Dual Functional Role of Membrane Depolarization/Ca^{2+} Influx in Rat Pancreatic B-Cell

YOSHIHIKO SATO, TORU AIZAWA, MITSUHISA KOMATSU, NAOMI OKADA, AND TAKASHI YAMADA

Transient exposure of rat pancreatic B-cell to 50 mM K^+ ($[K^+_{50}]$) makes exocytosis unresponsive to further depolarization, i.e., stimulation with 100 mM K^+ or 1 uM glyburide, which closes the ATP-sensitive K^+ (K^+_{ATP}) channel, simultaneously with $[K^+_{50}]$ does not produce any greater insulin secretion compared with $[K^+_{50}]$ alone. In sharp contrast, 16.7 mM glucose ($[G_{16.7}]$) applied simultaneously with $[K^+_{50}]$ elicits an insulin response markedly greater than that produced by $[K^+_{50}]$ alone, which is not attenuated by 100 uM diazoxide, an inhibitor of K^+_{ATP} channel closure. $[G_{16.7}]$-induced insulin secretion at the basal K^+ concn of 4.7 mM was greatly (93%) suppressed by 100 uM diazoxide. Insulin secretion induced by $[K^+_{50}]$ plus $[G_{16.7}]$ ($[K^+_{50} + G_{16.7}]$) was markedly suppressed (70%) by 1 uM nifedipine, a Ca^{2+}-channel blocker and was completely abolished by 2 mM 2-cyclohexen-1-one, which reportedly decreases reduced glutathione level and blocks glucokinase. This finding indicates that insulin release induced by $[K^+_{50} + G_{16.7}]$ is not due to leakage produced by toxic stimuli but to activation of exocytosis. When graded concentrations (25 and 50 mM) of K^+ were applied simultaneously with $[G_{16.7}]$ in the presence of 100 uM diazoxide, insulin response was clearly dependent on K^+ concentration, indicating that the physiological range of membrane depolarization also activates the glucose-responsive effector. Membrane depolarization/Ca^{2+} influx directly stimulates hormone exocytosis on one hand and activates the K^+_{ATP} channel-independent glucose-responsive effector or effectors on the other in the B-cell. The nature of the glucose-responsive effector or effectors remains to be established.
Diabetes **41:438–43, 1992**

From the Department of Geriatrics, Endocrinology and Metabolism, School of Medicine, Shinshu University, Nagano-ken, Japan.

Address correspondence and reprint requests to Toru Aizawa, MD, Department of Geriatrics, School of Medicine, Shinshu University, 3–1-1 Asahi, Matsumoto, Nagano-ken, Japan.

Received for publication 3 September 1991 and accepted in revised form 26 December 1991.

Although the mechanism of exocytosis is not fully understood, membrane depolarization, opening of the voltage-dependent Ca^{2+} channels (VDCC), and elevation of cytosolic free Ca^{2+} ($[Ca^{2+}]_i$) due to Ca^{2+} influx are considered important components of signal transduction in many endocrine cells (1–3). In the pancreatic B-cell, stimulation with glucose, the most important physiological secretagogue, accompanies membrane depolarization (4) due to closure of ATP-sensitive K^+ (K^+_{ATP}) channel (5); increased intracellular ATP produced by glucose metabolism is considered responsible for the closure of the channel (5). On the other hand, forced membrane depolarization by pharmacological agents including high concentration of K^+ elicits insulin secretion (6,7). Insulin secretion in response to high concentrations of glucose and K^+ is blocked by antagonists of VDCC (7). Thus, membrane depolarization/Ca^{2+} influx is considered a mediator of glucose-induced insulin secretion. However, glucose-induced insulin secretion shows a unique, biphasic temporal profile that cannot be reproduced by any other secretagogue (8,9). Therefore, a complex intracellular signal transduction is thought to ensue after glucose stimulation (8,9). In this study, we show that membrane depolarization/Ca^{2+} influx possesses dual function in the B-cell: direct stimulation of exocytosis and activation of glucose-responsive K^+_{ATP} channel–independent effector or effectors.

