Cationic Environment and Dynamics of Insulin Secretion III. Effect of the Absence of Potassium

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Summary. The absence of extracellular K^+ modifies glucose-induced insulin secretion. The dynamics of these changes was studied in isolated rat islets perfused with K^+ -deprived media containing various Na^+ concentrations. Omission of K^+ from the perfusate during stimulation with 300 mg glucose/100 ml did not cause significant changes in IRI secretion, when Na^+ concentration was normal (149 mM Na^+). If external Na^+ was partially substituted (24 mM Na^+) by choline, a decrease in the amplitude of the second phase of release was noted. Both phases were markedly depressed, when Na^+ was reduced to 2 mM. Removal of K^+ for 25 min before glucose stimulation resulted in an acceleration and potentiation of the early phase of release, contrasting with the progressive inhibition of the late one. This inhibition was slowly reversible 10 min after K^+ reintroduction. Under the same experimental conditions, decrease of Na^+ concen-

tration to 24 mM slowed and inhibited the first phase of secretion, and depressed further the second phase, without alteration of the dynamics of secretion. Glucose oxidation by islets was reduced by 35% after 2 h of incubation in a K^+ -free medium; this inhibition was more pronounced (65%) after 1 h, when the islets were previously perfused for 60 min in the absence of K^+ . From these results, it is suggested that changes in glucose-induced secretion observed in the absence of K^+ , are secondary to the intracellular modifications of Na^+ and K^+ concentrations due to the decreased activity of the Na^+/K^+ pump. They seem to be the result of two antagonistic effects: a facilitation of secretion by Na^+ accumulation and an inhibition by K^+ depletion.

Key words: Insulin secretion, glucose oxidation, isolated islets, perfusion, glucose, potassium, sodium.

Early investigations using static incubations of rabbit pancreatic slices demonstrated that insulin secretion was stimulated, at a low glucose concentration, by removal of potassium from the extracellular medium [1, 2]. These experiments were confirmed by studies using organ culture of fetal rat pancreas [3]. In this preparation, omission of potassium promoted insulin release in the absence of glucose and markedly potentiated the stimulant effect of glucose and/or caffeine.

On the basis of these findings, it was concluded that the accumulation of intracellular sodium, resulting from the blockade of the Na^+/K^+ pump by the absence of external potassium, could make substantial contribution to the facilitation of insulin secretion [2].

The investigations reported here were aimed at characterizing further the interrelations between potassium and sodium in the regulation of insulin releasing mechanisms. This was attempted by studying the effects of potassium deprivation upon the dynamics of glucose-induced secretion from rat islets perfused with media containing normal or low sodium concentrations. Some of these results have been reported in a preliminary form [4].

Material and Methods

Insulin Secretion

Specific details for the perfusion technique of isolated islets have been described in our earlier publications [5, 6]. Briefly, two groups of 27 islets of Langer-

hans obtained from fed male rats by the collagenase method [7] were perfused in parallel, allowing control and experimental conditions to be studied simultaneously. The Krebs-Ringer bicarbonate buffer (KRB) utilized as perfusion medium was conveyed to the twin-chambers at a rate of 2.8 ml/min. The effluent was collected at 30 s or 1 min intervals, and fractions in which immunoreactive insulin (IRI) content was to be measured were frozen until assay. KRB contained 0.5% bovine serum albumin and unless otherwise specified 50 mg glucose/100 ml. Its normal ionic composition was (in mM): Na^+ 143.3, K^+ 6.0, Ca^{++} 2.5, Mg^{++} 1.2, Cl^- 126.0, $H_2PO_4^-$ 1.2, SO_4^{--} 1.2 and HCO_3^- 24.6. K^+ -free media were prepared by substituting KCl and KH_2PO_4 by equivalent amounts of NaCl and NaH_2PO_4 . K^+ concentration in these media was lower than 1 μ M. In some experiments, Na^+ concentration was reduced to 24 or 1–2 mM by isosmotically replacing NaCl alone or with $NaHCO_3$ by corresponding choline salts. KRB was constantly gassed with a mixture of O_2/CO_2 (94/6%), so as to maintain pH at 7.4 during the whole experiment.

The usual experimental design started by a 25 min equilibration period (min –25 to 0), continued with a 40 min stimulation period (min 0 to 40) and was terminated by a final equilibration period (min 40 to 60). K^+ was omitted from the medium during either the whole perfusion or the stimulation period only; except in one series of experiments, glucose concentration was increased from 50 to 300 mg/100 ml between min 0 and 40.

Whenever possible all chemicals were of the analytical reagent grade. D-glucose and choline chloride were purchased from Merck A.G. (Darmstadt, Germany) and choline bicarbonate from Fluka A.G. (Buchs, Switzerland).

expressed as picomoles (pmol) of glucose oxidized/islet, according to Ashcroft *et al.* [9]. In one series of experiments, large batches of islets were perfused for 1 h with KRB containing 6 or 0 mM K⁺, before incubation with labelled glucose.

