Different insulin-secretory responses to calcium-channel blockers in islets of lean and obese (*ob/ob*) mice

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The purpose of these experiments was to determine whether the activity of the voltage-dependent Ca^{2+} channel was modulated in the same manner in islets of the *ob/ob* mouse as in islets of homozygous lean mice of the same strain. The effect of agents that are known to alter the concentrations and movements of intracellular Ca^{2+} were investigated in relation to glucose-stimulated insulin secretion and in relation to the effect of forskolin. In islets of obese mice, verapamil and nifedipine both inhibited glucose-induced insulin release, nifedipine being the more potent inhibitor. Forskolin-stimulated secretion was inhibited either not at all (verapamil) or much less (nifedipine) in islets of the *ob/ob* mouse compared with those of lean mice. At basal glucose concentrations, verapamil initiated insulin secretion in islets of the *ob/ob* mouse and acted synergistically with forskolin to evoke a secretory activity that was 3-fold greater than that evoked by 20 mM-glucose. Nifedipine also initiated secretion at basal glucose concentrations and acted synergistically with forskolin, but its effect was considerably smaller than that of verapamil. A comparison of the effect of forskolin in the presence of Ca^{2+} -channel blockers and in the absence of Ca^{2+} suggests that, in the obese mouse, the operation of the voltage-dependent Ca^{2+} channel is impaired.

INTRODUCTION

The process of stimulus–secretion coupling in the pancreatic β -cell is mediated by an increase in cytosolic Ca^{2+} (Hellman, 1985). Glucose-induced secretion is completely dependent on the presence of extracellular Ca^{2+} , which is believed to enter the β -cell mainly through voltage-dependent Ca^{2+} channels (see Wollheim & Sharp, 1981). Ca^{2+} can also be mobilized from intracellular stores, a source that is believed to be important to the process of insulin release.

Activators of adenylate cyclase augment the concentrations of cyclic AMP in isolated islets and enhance insulin secretion (Sharp, 1979). Although cyclic AMP enhances the Ca^{2+} response, the mechanism is still poorly understood (Rasmussen, 1986*a,b*). It has been proposed that increases in cyclic AMP potentiate secretion by increasing the amount of Ca^{2+} arising from intracellular stores (Rasmussen, 1986*a,b*), by enhancing Ca^{2+} influx through the voltage-dependent Ca^{2+} channels (Henquin & Meissner, 1984*a,b,c*; Eddlestone *et al.*, 1985) and/or (by analogy with cardiac cells) by inducing the phosphorylation of Ca^{2+} -channel proteins, increasing the probability that the channel is opened (Osterrieder *et al.*, 1982; Cachelin *et al.*, 1983; Bean *et al.*, 1984; Brum *et al.*, 1984). In neural cells, there is also considerable evidence that cyclic AMP-dependent phosphorylation can decrease the conductance of the K^{+} channel (Ozawa & Sand, 1986). Any of these effects, if present in islets, would lead to enhanced insulin secretion. It is noteworthy, however, that studies with permeabilized islets or insulin-secreting tumour cells have shown that cyclic AMP may be able to enhance insulin secretion independently of the cytosolic Ca^{2+} concentration (Wollheim *et al.*, 1984; Rorsman & Abrahamsson, 1985; Tamagawa *et al.*, 1985; Jones *et al.*, 1986).

Although direct biochemical evidence for the presence

of voltage-dependent Ca^{2+} channels in islets is lacking, their involvement in insulin secretion has been supported by the use of Ca^{2+} -channel blockers of various types. Antagonists of both the phenylalkylamine (class II) type (Devis *et al.*, 1975; Malaisse *et al.*, 1976; Malaisse & Boschero, 1977; Wollheim *et al.*, 1978) and the diphenylalkylamine (class III) type (Malaisse & Boschero, 1977; Malaisse *et al.*, 1984) decrease $^{45}\text{Ca}^{2+}$ influx and insulin secretion. Moreover, the Ca^{2+} agonists increase Ca^{2+} uptake and insulin secretion (Malaisse-Lagae *et al.*, 1984; Malaisse & Mathias, 1985; Morgan *et al.*, 1985).