RESEARCH DESIGN AND METHODS

Pancreatic islets were obtained from adult male Wistar rats by collagenase dispersion (7,10). Insulin secretion was measured in perifusion and incubation experiments with Krebs-Ringer bicarbonate buffer (KRBB) containing 118.4 mM NaCl, 4.7 mM KCl, 1.3 mM $MgSO_4$, 1.2 mM KH_2PO_4, 1.9 mM $CaCl_2$, 3 mM glucose, and 25 mM

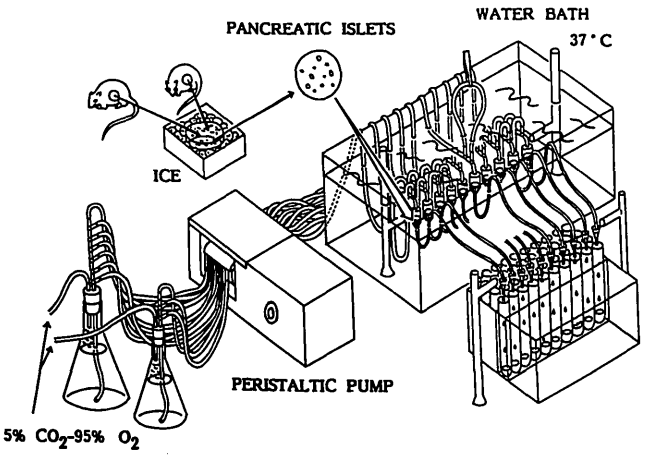

FIG. 1. Schema of multiple parallel perifusion system. Batch of 500 islets obtained from 3–4 rats were pooled and 50 islets each were packed into 10 small columns of identical size. All columns were perifused with a single peristaltic pump in parallel.

$NaHCO_3$ (equilibrated with 5% CO_2/95% O_2, pH 7.4) as described previously (7,11,12). Each addition was made without an adjustment of osmolarity, i.e., test substance was simply added to the basal KRBB. In incubation experiments, five islets were incubated with KRBB for 30 min at 37°C (preincubation), and the buffer was replaced with buffer containing secretagogues and incubated for another 20 or 30 min at 37°C (experimental incubation). In perifusion experiments, to facilitate quantitative analysis of the data, 10 columns filled with the identical batch of islets were perifused parallel (Fig. 1). Glyburide was obtained from Yamanouchi (Tokyo); diazoxide, *p*-nitrophenyl-α-D-glucopyranoside (PNP-Glu), and nifedipine were from Sigma (St. Louis, MO); and 2-cyclohexen-1-one was from Wako (Osaka, Japan). Insulin was measured by radioimmunoassay with commercially available kits (Eiken, Tokyo), with rat insulin (Novo, Copehagen) as a standard. Wilcoxon's signed-rank test was used for statistical analysis. $P < 0.05$ was significant.

RESULTS

As shown in Fig. 2*A*, 50 mM K^+ ($[K^+_{50}]$) and 16.7 mM glucose ($[G_{16.7}]$) elicit typical monophasic and biphasic insulin secretion, respectively. One hundred millimolar K^+ ($[K^+_{100}]$) also produced monophasic insulin secretion (Fig. 3). $[K^+_{100}]$ caused no greater insulin secretion than $[K^+_{50}]$ (Fig. 3). E_K values for various ambient K^+ concn were −85, −43, −25.7, and −8.4 mV for 4.7, 25, 50, and 100 mM K^+, respectively, and the actual membrane potentials measured at these concentrations of ambient K^+ in the presence of stimulatory concentration of glucose were less negative (13) than the E_K values. We know that some fraction of VDCC is open at these levels of membrane potentials (14). However, only a minor fraction of Ca^{2+} channels is available for activation by further depolarization by 100 mM K^+ in the presence of 50 mM K^+ and 3 mM glucose (14); we expect it to be only a few more millivolts (15). Thus, the data on insulin release obtained in our experiments are compatible with previous observations made in other electrophysiological experiments (12–15). Inability of $[K^+_{100}]$ to produce any