Table 1. Total IRI output from perfused islets in the absence of extracellular K⁺ during the stimulation period

Line no	n	Composition of perfusion media						IRI released during stimulation			
		Equilibration			Stimulation			1st phase	P	2nd phase	P
		Glucose (mg/100 ml)	K ⁺ (mM)	Na ⁺ (mM)	Glucose (mg/100 ml)	K ⁺ (mM)	Na ⁺ (mM)	min 0.5–5.5 (ng/islet)		min 5.5–40 (ng/islet)	
1	4	50	6	143	50	6	143	0.00±0.02	NS	0.40±0.13	NS
2		50	6	143	50	0	149	0.06±0.03		0.01±0.30	
3	6	50	6	143	300	6	143	0.29±0.07	NS	7.49±1.24	NS
4		50	6	143	300	0	149	0.46±0.11		6.77±1.21	
5	4	50	6	143	300	0	149	0.71±0.13	NS	8.30±1.25	<0.02
6		50	6	24	300	0	24	0.59±0.07		5.13±0.99	
7	4	50	6	143	300	0	149	0.63±0.10	<0.02	7.61±0.56	<0.001
8		50	6	1	300	0	2	0.18±0.04		0.87±0.20	

Paired groups of islets were perfused either in the presence of 6 mM K⁺ or in its absence, as indicated in the central part of the table; glucose and Na⁺ concentrations of the perfusate are also mentioned.

Results are expressed as the mean±SEM of n experiments. Statistical significance (P) refers to the comparison between the control group (1st line of each pair) and the experimental group (2nd line). NS = not significant.

IRI content of the effluent fractions was measured by a double antibody immunoassay [8] against standard solutions of rat insulin (21 IU/mg; generous gift of Dr. J. Schlichtkrull, Novo Research Institute, Copenhagen, Denmark). Results were expressed as ng IRI released/min/islet. The total amounts of IRI secreted during the first (min 0.5 to 5.5) and second (min 5.5 to 40) phases of secretion in response to glucose were calculated by summation of all individual secretion rates after correction for the time of collection and subtraction of basal rate of release [5]. Negative values obtained by this mode of calculation mean that the rate of secretion continued to decline after the end of the equilibration period. Statistical significance of differences between means of experimental and control groups was assessed by Student's *t* test for paired data.

Glucose Oxidation

Batches of 10 islets were incubated in 100 µl KRB (300 mg glucose/100 ml, 0 or 6 mM K⁺) supplemented with 0.2 µCi D-glucose-U-¹⁴C (The Radiochemical Centre, Amersham, England). The polyethylene tubes (volume:0.4 ml) containing the medium were placed inside stoppered glass tubes (75×11 mm). After 1 or 2 h of incubation at 37°C in a shaking incubator (140 strokes/min), 0.6 ml of hyamine was injected into the outer vessel and CO₂ was liberated into the hyamine by injection of 50 µl of 0.5 M HCl in the polyethylene tube. The incubation vials were shaken at 37°C for 2 further hours. Hyamine was transferred into 10 ml of Instagel (Packard Instrument Co, Downers Grove, Illinois, U.S.A.) and the radioactivity measured by liquid scintillation spectrometry. Blank incubations were carried out for both types of medium. Results are

Fig. 1. Effect of the absence of extracellular potassium during the stimulation period upon IRI secretion induced by 300 mg glucose/100 ml (G 300). Sodium concentration of the perfusate was either normal (●—●) or decreased to 24 mM by partial replacement by choline (○—○). In this and the following figures, the shaded area delimits 2 SEM of the mean IRI response to 300 mg glucose/100 ml obtained in 75 control experiments with a medium of normal cationic composition. Only the last 10 min of the equilibration period are shown. Each point represents the mean±SEM of 4 experiments

Results

1. Insulin Secretion

Effect of the absence of extracellular potassium during the stimulation period. In the continuous presence of a

low glucose concentration (50 mg/100 ml), removal of external K^+ from the medium, between min 0 and 40, did not significantly augment IRI release (Table 1, lines 1 and 2). Although some individual secretion rates were statistically elevated¹ during the absence of K^+ , the total amount of IRI secreted was not different from that of islets maintained in normal KRB.

Fig. 2. Effect of the absence of extracellular potassium during the stimulation period upon IRI secretion induced by 300 mg glucose/100 ml. Perfusion medium contained either a normal sodium concentration (●—●) or 2 mM sodium (○—○). Each point represents the mean $\pm$ SEM of 4 experiments

When removal of K^+ coincided with an elevation of glucose concentration from 50 to 300 mg/100 ml, a slight increment of the first phase and reduction of the second phase of the response to the sugar were observed. Though these modifications failed to achieve statistical significance (Table 1, lines 3 and 4), it should be noted that the secretion rates tended to decline rapidly after 25 min of absence of K^+ from the medium (Figs. 1 and 2; compare the solid lines with the shaded area).

When Na^+ had been partially substituted by choline (24 mM Na^+) since the beginning of the equilibration period, the first phase of secretion in response to glucose in a K^+ -free medium was unaffected, but the second phase was decreased by about 40% (Fig. 1; Table 1, lines 5 and 6). If Na^+ was omitted from the perfusion medium (2 mM Na^+)², both phases of secretion were markedly diminished (Fig. 2; Table 1, lines 7 and 8).