The obese mouse is characterized by an exaggerated insulin-secretory response (Dubuc, 1976; Lavine *et al.*, 1977; Dunbar & Walsh, 1980; Ahren & Lundquist, 1982; Dalpé-Scott *et al.*, 1983). Insulin secretion in these islets is extremely sensitive to low concentrations of forskolin and less dependent on the provision of normal concentrations of extracellular Ca^{2+} than in the islets of lean mice (Black *et al.*, 1986; M. A. Black, H. M. Heick & N. Bégin-Heick, unpublished work). The reports indicating abnormal electrical activity and ionic permeabilities in the islets of obese mice of the Norwich colony (Rosario *et al.*, 1985) prompted us to examine the effects of drugs that alter the Ca^{2+} -channel activity on insulin secretion in islets of obese mice.

EXPERIMENTAL

Materials

Collagenase and hyaluronidase were from Worthington Biochemical Corp. (via Cooper Biomedical, Mississauga, Ontario, Canada); bovine serum albumin (fraction V, radioimmunoassay grade), verapamil and nifedipine were from Sigma Chemical Co., St. Louis, MO, U.S.A.; forskolin was from Calbiochem–Behring, San Diego, CA, U.S.A. (via Terochem Laboratories,

Table 1. Effect of verapamil on glucose-induced and forskolin-enhanced insulin secretion

Groups of three to four islets were preincubated at 3 mM-glucose and incubated at 20 mM-glucose as described under 'Methods'. Where indicated, verapamil or forskolin was added during the incubation period. Insulin secretion is reported as a percentage of the total insulin content of the islets. The data are means $\pm$ S.E.M. for $n = 10$ –20 observations. The level of significance (P value) of the differences between samples incubated without and with verapamil is indicated by the numbers in parentheses. Forskolin produced a significant effect in each case.

Mice	Insulin secreted (%)			
	Forskolin (μ M) ...		Verapamil (μ M) ...	
	0	50	0	50
Lean	0.9 $\pm$ 0.20	0.4 $\pm$ 0.04 (0.058)	15.3 $\pm$ 2.50 (< 0.001)	4.9 $\pm$ 0.98
Obese	10.1 $\pm$ 0.59 (< 0.04)	8.0 $\pm$ 1.02	42.7 $\pm$ 2.31 (> 0.2)	40.3 $\pm$ 3.89

Toronto, Ontario, Canada). CGP-28392 was a gift from Dr. H. Kuhn, Ciba-Geigy, Basel, Switzerland.

Animals

Male C57BL/6J-*ob/ob* mice and their lean controls (+ / +) were obtained from the Jackson Laboratory, Bar Harbor, ME, U.S.A. They were maintained on Purina Chow and water *ad libitum* and used in experiments when between 8 and 12 weeks of age.

Methods

Preparation of islets. The splenic portion of the pancreas was removed and the islets were prepared by collagenase digestion as described in detail previously (Dalpé-Scott *et al.*, 1983).

Determination of insulin secretion. Unless otherwise noted in the legends, islets were preincubated (45 min) and incubated (60 min) at 37° in Krebs-Ringer bicarbonate buffer (see below) under O₂/CO₂ (19:1). At the end of the incubation period, the medium was removed and frozen for subsequent determination of insulin content. The islets were extracted with acid ethanol and the extract was saved for insulin determination (Dalpé-Scott *et al.*, 1982). The results are expressed as the fraction of insulin secreted {[insulin secreted/(insulin remaining in the islets + insulin secreted)] $\times$ 100}. The absolute amount of insulin secreted is a function of the total insulin content of the islet. Whereas in lean mice islets are of fairly uniform size and degree of granulation, in obese mice both the size of the islet and the degree of granulation vary considerably, leading to large differences between samples in the absolute amount secreted. When expressed on the basis of the fractions of insulin secreted, these differences are less and the agreement between samples is much better. From measurement of several hundred islets, the average insulin content of the islets of lean mice is 32 ng, compared with 20 ng for the islets of obese mice.

Incubation medium. The composition of the Krebs-Ringer bicarbonate was as described previously (Black *et al.*, 1986). The drugs used were added as ethanol solutions, and, in the case of dantrolene, as a dimethyl sulphoxide solution. The same amount of ethanol or

dimethyl sulphoxide was added to the control tubes. When the dihydropyridine compounds (nifedipine and CGP-28392) were used, care was taken to minimize exposure to light as much as possible.

Statistical analysis. This was done by analysis of variance or by unpaired t test. The significance of differences between means is given in the individual Tables and Figures.

RESULTS

Glucose-induced insulin secretion was decreased in the presence of 50 μ M-verapamil in the islets of both lean and obese mice. These experiments show that verapamil had a similar effect on glucose-induced insulin secretion in islets of lean and obese mice. In contrast, in the presence of forskolin, 50 μ M-verapamil effectively inhibited secretion (by 72 %) in the islets of lean mice, but had no effect in the islets of obese mice (Table 1).