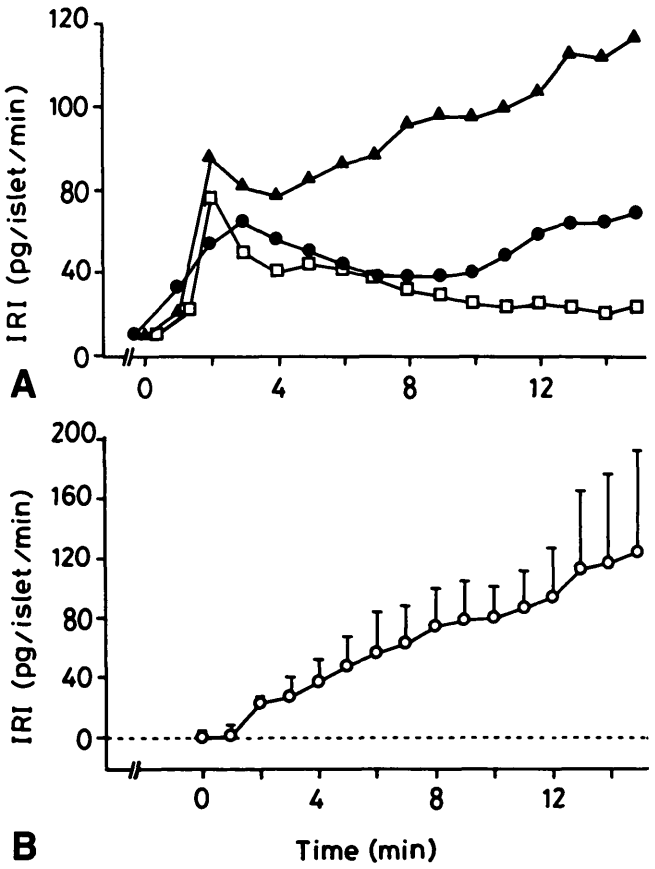

FIG. 2. *A*: Immunoreactive insulin (IRI) secretion in response to 16.7 mM glucose ($[G_{16.7}]$ (●), 50 mM K^+ ($[K^+_{50}]$ (□), and ($[K^+_{50} + G_{16.7}]$ (▲). *B*: subtraction of the response to $[K^+_{50}]$ from that to $[K^+_{50} + G_{16.7}]$ yields gradually increasing IRI secretion. Data shown were obtained with the same batch of islets (for details of experimental procedure, see METHODS). For clarity, mean values of 3–5 columns are shown, and SD (86% of the mean at most) has been omitted in all perifusion results except in *B*. Stimulation is started at time 0 min after stabilization of basal secretion in all perifusion experiments. Conversion factor of IRI to SI units is 0.1739 (pg/islet/min→nmol/islet/min).

greater insulin secretion than $[K^+_{50}]$ alone is not due to increased osmolarity because a stepwise elevation of K^+ from 4.7 to 50 mM and then from 50 to 100 mM caused an unequivocal insulin release at the second step-up (Fig. 3). Because a rapid inactivation of VDCC takes place with persistent depolarization (16), the second step-up of K^+ concn from 50 to 100 mM is thought to transiently reopen the VDCC.

One micromolar glyburide ([Glyb]) caused a rather sustained monophasic insulin release (Fig. 4); the relatively small response to 1 uM Glyb is due to the fact that ambient glucose concentration is subthreshold (3 mM). We intentionally avoided using a stimulatory concentration of glucose with Glyb because the interaction of sulfonylurea and glucose, which occurs only with a stimulatory concentration of glucose, makes the data more difficult to interpret. Simultaneous stimulation of the B-cell with $[K^+_{50}]$ and 1 uM glyburide ($[K^+_{50}$ + Glyb]) produced no greater insulin secretion than $[K^+_{50}]$ alone.

Thus, further depolarization by higher concentration of K^+ ($[K^+_{100}]$) or by closure of K^+_{ATP} channel ([Glyb]) cannot additionally stimulate exocytosis in the B-cell

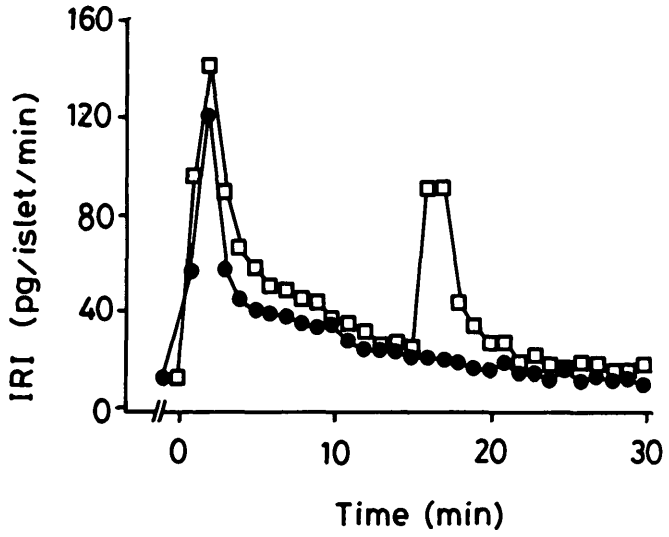

FIG. 3. Comparison of immunoreactive insulin (IRI) response to 100 and 50 mM K^+. K^+ concentration was raised at time 0 min from 4.7 to 100 mM (●) or 4.7 to 50 mM (□) and then 50 to 100 mM (□) 15 min later. Conversion factor of IRI to SI units is 0.1739 (pg/islet/min→nmol/islet/min).

when these stimuli are applied simultaneously with $[K^+_{50}]$.

