Notes

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Effects of Potassium Ions on Insulin Release in the Rat from the Perifused Islets of Langerhans with Slow-rise Glucose Stimulation

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The role of extracellular potassium ions in the mechanism of glucose-induced insulin release in the rat was investigated by both dynamic and kinetic analyses of insulin release from islets of Langerhans by slow-rise glucose stimulation in the presence of 6.2 or 12.4 mM K^+, or in its absence.

The dose-response curves were sigmoidal in profile with a tendency to show a similar maximal rate of insulin release, but the K_m values were very different. In particular, removal of potassium ions from the medium reduced the K_m value from 8.9 mM (control) to 3.7 mM glucose. Hill's constant (n) was reduced from 5.7 (control) to 4.2 in the absence of K^+ and 4.4 in the presence of 12.4 mM K^+. The Hill plots were linear with slopes of 4.2 (0 mM K^+) and 5.7 (6.2 mM K^+), respectively, whereas in the presence of 12.4 mM K^+ the Hill plot consisted of two straight lines with a break at 4.7 mM glucose ($n=1.4$ and 4.4).

In conclusion, it is suggested that potassium ions in the medium play important roles not only as an electrolyte in connection with the integrity of the B cell membrane, but also as a heterotropic inhibitory effector in the allosteric interaction between the glucoreceptors, leading to a depression of insulin release at low glucose concentrations.

Keywords——insulin release; pancreatic islets; B cell membrane; glucoreceptor; perifusion; slow-rise glucose stimulation; depolarization; Hill's constant

Introduction

It is well known that the glucose-induced insulin release is regulated by variations of the extra- and intracellular cationic environments, involving Ca^{2+}, Na^+ and K^+.[2] In particular, removal of potassium ions from the medium depolarizes the B cell membrane to increase the accumulation of intracellular Na^+, and thus induces insulin release in response to glucose at concentrations lower than the physiological threshold (4.2 mM) for insulin release.[3] On the other hand, potassium ions at concentrations higher than 9 mM also depolarize the B cell membrane to cause a transient increase in free Ca^{2+} accumulation in the cytosol.[4]

In our previous study, we demonstrated that rat islets perifused with a slowly increasing level of glucose secrete corresponding amounts of insulin response to change of glucose concentration of up to 0.22 mM/min.[5] Curry suggested that insulin release on glucose stimulation of this type may not be accompanied by higher depolarization of the B cell membrane.[6]

In order to investigate further the role of potassium ions in the insulin release mechanism, we have now examined the effects of potassium ions upon the dynamics of insulin release by

1) Location: *1-1, Mukogawa-cho, Nishinomiya 663, Japan.*
2) J.C. Henquin and A.E. Lambert, *Diabetes*, **23**, 933 (1974); R.D.G. Milner and C.N. Hales, *Biochim. Biophys. Acta* (Amst.), **135**, 375 (1967).
3) C.N. Hales and R.D.G. Milner, *J. Physiol.*, **194**, 725 (1968).
4) P.M. Dean and E.K. Matthews, *J. Physiol.*, **210**, 265 (1970).
5) K. Kobayashi, Y. Tsumura, H. Ise, S. Kagawa, and A. Matsuoka, *Chem. Pharm. Bull.* (Tokyo), **25**, 2112 (1977).
6) D.L. Curry, *Am. J. Physiol.*, **221**, 324 (1971).

slow-rise glucose stimulation from rat islets perifused with media containing K^+ concentrations higher or lower than the physiological level.

Materials and Methods

Reagents——D-Glucose and theophylline were products of Wako Pure Chemical Industries, Ltd., Japan. Bovine serum albumin (fraction V) was purchased from The Armour Laboratories, U.S.A., and collagenase from Sigma Chemical Co., U.S.A.

Isolation of Islets——Pancreatic islets of Langerhans were isolated from overnight-fasted male Wistar rats weighing 120—200 g by the method of Lacy and Kostianovsky.[7] On average, about 120 islets (diameter, 200—250 μ) were obtained from one animal. One hundred and fifty islets of comparable size collected from three animals were used for a series of experiments.

Perifusion Media——The medium for all experiments was Krebs-Henseleit bicarbonate buffer (pH 7.35) supplemented with 0.5% bovine serum albumin and 1 mM theophylline, and equilibrated with a mixture of O_2 and CO_2 (95 : 5, v/v). A K^+-depleted medium was prepared by replacing KCl and KH_2PO_4 with equivalent amounts of NaCl, and a medium containing 12.4 mM K^+ was prepared by addition of an equivalent amount of KCl to Krebs-Henseleit bicarbonate buffer.