Further experiments showed that the inhibitory effect of verapamil on forskolin-enhanced secretion was dose-dependent in islets of lean mice, but a typical dose/response curve was not obtained in the islets of obese mice (see Fig. 1a). To determine whether the failure of verapamil to inhibit secretion in the presence of forskolin in the islets of obese mice was related to the ability of forskolin to induce secretion at non-stimulatory glucose concentrations (Black *et al.*, 1986; M. A. Black, H. M. Heick & N. Bégin-Heick, unpublished work), the effects of verapamil at basal glucose concentrations were examined (Table 2). Verapamil (50 μ M) had no significant effect on secretion at basal (3 mM) glucose concentrations in islets of lean mice. Surprisingly, under the same conditions verapamil produced a large stimulatory effect in islets of obese mice. Furthermore, forskolin and verapamil acted synergistically to stimulate insulin secretion at basal glucose concentrations in both types of islets, although the magnitude of the effect was much less in the islets of lean (2 $\times$ activity with forskolin alone) than those of obese mice (9 $\times$ activity with forskolin alone).

Since at basal glucose concentrations 50 μ M-verapamil could actually induce secretion as effectively as 20 mM-glucose in islets of obese mice and, with forskolin,

Fig. 1. Effect of increasing concentrations of verapamil on insulin secretion

Islets of lean mice (○) and obese mice (●) were preincubated at 3 mM-glucose and incubated at 20 mM-glucose (a) or 3 mM-glucose (b) with 10 μM-forskolin and the concentrations of verapamil indicated on the abscissa. Each data point represents the mean ± S.E.M. for 4–12 observations. The results are expressed as insulin secreted as a percentage of total islet insulin content. In (a) the left ordinate corresponds to the values for lean mice, and the right to those for obese mice.

synergistically increase secretion even further, we examined the effect of different concentrations of verapamil on forskolin-stimulated insulin secretion in islets of obese mice. As shown in Fig. 1(b), this stimulatory effect occurred at concentrations above 5 μM, and its magnitude was dose-dependent. Since this effect of verapamil was slight in islets of lean mice, it is concluded that the unusual response to verapamil observed in the islets of obese mice was not due to non-specific interactions, which would be expected to be similar in both types of islets, but to a functional difference in the two types of islets.

Because nifedipine is supposed to act on the voltage-dependent Ca²⁺ channel at a site different from that of verapamil (Stanfield, 1986), it was used to study further

the secretory abnormalities in islets of obese mice. The purpose of employing this agent was two-fold: first, to study the issue of a decreased requirement for extracellular Ca²⁺ in the islets of obese mice, and secondly, to see if the stimulatory effects of verapamil at 3 mM-glucose could be duplicated by a different Ca²⁺ blocker.

The results in Table 3 show that nifedipine inhibited glucose-induced secretion much more efficiently in islets of obese than of lean mice. Therefore, in contrast with verapamil, nifedipine could effectively block the entry of Ca²⁺ through voltage-dependent channels in islets of obese mice, and thus inhibit insulin secretion. In the presence of forskolin, however, nifedipine was much less effective in the islets of obese than of lean mice.

At 3 mM-glucose (Table 4), nifedipine by itself produced a 3.5-fold stimulation of secretion in the islets of obese mice. Nifedipine acted synergistically with forskolin in enhancing secretion and, like verapamil, the effect was considerably greater in the islets of obese than of lean mice.

The action of the dihydropyridine Ca²⁺-channel agonist CGP-28392 was examined next (Table 5). In islets of both lean and obese mice, this agent enhanced insulin secretion stimulated by 10 mM- and 20 mM-glucose. In the islets of lean mice [and in those of rats, as reported by Malaisse & Mathias (1985)], this compound did not stimulate secretion at 3 mM-glucose. In contrast, at 3 mM-glucose in the islets of obese mice, stimulation occurred which was similar in magnitude to that produced by nifedipine at that concentration of glucose (cf. Table 4), suggesting a similar mechanism.

The effect of trifluoperazine was examined (Table 6) in order to see whether or not it could produce effects similar to those of the more specific Ca²⁺-channel antagonists, verapamil and nifedipine. Like nifedipine, trifluoperazine inhibited glucose-induced and forskolin-potentiated insulin secretion in islets of obese mice, but it had little effect on these processes in the islets of lean mice. At 3 mM-glucose, trifluoperazine stimulated insulin secretion and, like verapamil and nifedipine, it acted synergistically with forskolin in the islets of lean and obese mice.