In sharp contrast, stimulation of the B-cell with $[K^+_{50} + G_{16.7}]$ caused a biphasic insulin release (Fig. 2*A*); the response during the initial period (corresponding to the 1st-phase insulin response to glucose alone, 0–6 min) was less than the addition of responses to each stimulation, but the response during the later period (2nd-phase insulin response, after 7 min) was significantly greater than the simple addition of responses to the two stimuli (Fig. 2). As a result, a subtraction of insulin response induced by $[K^+_{50}]$ alone from that induced by $[K^+_{50} + G_{16.7}]$ resulted in a gradually increasing insulin secretion (Fig. 2*B*), which temporally resembles the second phase of glucose-induced insulin secretion (Fig. 2*A*).

Diazoxide, a hyperglycemic sulfonamide (17), suppresses glucose-induced insulin release in a dose-related manner and does not attenuate K^+ depolarization–induced insulin release (18). This was confirmed in our laboratory; at a concn of 100 uM, diazoxide greatly suppressed insulin secretion in response to $[G_{16.7}]$ (93%) but did not inhibit $[K^+_{50}]$-induced insulin release, which indicates that the closure of K^+_{ATP} channel is of primary importance for glucose-induced but not K^+ depolarization–induced insulin release. To test whether the additional insulin secretion produced by $[G_{16.7}]$, in addition to $[K^+_{50}]$-induced response, is mediated by closure of the K^+_{ATP} channel, the B-cell was pretreated with 100 uM diazoxide and stimulated with $[K^+_{50} + G_{16.7}]$. Diazoxide did not effect $[K^+_{50} + G_{16.7}]$-induced insulin release (Table 1, *experiment I, group 2* vs. *4*).

Effects of other drugs on the insulin release in response to $[K^+_{50} + G_{16.7}]$ were also tested to see whether VDCC, putative glucoreceptor, glucokinase, and sulfohydryl group–dependent exocytotic process are involved in this pathway of B-cell activation. The response was markedly suppressed by 1 uM nifedipine (70%), a blocker of VDCC; however, the response was significantly greater than insulin secretion produced by $[K^+_{50}]$ alone in the presence of nifedipine (Table 1, *experiment II, group 3* vs. *5*). Insulin release induced by $[K^+_{50} + G_{16.7}]$ was completely abolished by 2 mM 2-cyclohexen-1-one (Table 1), which reportedly inhibits glucokinase (19) and exocytotic process by lowering reduced glutathione (20). Pretreatment of the B-cell with PNP-Glu, a putative inhibitor of "glucoreceptor" of the B-cell (21–23), did not effect $[K^+_{50} + G_{16.7}]$-induced insulin release (Fig. 5).

When the B-cell was stimulated with graded concentration of K^+ and $[G_{16.7}]$ in the presence of 100 uM diazoxide, insulin secretion was clearly dependent on the concentration of K^+ (Fig. 6); i.e., high K^+-induced activation of the secretion (the difference between high K^+ alone and high K^+ plus $[G_{16.7}]$) was detectable at K^+ concn of 50 and 25 mM (Fig. 6).

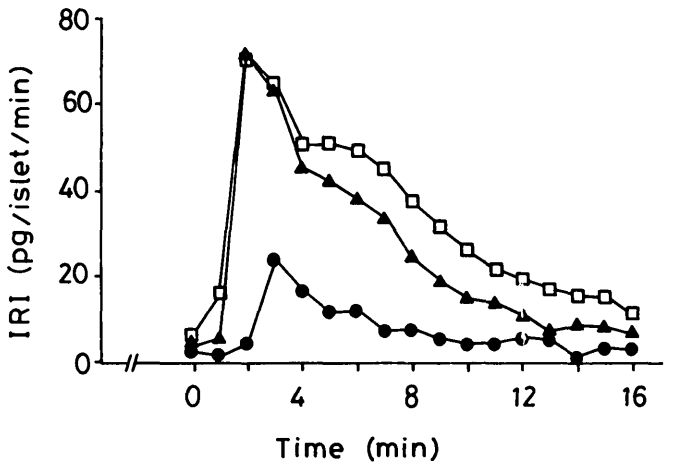

FIG. 4. Immunoreactive insulin (IRI) release in response to 1 uM glyburide (●), 50 mM K^+ plus 1 uM glyburide (▲), and 50 mM K^+ alone (□). Conversion factor of IRI to SI units is 0.1739 (pg/islet/min→nmol/islet/min).