Perifusion——Fifty islets each were perifused in a plastic flow cell maintained at a constant temperature (37°); the average flow rate of the perifusate was 0.5 ml/min. After a 30-min equilibration with one of three glucose-depleted media containing various concentrations of potassium, perifusion was carried out for 120 min under slow-rise stimulation with glucose at a rate of 0.10 mM/min. The multichamber technique was used in a series of experiments to prevent between-experiment variations. Namely, the chambers were simultaneously perifused with media containing 6.2 or 12.4 mM K^+ or not containing K^+.

Measurements——Insulin (immunoreactive insulin, IRI) and glucose contents in the effluent fraction were measured by doubleantibody radioimmunoassay (Insulin RIA kits, Dainabot Radioisotope Laboratories, Ltd., Japan) and with a glucose analyzer (ERA 2001, Beckman Instruments Inc., U.S.A.), respectively.

Calculations——All kinetic analyses for the rate of insulin release were performed using Hill's equation, as described in detail in our previous report,[8] and statistical comparisons were done using Student "t" test.

Results and Discussion

Dynamics of Insulin Release from Perifused Rat Islets with Slow-rise Glucose Stimulation

As shown in Fig. 1, insulin release in the presence of 6.2 mM K^+ was initially observed at about 6 mM glucose and then gradually increased in parallel with the increase in glucose level, reaching a plateau (0.53±0.01 μU/ml/islet/min) at around 13 mM glucose. On the other hand, insulin release in the K^+-depleted medium occurred immediately after the beginning of perifusion, increased at the greatest rate during the period from 30 to 60 min and afterwards remained constant (0.52±0.03 μU/ml/islet/min) at over 9 mM glucose.

Moreover, in the presence of 12.4 mM K^+ insulin release was higher ($p<0.01$) than that in the presence of 6.2 mM K^+ during the period of perifusion from 0 to 40 min. Afterwards, insulin release gradually increased, reaching a plateau (0.43±0.03 μU/ml/islet/min) which was lower than that in the other two media ($p<0.01$).

Dose Response Curves for the Rate of Insulin Release

The dose-response relationships between the rate of insulin release and the glucose concentration during slow-rise glucose stimulation in the three different media are shown in Fig. 2. Each dose-response curve showed a sigmoidal profile, with a K_m value of 3—4 mM (0 mM K^+), 6—7 mM (6.2 mM K^+) or 8—9 mM (12.4 mM K^+). Both the presence 12.4 mM K^+ and its absence induced supra-basal insulin secretion even at glucose concentrations lower than 2.8 mM, as distinct from the presence of 6.2 mM K^+.

The enhancing effect of K^+ deprivation on insulin response to glucose has been considered to be due to increased accumulation of free Ca^{2+} in the cytosol,[2] and activation of the micro-

7) P.E. Lacy and M. Kostianovsky, *Diabetes*, **16**, 35 (1967).
8) K. Kobayashi, H. Ise, Y. Tsumura, S. Kagawa, and A. Matsuoka, *Endocrinol. Japon.*, **24**, 367 (1977).

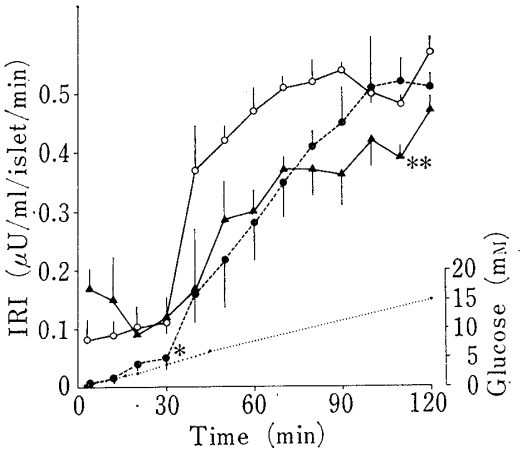

Fig. 1. Dynamics of Insulin Release from Perifused Rat Islets with Slow-rise Glucose Stimulation in Media Containing Various K^+ Concentrations

After a control equilibration with glucose-depleted medium containing 6.2 or 12.4 mM K^+, or without K^+ for 30 min perifusion was carried out for 120 min under slow-rise glucose stimulation in the presence of 6.2 mM K^+ (●---●), 12.4 mM K^+ (▲—▲) or its absence (○—○). The results are the means±S.E.M. of three experiments. The dotted line shows average levels of glucose in the perifusate collected at 2-min intervals during the period of perifusion. * $p<0.01$ *vs.* 0 or 12.4 mM K^+, ** $p<0.01$ *vs.* 0 or 6.2 mM K^+.