One of the suggested effects of forskolin on insulin secretion (via cyclic AMP) is to mobilize stored Ca²⁺. The effect of dantrolene was therefore studied. The results in Table 7 show that, in the islets of obese mice,

Table 2. Effect of verapamil on basal and forskolin-stimulated insulin secretion

Islets were preincubated and incubated in a medium containing 3 mM-glucose and verapamil and forskolin as indicated. The data are means ± S.E.M. for the numbers of observations given in parentheses; ^a indicates that the effect of verapamil is significant ($P < 0.001$), ^b that the effect of forskolin is significant ($P < 0.001$), and ^c that the effect of forskolin and verapamil are significant ($P < 0.002$).

	Insulin secreted (%)			
	Forskolin (μM) ... 0		10	
	Verapamil (μM) ... 0	50	0	50
Mice				
Lean	0.2 ± 0.03 (12)	0.2 ± 0.04 (7)	0.3 ± 0.10 (7)	0.7 ± 0.08 ^c (8)
Obese	0.3 ± 0.03 (15)	9.7 ± 0.24 ^a (4)	1.7 ± 0.33 ^b (8)	18.3 ± 2.22 ^c (8)

Table 3. Effect of nifedipine on glucose-induced and forskolin-enhanced insulin secretion

Groups of islets were preincubated at 3 mM-glucose and incubated at 20 mM-glucose as described under 'Methods'. Where indicated, nifedipine or forskolin was added during the incubation period. Insulin secretion is reported as a percentage of the total insulin content of the islets. The data are means $\pm$ S.E.M. for the numbers of observations given in parentheses: * indicates a significant difference ($P < 0.001$) between samples incubated without and with nifedipine. The values with forskolin + glucose are significantly different from those with glucose alone.

Mice	Forskolin (μ M) ...	Nifedipine (μ M) ...	Insulin secreted (%)			
			0		10	
			0	50	0	50
Lean			0.6 $\pm$ 0.08 (11)	0.3 $\pm$ 0.05 (10)	5.9 $\pm$ 1.3 (9)	1.6 $\pm$ 0.18* (9)
Obese			7.8 $\pm$ 0.78 (7)	0.8 $\pm$ 0.15* (7)	42.5 $\pm$ 2.8 (7)	21.2 $\pm$ 1.08* (7)

Table 4. Effect of nifedipine on basal and forskolin-stimulated insulin secretion

Islets were preincubated and incubated in a medium containing 3 mM-glucose with nifedipine and forskolin as indicated. The data are means $\pm$ S.E.M. for the numbers of observations in parentheses: * indicates that the effect of nifedipine is significant ($P < 0.05$), ^b that the effect of forskolin is significant ($P < 0.001$) and ^c that the combined effects of forskolin and nifedipine are significantly different ($P < 0.001$) from either alone.

Mice	Forskolin (μ M) ...	Nifedipine (μ M) ...	Insulin secreted (%)			
			0		10	
			0	50	0	50
Lean			0.6 $\pm$ 0.11 (10)	0.5 $\pm$ 0.09 (10)	0.4 $\pm$ 0.07 (10)	1.3 $\pm$ 0.15 ^c (15)
Obese			0.4 $\pm$ 0.08 (9)	1.6 $\pm$ 0.28* (12)	1.1 $\pm$ 0.29 ^b (7)	11.8 $\pm$ 1.54 ^c (16)

Table 5. Effect of Ca²⁺-channel agonist on insulin secretion, stimulated by different glucose concentrations

Islets were preincubated at 3 mM-glucose and incubated with the concentrations of glucose indicated, without and with 50 μ M of the Ca²⁺-channel agonist CGP-28392. The data are means $\pm$ S.E.M. for the numbers of observations given in parentheses. The level of significance of the difference between samples with and without CGP-28392 is given by the P value in brackets beneath each set of values.

Mice	Glucose (mM) ... CGP-28392	Insulin secreted (%)		
		3	10	20
Lean	—	0.2 (2)	0.1 $\pm$ 0.02 (3)	0.6 $\pm$ 0.16 (4)
	+	0.1 $\pm$ 0.03 (3) [> 0.2]	0.4 $\pm$ 0.19 (3) [0.04]	1.0 $\pm$ 0.14 (3) [0.05]
Obese	—	0.3 $\pm$ 0.04 (3)	2.3 $\pm$ 0.63 (3)	5.3 $\pm$ 0.64 (4)
	+	1.9 $\pm$ 0.26 (3) [0.001]	5.9 $\pm$ 1.40 (3) [0.02]	7.4 $\pm$ 0.52 (4) [0.01]

dantrolene had a small inhibitory effect on glucose-induced insulin secretion, but that it had little effect on forskolin-potentiated secretion. This shows that the presence of forskolin could effectively mask any inhibitory effect of dantrolene.