DISCUSSION

In this study, we have clearly demonstrated the dual functional role of membrane depolarization/Ca^{2+} influx in the pancreatic B-cell. One hundred millimolar K^+ depolarizes the B-cell membrane stronger than $[K^+_{50}]$ (13,14). However, the two concentrations of K^+ produced an identical insulin response, indicating that the direct stimulation of insulin exocytosis by membrane depolarization/Ca^{2+} influx is already maximum at $[K^+_{50}]$. Further depolarization does not cause additional insulin release unless it is applied at a later time in which the Ca^{2+} channels regain responsiveness to the step-up of external K^+. In agreement with this finding, insulin response to $[K^+_{50}$ + Glyb] was identical to $[K^+_{50}]$ alone. Because sulfonylurea closes K^+_{ATP} channel and depolarizes the B-cell to stimulate insulin secretion (24,25), it is expected that the drug is ineffective when the secretory machinery is unresponsive to further depolarization due to presence of $[K^+_{50}]$. We tested the effect of sulfonylurea because the mechanism of B-cell depolarization is different be-

Table 1
Effects of diazoxide, nifedipine, and 2-cyclohexen-1-one on insulin release

Experiment	Pretreatment		Experimental incubation		Insulin release (nmol/islet)*
	Drug	Concentration	Glucose (mM)	K^+ (mM)	
I					
Group					
1	None		3	4.7	100.5 ± 37.3
2	None		16.7	50	676.2 ± 151.4
3	Diazoxide	100 uM	3	4.7	70.3 ± 15.2
4	Diazoxide	100 uM	16.7	50	632.6 ± 176.0†
5	2-Cyclohexen-1-one	2 mM	3	4.7	104.3 ± 34.5
6	2-Cyclohexen-1-one	2 mM	16.7	50	96.5 ± 31.3
II					
Group					
1	None		3	4.7	54.9 ± 19.1
2	None		3	50	121.0 ± 37.9
3	Nifedipine	1 uM	3	50	64.3 ± 29.1
4	None		16.7	50	326.7 ± 62.2
5	Nifedipine	1 uM	16.7	50	92.0 ± 13.6‡

Data are means ± SD of 10–13 determinations. For each experiment, the same batch of islets were used. Drugs used for pretreatment are present throughout experimental incubation. During pretreatment, glucose and K^+ concn were 3 and 4.7 mM, respectively. For details of experimental procedure, see METHODS.
*Experimental incubation was carried out for 30 (experiment I) or 20 (experiment II) min.
†Not significantly different from the value of group 2 (experiment I).
‡$P < 0.05$ vs. group 3 (experiment II).

tween sulfonylurea and high K^+, the former depolarizes the B-cell through its inhibitory action on K^+_{ATP} channel, whereas high K^+ clamps the membrane voltage to the value of E_K.

In sharp contrast to sulfonylurea and increasing K^+ concentration, stimulation with high concentration of glucose applied simultaneously with $[K^+_{50}]$ caused significantly greater insulin response than that produced by $[K^+_{50}]$ alone. These data indicate that glucose stimulates B-cell in a qualitatively different manner compared with sulfonylurea and K^+ depolarization under the conditions examined. The activation of K^+_{ATP} channel–independent, glucose-responsive effector by membrane depolarization was clearly detectable with a lower (25-mM) K^+ concentration as well. This finding implies that weaker membrane depolarization induced by 25 mM K^+ than that produced by $[K^+_{50}]$ (4,13) possesses such a role, although 25 mM K^+ causes significant depolarization of the B-cell compared with the normal basal membrane potentials (26). We tried to delineate the nature of K^+_{ATP} channel–independent, glucose-responsive effector in the following experiments; however, it remains unestablished (see below). For the mechanism of K^+_{ATP} channel–independent B-cell activation by glucose, the

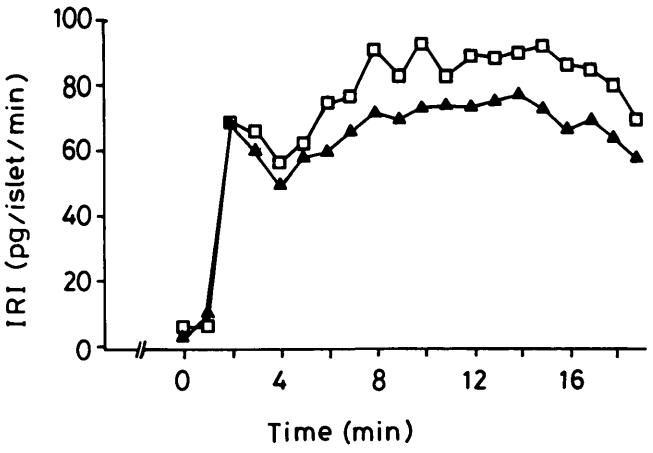

FIG. 5. Effect of *p*-nitrophenyl-α-D-glucopyranoside (PNP-Glu) on immunoreactive insulin (IRI) release in response to 50 mM K^+ plus 16.7 mM glucose ($[K^+_{50} + G_{16.7}]$). Responses to $[K^+_{50} + G_{16.7}]$ in the absence (▲) or presence (□) of 16.7 mM PNP-Glu are shown. Conversion factor of IRI to SI units is 0.1739 (pg/islet/min→nmol/islet/min).