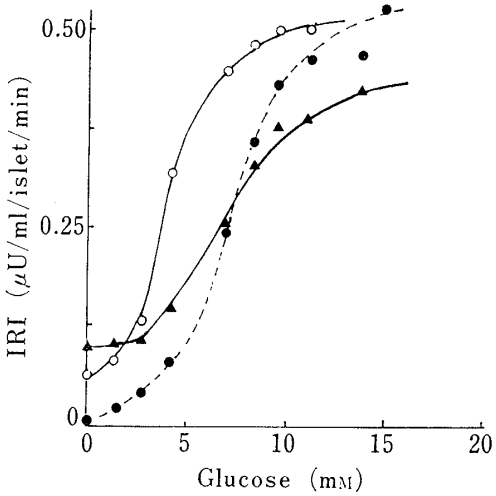

Fig. 2. Effects of Various K^+ Concentrations upon the Dose-response Curves for the Rate of Insulin Release

Each point was derived from the dynamics of insulin release (Fig. 1) and is the mean of three experiments (●---●, 6.2 mM K^+; ▲—▲, 12.4 mM K^+; ○—○, 0 mM K^+).

tubule-microfilamentous system leading to more active translocation of the B granules toward the cell membrane.[9]

It has been reported by Dean *et al.* that the depolarization of the B cell membrane was similar in the presence of 12.4 mM K^+ and in its absence.[4] As distinct from the absence of K^+, higher extracellular concentrations of K^+ have been reported to increase the accumulation of intracellular K^+ through activation of the Na^+/K^+ ATPase and to decrease the intracellular concentration of Na^+ in the muscle cells of several animals.[10] Thus, a higher concentration of K^+ may reduce the effect of glucose upon insulin release from the perfused rat islets during the period of perifusion.

The present results, *i.e.*, an increase in the rate of insulin release in the early stage (within 40 min) of perifusion and a reduction of the maximal rate, seem to be consistent with the above considerations.

Kinetic Analysis of Insulin Release by Means of Hill's Equation

Kinetic constants for the rate of insulin release from perifused rat islets under slow-rise glucose stimulation are summarized in Table I. The K_m value for insulin release was 3.7 mM glucose in the K^+-depleted medium, being significantly smaller than that (8.9 mM) of the control ($p<0.05$). Hill's constant (n) was calculated as 5.7 in the presence of 6.2 mM K^+, while in the presence of 12.4 mM K^+ and in its absence Hill's constant decreased to 4.2 and 4.4, respectively.

In our previous report, we showed that Hill's constant under square-wave glucose stimulation was 3.1 for perifused rat islets and 2.4 for perfused rat pancreas in the presence of 6.2 mM K^+, and it was only 1.2 for the perfused rat pancreas in the absence of K^+.[8]

9) P.E. Lacy, S.L. Howell, D.A. Young, and C.J. Fink, *Nature*, (London), **219**, 1177 (1968).
10) D. Horowoz and C.J. Gerber, *J. Gen. Physiol.*, **48**, 489 (1965); H.A. Fozzard and D.M. Kipnis, *Science*, **156**, 1257 (1967).

Table I. Effects of Various K^+ Concentrations in the Medium upon Kinetic Constants of Insulin Release

Condition	n	log K (mM^{-n})	K_m (mM)	V_m (μU/ml/islet/min)	N
6.2 mM K^+	5.7±1.1	−5.3±1.8	8.9±2.7	0.53±0.01	3
0 mM K^+	4.2±1.0****	−2.3±1.1***	3.7±1.3**	0.52±0.03*****	3
12.4 mM K^+	4.4±0.2***	−3.6±0.2****	6.4±0.2****	0.43±0.03*	3
	1.4±0.4†				

* $p<0.01$, ** $p<0.05$, *** $p<0.10$, **** $p<0.20$, ****** not significant, compared with the mean value of experiments with medium containing 6.2 mM K^+. † indicates the value calculated from the rate of insulin release at glucose levels below 4.7 mM. Each kinetic constant is the mean value (±S.E.M.) calculated from Hill's equation; $\log \frac{v}{V_m - v} = n \log (S) + \log K$ (V_m and v are the maximal rate and rate of insulin release, respectively; n, K and (S) represent Hill's constant, the equilibrium constant between glucose and binding site, and glucose concentration, respectively). K_m was calculated from the following equation; $K_m = 10^{\frac{-\log K}{n}}$.[8]

Under the slow-rise glucose stimulation tested here, Hill's constant was 5.7 in the presence of 6.2 mM K^+, but fell to 4.2 in the presence of 12.4 mM K^+ or 4.4 in the absence of K^+. Accordingly, a slowly increasing level of glucose gave larger values of Hill's constant than square-wave glucose stimulation, either in the presence of 6.2 mM K^+ or in its absence.