DISCUSSION

Hess *et al.* (1984) have proposed that the gating of the voltage-dependent Ca²⁺ channel switches between different modes and that the β -adrenergic agonist iso-

Table 6. Effect of trifluoperazine on insulin release

Islets were preincubated at 3 mM-glucose and incubated at either 3 mM- or 20 mM-glucose, without and with 100 μ M-forskolin and 20 μ M-trifluoperazine (TFP) as indicated. When added, TFP was present during both the preincubation and the incubation periods. The data represent means $\pm$ S.E.M. for the numbers of observations in parentheses: * forskolin significantly stimulated insulin release at a given concentration of glucose; ^b TFP significantly increased insulin release; ^c TFP significantly decreased insulin release. The level of significance was $P < 0.01$ in each case.

		Insulin secreted (%)			
[Glucose]	TFP (μ M) ... Forskolin (μ M) ... Mice	0	20	0	20
		0	0	100	100
3 mM	Lean	0.4 $\pm$ 0.1 (13)	1.3 $\pm$ 0.3 ^b (16)	0.6 $\pm$ 0.1 (7)	4.4 $\pm$ 1.2 ^b (7)
	Obese	1.0 $\pm$ 0.3 (12)	4.2 $\pm$ 0.6 ^b (15)	2.7 $\pm$ 0.3 ^a (4)	13.9 $\pm$ 1.2 ^b (5)
20 mM	Lean	1.1 $\pm$ 0.4 (14)	1.1 $\pm$ 0.4 (14)	5.3 $\pm$ 1.0 ^a (8)	5.1 $\pm$ 1.5 ^a (5)
	Obese	11.0 $\pm$ 1.2 (14)	4.4 $\pm$ 0.8 ^c (13)	49.3 $\pm$ 4.5 ^a (4)	23.6 $\pm$ 4.2 ^c (5)

Table 7. Effect of dantrolene on insulin secretion

Islets were preincubated with 3 mM-glucose and incubated with 20 mM-glucose for 10 min. Where indicated, the medium contained forskolin and/or dantrolene. The data are means $\pm$ S.E.M. for four observations: * denotes a significant effect of dantrolene ($P = 0.022$).

		Insulin secreted (%)			
Mice	Forskolin (μ M) ...	0		10	
	Dantrolene (μ M) ...	0	100	0	100
Lean		0.3 $\pm$ 0.12	0.2 $\pm$ 0.05	0.6 $\pm$ 0.09	0.5 $\pm$ 0.08
Obese		2.0 $\pm$ 0.16	1.3 $\pm$ 0.19*	5.1 $\pm$ 0.74	6.8 $\pm$ 0.70

prenaline, acting via cyclic AMP, increases the number of functional channels by recruitment of channels from mode 0 to mode 1. This change in the properties of the channel is believed to be due to a cyclic AMP-induced phosphorylation (Osterrieder *et al.*, 1982; Cachelin *et al.*, 1983; Bean *et al.*, 1984; Brum *et al.*, 1984). Likewise, it has been shown that cyclic AMP can lead to depolarization of membranes by promoting the phosphorylation of the Ca²⁺-activated K⁺ channels (Dunlap, 1985; Choquet *et al.*, 1987; Grega *et al.*, 1987). Similar changes in channel activity in islets would result in enhanced insulin secretion, by enhancing the influx of Ca²⁺ and decreasing the efflux of K⁺, keeping the membrane in a more depolarized state. It is thus possible that forskolin alters insulin secretion via these mechanisms, since glucose-induced electrical activity is increased by forskolin in mouse islets (Henquin & Meissner, 1984a; Eddlestone *et al.*, 1985), and the only important action of forskolin in cells is believed to be that on the adenylate cyclase system (Seamon & Daly, 1986).

In the presence of a Ca²⁺-channel blocker, the stimulatory effect of forskolin should be greatly attenuated, because of the decrease in activity of the Ca²⁺ channel. The effects of verapamil and nifedipine on

glucose-induced insulin secretion (Tables 1 and 3) can be explained on that basis. The difference in the effectiveness of the two drugs could be due to the fact that they interact at different sites in the Ca²⁺ channel (Schramm & Toward, 1985; Spedding, 1985; Stanfield, 1986), have different potencies (cf. Schramm *et al.*, 1983), or that verapamil interacts at other sites, such as with the Ca²⁺-activated K⁺ channel (Lebrun *et al.*, 1981; Henquin *et al.*, 1982), in addition to the voltage-dependent Ca²⁺ channel.