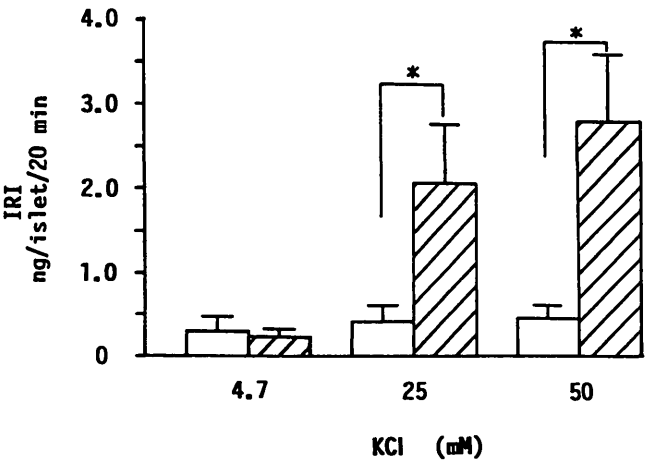

FIG. 6. Effects of graded concentration of K^+ on immunoreactive insulin (IRI) secretion in the presence or absence of 16.7 mM glucose. Data are means ± SD of 10–12 determinations. Islets were preincubated with 100 uM diazoxide at the ambient K^+ and glucose concn of 4.7 and 3 mM, respectively. Subsequently, they were incubated for 20 min (experimental incubation) with the indicated concentration of K^+ with (*hatched columns*) or without (*open columns*) 16.7 mM glucose in the continuous presence of 100 uM diazoxide. IRI secreted during experimental incubation was measured. *$P < 0.01$. See METHODS for details of experimental procedure. Conversion factor of IRI to SI units is 0.1739 (ng→umol).

following sequence of events might be possible. Glucose per se or its metabolism results in modulation of L-type Ca^{2+} channels, thereby increasing initial frequency of opening at any depolarizing potential. A modulation by glucose of dihydropyridine-sensitive Ca^{2+} channels has been reported in mouse B-cell (27).

Insulin release in response to $[K^+_{50} + G_{16.7}]$ was completely resistant to diazoxide, a hyperglycemic sulfonamide that inhibits K^+_{ATP} channel closure (17). Thus, glucose-generated signal or signals responsible for this action are not traveling through this pathway, the closure of K^+_{ATP} channel.

On the other hand, insulin release in response to $[K^+_{50} + G_{16.7}]$ was greatly suppressed (70%) by 1 uM nifedipine, a blocker of VDCC, although the response was clearly greater than that induced by $[K^+_{50}]$ alone in the presence of 1 uM nifedipine. We have previously reported that 1 uM (or even 10 uM) nifedipine does not completely abolish insulin release induced by $[K^+_{50}]$ (7) and concluded that a fraction of the rat B-cell VDCC is resistant to dihydropyridine; whereas, 1 uM nifedipine almost completely abolishes $[G_{16.7}]$-induced insulin release at the ambient K^+ concn of 4.7 mM, indicating that glucose-responsive L-type Ca^{2+} channels are effectively blocked by this concentration of nifedipine (7). Thus, the data clearly indicate that residual $[K^+_{50}]$-induced VDCC opening/Ca^{2+} influx in the presence of 1 uM nifedipine (7) causes direct stimulation of insulin release on one hand and activates the glucose-responsive effector coupled to the process of exocytosis on the other.

2-Cyclohexen-1-one was thought to inhibit insulin release by lowering the reduced glutathione levels (20). Miwa et al. (19) reported that 2-cyclohexen-1-one also inhibits glucokinase and speculated that the blockade of glucose-induced insulin release by 2-cyclohexen-1-one is primarily due to blockade of this enzyme (19). In this study, insulin release in response to $[K^+_{50} + G_{16.7}]$ was completely abolished by 2 mM 2-cyclohexen-1-one. The data imply that 2-cyclohexen-1-one abolishes insulin response induced by $[G_{16.7}]$ and that induced by K^+ depolarization. Because insulin release in response to K^+ depolarization is not dependent on glucokinase, we consider the drug not only an inhibitor of glucokinase but also a global inhibitor of exocytosis, presumably through its action on reduced glutathione (20). Therefore, the results of experiments with 2-cyclohexen-1-one do not necessarily support the idea that glucose metabolism is required for this process. Nevertheless, inhibition by 2-cyclohexen-1-one and nifedipine of insulin release induced by $[K^+_{50} + G_{16.7}]$ indicates that the response is not due to "leakage" of insulin but to activation of exocytosis.