A square-wave glucose stimulation has been demonstrated to depolarize strongly the B cell membranes as distinct from slow-rise stimulation.[6,8] It is also known that pancreatic B cell membrane depolarization causes the rapid burst of insulin release in the early stage of glucose stimulation.[11] These findings suggest that a reduction in Hill's constant may be related to an increase in the extent of depolarization of the B cell membranes.

Gomez demonstrated that a rapid change of potassium concentration in a medium without glucose caused a rapid burst of insulin release from the perfused rat pancreas, followed by an immediate decline to almost basal levels, whereas no burst of insulin release was observed when the potassium concentration was slowly increased.[11] In the present study, the rapid burst of insulin release had already declined to basal levels after 30-min control equilibration with glucose-depleted medium containing 0 or 12.4 mM K^+, so the insulin profiles seen here should be due not to depolarization of the B cell membrane but to the slow-rise glucose stimulation.

Furthermore, the decrease in Hill's constant observed in the presence of 12.4 mM K^+ or in its absence might be associated with a reduction in allosteric interaction relative to potassium at the physiological concentration.

As shown in Fig. 3, Hill plots obtained on perifusion with media containing 6.2 mM K^+ and without K^+ were linear ($\gamma > 0.99$) with slopes of 4.2 and 5.7 respectively, while in the presence of 12.4 mM K^+ the Hill plot consisted of two straight

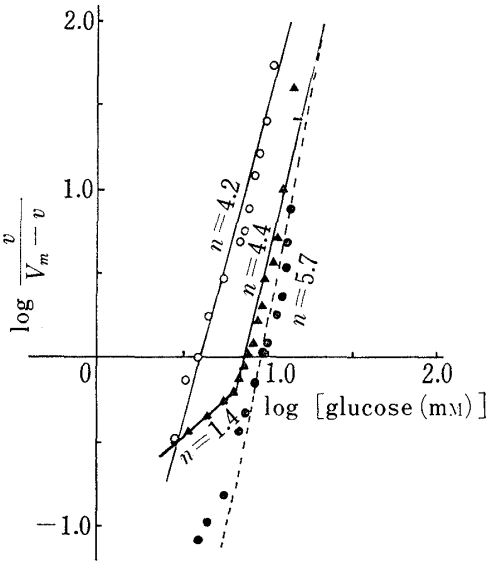

Fig. 3. Hill Plots for the Rate of Insulin Release from Perifused Rat Islets with Slow-rise Glucose Stimulation in Media Containing Various K^+ Concentrations

Each point was calculated from the dose-response curves shown in Fig. 2 and is the mean of three experiments (●---●, 6.2 mM K^+; ▲—▲, 12.4 mM K^+; ○—○, 0 mM K^+).

11) M. Gomez and D.L. Curry, *Endocrinol.*, **92**, 1126 (1973).

lines with a break at 4.7 mM glucose ($\gamma > 0.99$, $n = 1.4$ and 4.4). These results suggest a rapid change in the interaction between glucoreceptors may occur at 4.7 mM glucose.

An analogous plot ($n = 2.3$ and 6.2) with a break at 8.3 mM glucose was also observed under slow-rise stimulation by β-D-glucose.[12] We have suggested that the substrate-glucoreceptor complex formed between the β anomer and the glucoreceptor may be incompletely bound below 8.3 mM glucose, and may become fully bound through a change of the glucoreceptor conformation on raising the β anomer concentration. Similarly, the presence of 12.4 mM K^+ in the medium has been suggested to change the receptor conformation.[12]

Furthermore, in parallel with the increase of K^+ concentration from 0 to 6.2 mM, the dose-response curves, which tended to show similar maximal rates of insulin release, moved to higher glucose concentrations. These profiles caused by K^+ resemble the actions of some allosteric enzymes in the presence of a negative effector, moving the dose-response curve to a higher concentration of the substrate without changing the maximal rate of the reaction.

It is concluded that potassium ions in the extracellular medium may be characterized as a heterotropic inhibitory effector of the glucoreceptors on the B cell membrane, and may play a role in the supression of insulin release by glucose, especially at lower concentrations of glucose.

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