (1994) Endocrinology 135, 365–372
35. Pollo, A., Lovallo, M., Biancardi, E., Sher, E., Soci, C., and Carbone, E. (1993) Pflugers Arch. 423, 462–471
36. Yan, H. M., Landis, C., Tchao, N., Wang, J., Rodd, G., Hanahan, D., Bourne, H. R., and Grodsky, G. M. (1994) Endocrinology 134, 42–47
37. Lledo, P. M., Somasundaram, B., Morton, A. J., Emson, P. C., and Mason, W. T. (1992) Neuron 9, 943–954
38. Kung, C., Preston, R. R., Maley, M. E., Ling, K. Y., Kanabrocki, J. A., Seavey, B. R., and Saimi, Y. (1992) Cell Calcium 13, 413–425
39. Saimi, Y., and Kung, C. (1994) FEBS Lett. 350, 155–158
40. Wenham, R. M., Landt, M., Walters, S. M., Hidaka, H., and Easom, R. A. (1992) Biochem. Biophys. Res. Commun. 189, 128–133
41. Li, G., Hidaka, H., and Wollheim, C. B. (1992) Mol. Pharmacol. 42, 489–498
42. Arkhammar, P., Juntti-Berggren, L., Larsson, O., Welsh, M., Nanberg, E., Sjoblom, A., Kalyer, M., and Berggren, P. O. (1994) J. Biol. Chem. 269, 2743–2749
43. Hedeskov, C. J., Thams, P., Gambel, M., Malik, T., and Capito, K. (1991) Mol. Cell. Endocrinol. 78, 187–195
44. Chafouleas, J. G., Bolton, W. E., Hidaka, H., Boyd, A. E., III, and Means, A. R. (1982) Cell 28, 41–50
45. Means, A. R. (1988) Recent Prog. Horm. Res. 44, 223–262
46. Sudhof, T. C. (1995) Nature 375, 645–653
Alterations in Calcium Channel Currents Underlie Defective Insulin Secretion in a Transgenic Mouse
Chung-Ren Jan, Thomas J. Ribar, Anthony R. Means and George J. Augustine

J. Biol. Chem. 1996, 271:15478-15485.
doi: 10.1074/jbc.271.26.15478

Access the most updated version of this article at http://www.jbc.org/content/271/26/15478

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 45 references, 12 of which can be accessed free at http://www.jbc.org/content/271/26/15478.full.html#ref-list-1