A competitive inhibitor of putative glucoreceptor, PNP-Glu (21,22), was also without effect on the K^+_{ATP} channel–independent branch of glucose-induced signal transduction. Therefore, PNP-Glu–sensitive glucoreceptor may not be involved in this path, or, alternatively, the drug may not be as powerful an inhibitor of the glucoreceptor as previously reported (23); we see only marginal suppression by PNP-Glu of insulin response to high glucose alone (data not shown). Because biochemical evidence for B-cell glucoreceptor is lacking, clarification of the problem needs further investigation.

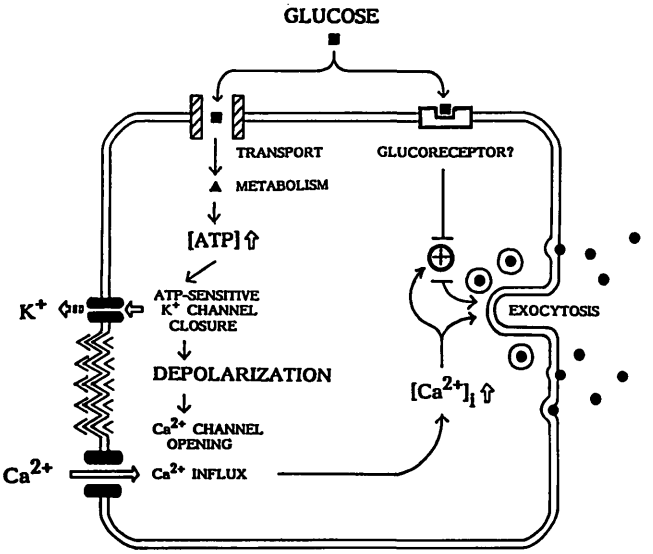

FIG. 7. Schema for dual functional role of membrane depolarization/Ca^{2+} influx. Depolarization is caused by K^+_{ATP} channel closure due to increase of ATP. Opening of the voltage-dependent Ca^{2+} channel ensue and cytosolic free Ca^{2+} ($[Ca^{2+}]_i$) rises as a result of Ca^{2+} influx. It stimulates exocytosis through Ca^{2+}-dependent mechanisms on one hand and induces the coupling of another glucose-responsive effector and the process of exocytosis on the other.

For the mechanism of glucose-induced insulin secretion, we showed that the B-cell is equipped with K^+_{ATP} channel–independent, glucose-responsive effector or effectors. Nevertheless, glucose-induced insulin secretion with ambient nondepolarizing K^+ concn of 4.7 mM is mostly abolished by 100 μM diazoxide. This implies that, for the activation of the K^+_{ATP} channel–independent effector, the signal generated by membrane depolarization, most likely elevation of $[Ca^{2+}]_i$, is absolutely necessary. Based on these data, we propose that glucose-induced biphasic insulin secretion results from the activation of two mutually interrelated signal transduction pathways (Fig. 7). One pathway is direct stimulation of exocytosis due to $[Ca^{2+}]_i$ elevation and the other is stimulation through the K^+_{ATP} channel–independent, glucose-responsive effector or effectors; for the coupling of the latter effector or effectors and exocytosis, $[Ca^{2+}]_i$ elevation is mandatory (Fig. 7). Because glucose elicits mostly second-phase response when the former pathway is inactivated by high concentration of K^+ (Fig. 2*B*), the latter pathway appears primarily responsible for the second-phase insulin release, whereas, the direct stimulation of exocytosis by elevated $[Ca^{2+}]_i$ may be closely related to the first-phase response.

ACKNOWLEDGMENTS

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

We wish to thank Drs. M. Kakei, S. Ozawa, and A. Niki for constructive discussion and J. Miyazawa and K. Murase for expert technical and secretarial assistance.