In islets of lean mice, forskolin-enhanced insulin secretion was effectively blocked by both nifedipine (by 82%; Table 3) and verapamil (by 73%; Table 1). The degree of inhibition produced by the antagonists is compatible with the data obtained in Ca²⁺-deprived media (90% inhibition; M. A. Black, H. M. Heck & N. Bégin-Heick, unpublished work). In contrast, incubation in Ca²⁺-deprived media decreased forskolin-enhanced secretion by 83% (M. A. Black, H. M. Heck & N. Bégin-Heick, unpublished work) in islets of obese mice, but verapamil was ineffective, and nifedipine was only partially effective (46% inhibition), in inhibiting secretion. These data show that in the islets of lean mice the blockers were effective in preventing Ca²⁺ influx,

since they decreased secretion as effectively as did the removal of Ca^{2+} . In the obese mice, the removal of Ca^{2+} was considerably more effective than the blockers in decreasing secretion, demonstrating either that the channels themselves are less effectively blocked by the drugs, or that secretion can occur despite a blocked channel. Neither alternative is consistent with a normal process.

The finding that at basal glucose concentrations nifedipine was an effective inducer of insulin secretion in islets of lean and obese mice, whereas verapamil had an even more pronounced effect, but only in islets of obese mice, was surprising. In the absence of glucose, these compounds appeared to be able to act as Ca^{2+} agonists, an effect that has not previously been described in islet tissue. At basal glucose concentrations, the channels should be primarily in the 'closed' state (in mode 1). It is possible that an antagonist interacting with the Ca^{2+} channel in that state is able to switch and stabilize it into mode 2, increasing Ca^{2+} entry and insulin secretion. The dihydropyridine antagonists have been found to behave as agonists under certain conditions (Hess *et al.*, 1984); furthermore, there are types of dihydropyridines (CGP-28392, BAY-k8644) that are agonists and increase the probability that the Ca^{2+} channel remains in the 'open' state (Schramm *et al.*, 1983; Erne *et al.*, 1984). The finding that CGP-28392 enhanced insulin secretion in islets supports the hypothesis that this compound prolongs the effect of glucose and increases Ca^{2+} influx through the channels, and the finding that CGP-28392 induced secretion at basal glucose concentrations in the islets of the obese mouse is further evidence for a lack of normal control in this animal.

Although the analogy between the dihydropyridine agonists and antagonists may be an adequate explanation for the action of nifedipine, it is unlikely that it could be invoked to explain the strong stimulatory effect of verapamil at basal glucose concentrations in the islets of obese mice. Lebrun *et al.* (1981) and Henquin *et al.* (1982) have reported that the phenylalkylamines can decrease K^+ permeability directly, theoretically enhancing Ca^{2+} influx and causing a stimulation rather than an inhibition of insulin secretion. If K^+ permeability is already decreased in islets of the obese mouse, as reported by Rosario *et al.* (1985) and Rosario (1985), exposure to verapamil would decrease it further and cause a stimulation rather than an inhibition of secretion at basal glucose concentrations; on that basis, one would predict that the stimulation would be enhanced even further in the combined presence of verapamil and forskolin, since the increased cyclic AMP concentrations would further decrease K^+ permeability and increase the probability of opening of the Ca^{2+} channel. This is indeed what was observed in the islets of obese mice, but to a much lesser extent in the islets of lean mice. Although cyclic AMP concentrations do not rise as high in the islets of obese as of lean mice in response to forskolin (Black *et al.*, 1986; M. A. Black, H. M. Heick & N. Bégin-Heick, unpublished work), the increase in cyclic AMP induced by forskolin may be sufficient to exacerbate an already abnormal process. This explanation provides a reasonable framework to explain the lack of effect of verapamil on glucose-induced secretion enhanced by forskolin in the islets of obese mice, the stimulatory effect of verapamil at basal glucose concentrations, the synergistic effect of the drug with

forskolin and the apparent enhanced sensitivity of the insulin-secretory process to Ca^{2+} in the islets of the obese mouse. Direct measurements of intracellular Ca^{2+} concentrations and work with permeabilized islets may help to clarify the mechanisms further.

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