REFERENCES

1. Douglas WW: Stimulus secretion coupling: the concept and clues from chromaffin and other cells. *Br J Pharmacol* 34:451–74, 1968
2. Kaczorowski GJ, Vandlen RL, Katz GM, Reuben JP: Action potentials occur in cells from the normal anterior pituitary gland and are stimulated by the hypophysiotropic peptide thyrotropin-releasing hormone. *J Membrane Biol* 71:109–18, 1983
3. Ozawa S, Sando O: Electrophysiology of excitable endocrine cells. *Physiol Rev* 66:887–952, 1986
4. Dean PM, Matthews EK: Glucose-induced electrical activity in pancreatic islet cells. *J Physiol* 210:255–64, 1970
5. Cook DL, Satin LS, Ashford MLJ, Hales CN: ATP-sensitive K^+ channels in pancreatic β-cells: spare-channel hypothesis. *Diabetes* 37:495–98, 1988
6. Henquin JC, Lambert AE: Cationic environment and dynamics of insulin secretion. II. Effect of a high concentration of potassium. *Diabetes* 23:933–42, 1974
7. Komatsu M, Yokokawa N, Takeda T, Nagasawa Y, Aizawa T, Yamada T: Pharmacological characterization of the voltage-dependent calcium channel of pancreatic B-cell. *Endocrinology* 125: 2008–14, 1989
8. Prentki M, Matschinsky FM: Ca^{2+}, cAMP, and phospholipid-derived messengers in coupling mechanisms of insulin secretion. *Physiol Rev* 67:1185–248, 1987
9. Hedeskov CJ: Mechanism of glucose-induced insulin secretion. *Physiol Rev* 60:442–508, 1980
10. Lacy PE, Kostianovsky M: Method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes* 16:35–39, 1967
11. Aizawa T, Kobayashi M, Komiya I, Hiramatsu K, Sato A, Yamada T: Increased sensitivity of the thyrotroph to thyrotropin releasing hormone in rats with hypothalamic hypothyroidism. *Endocrinology* 114:8–13, 1984
12. Aizawa T, Hinkle PM: Thyrotropin-releasing hormone rapidly stimulates a biphasic secretion of prolactin and growth hormone in GH_4C_1 rat pituitary tumor cells. *Endocrinology* 116:73–82, 1985
13. Atwater I, Ribalet B, Rojas E: Cyclic changes in potential and resistance of the β-cell membrane induced by glucose in islets of Langerhans from mouse. *J Physiol* 278:117–39, 1978
14. Satin LS, Cook DL: Calcium current inactivation in insulin-secreting cells is mediated by calcium influx and membrane depolarization. *Pfluegers Arch Eur J Physiol* 414:1–10, 1989
15. Hopkins WF, Satin LS, Cook DL: Inactivation kinetics and pharmacology distinguish two calcium currents in mouse pancreatic B-cells. *J Membrane Biol* 119:229–39, 1991
16. Chin JH: Differential sensitivity of calcium channels to dihydropyridines: the modulated receptor hypothesis. *Biochem Pharmacol* 35:4115–20, 1986
17. Trube G, Rorsman P, Ohno-Shosaku T: Opposite effects of tolbutamide and diazoxide on the ATP-dependent K^+ channel in mouse pancreatic β-cells. *Pfluegers Arch Eur J Physiol* 407:493–39, 1986
18. Henquin JC, Charles S, Nenquin M, Mathot F, Tamagawa T: Diazoxide and D600 inhibition of insulin release: distinct mechanisms explain the specificity for different stimuli. *Diabetes* 31:776–83, 1982
19. Miwa I, Murata T, Mitsuyama S, Okuda J: Participation of glucokinase inactivation in inhibition of glucose-induced insulin secretion. *Diabetes* 39:1170–78, 1990
20. Malaisse WJ, Garcia-Morales P, Gomis R, Dufrane SP, Mathias PCF, Valverde I, Sener A: The coupling of metabolic to secretory events in pancreatic islets: inhibition by 2-cyclohexen-1-one of the secretory response to cyclic AMP and cytochalasin B. *Biochem Pharmacol* 35:3709–17, 1986
21. Jakinovich W Jr: Methyl 4,6-dichloro-4,6-dideoxy-α-D-galactopyranoside: an inhibitor of sweet taste responses in gerbils. *Science* 219:408–10, 1983
22. Vlahopoulos V, Jakinovich W Jr: Antagonism of the gerbil's sucrose taste response by *p*-nitrophenyl-α-D-glucopyranoside and chloramphenicol. *J Neurosci* 6:2611–15, 1986
23. Niki A, Niki H, Hashioka T: An inhibitor of sweet taste response inhibits glucose-induced insulin release. *Biomed Res* 10:169–72, 1989
24. Gorus FK, Schuit FC, In't Veld PA, Gepts W, Pipeleers DG: Interaction of sulfonylureas with pancreatic β-cells: a study with glyburide. *Diabetes* 37:1090–95, 1988
25. Amoroso S, Schmid-Antomarchi H, Fosset M, Lazdunski M: Glucose, sulfonylureas, and neurotransmitter release: role of ATP-sensitive K^+ channels. *Science* 247:852–54, 1990
26. Dawson CM, Atwater I, Rojas E: The response of pancreatic β-cell membrane potential to potassium-induced calcium influx in the presence of glucose. *J Exp Physiol* 69:819–30, 1984
27. Smith PA, Rorsman P, Ashcroft FM: Modulation of dihydropyridine-sensitive Ca^{2+} channels by glucose metabolism in mouse pancreatic β-cells. *Nature* (Lond) 342:550–53, 1989