Effect of the absence of extracellular potassium during the whole experiment. As shown in Fig. 3, omission of K^+ from the perfusate already during the equilibration period increased both the rapidity and the amplitude of the early secretory response to glucose stimulation

applied 25 min later (min 0). Thus individual secretion rates were significantly higher ($p < 0.05$) in the absence of K^+ than in its presence at min 1.5, 2.5 and 3, and the first significant elevation of release above basal levels was noted 30 s earlier in the experimental group (min 1.5) than in the controls. Moreover, the amount of IRI secreted during the first 5.5 min of stimulation was augmented by approximately 40% (Table 2, lines 1 and 2).

By contrast, such K^+ -deprivation not only prevented the secondary rise in secretion normally evoked by glucose, but progressively inhibited IRI release with time (Figs. 3 and 4; Table 2, lines 1 and 2). Increase of K^+ concentration from 0 to 6 mM (min 30), while keeping 300 mg glucose/100 ml did not prevent the secretion rate from declining further during the following 10 min; thereafter the rate of secretion progressively rose to reach 0.16 ± 0.02 ng/min/islet at the end of stimulation (min 60), a value not significantly lower than that of 0.21 ± 0.02 ng/min/islet observed in the controls (Fig. 3).

Fig. 3. Reversibility of the effect of extracellular potassium deprivation upon IRI secretion induced by 300 mg glucose/100 ml. Potassium was absent from the perfusate between min -25 and min 30 (○—○) at which time its concentration was restored to normal. Each point represents the mean $\pm$ SEM of 4 experiments

Decrease of Na^+ to 24 mM during the whole experiment inhibited both phases of glucose-induced release observed in the absence of K^+ , without major alteration of the secretory pattern (Fig. 4; Table 2, lines 3 and 4). In addition, it delayed the first significant rise in secretion above baseline to min 3 as compared with min 1.5 in the presence of 149 mM Na^+ (Fig. 4). In the presence of 2 mM Na^+ , the two phases of secretion in response to glucose stimulation were diminished by 80% (Table 2, lines 5 and 6) and exhibited a dynamics similar to that of the experimental condition illustrated by Fig. 2.

2. Glucose Oxidation

As shown in Table 3, the amount of glucose oxidized by the islets was not altered after 60 min of in-

1 ($p < 0.05$) on min 2, 2.5 and from min 27 to 39.

2 The residual Na^+ concentration was due to the presence of 1.2 mM NaH_2PO_4 in the medium and contamination by the preparation of bovine serum albumin.

cubation in a K^+ -free medium; it was reduced by about 35% after 2 h in the absence of K^+ . By contrast, if the islets were first perfused for 60 min with a K^+ -deprived medium, a 65% inhibition of glucose oxidation was already evidenced at the end of the first hour of incubation.

Discussion

The validity of perfused rat islets to analyze the dynamics of IRI secretion has been established by Lacy *et al.* [10, 11] and confirmed in our previous re-

sence of extracellular K^+ affects the secretory response to glucose in a manner dependent on its duration and on the concentration of Na^+ ; moreover, K^+ omission can modify the two phases of release in opposite directions under some experimental conditions. Despite the rapidity of K^+ withdrawal and of its reintroduction in the perfusate, the observed modifications of IRI secretion were delayed and progressive. Indeed, changes in glucose-induced secretion could not be detected until 25 min of K^+ deprivation (Figs. 1 and 2), and the inhibition of the second secretory phase was not corrected during the first 10 min following

Table 2. Total IRI output from perfused islets in the absence of extracellular K^+ during the whole experiment

Line no	n	Composition of perfusion media						IRI released during stimulation			
		Equilibration			Stimulation			1st phase min 0.5–5.5 (ng/islet)	P	2nd phase min 5.5–40 (ng/islet)	P
		Glucose (mg/100 ml)	K^+ (mM)	Na^+ (mM)	Glucose (mg/100 ml)	K^+ (mM)	Na^+ (mM)				
1	4	50	6	143	300	6	143	0.40 ± 0.06	<0.05	8.42 ± 0.59	<0.005
2		50	0	149	300	0	149	0.55 ± 0.06		2.66 ± 0.18	
3	4	50	0	149	300	0	149	0.86 ± 0.10	<0.02	4.68 ± 0.49	<0.005
4		50	0	24	300	0	24	0.27 ± 0.06		1.22 ± 0.39	
5	4	50	0	149	300	0	149	0.76 ± 0.05	<0.001	4.87 ± 0.34	<0.001
6		50	0	2	300	0	2	0.14 ± 0.01		0.88 ± 0.11	

Paired groups of islets were perfused either in the presence of 6 mM K^+ or in its absence, as indicated in the central part of the table; glucose and Na^+ concentrations of the perfusate are also mentioned.

Results are expressed as the mean $\pm$ SEM of n experiments. Statistical significance (P) refers to the comparison between the control group (1st line of each pair) and the experimental group (2nd line).

Fig. 4. Effect of the absence of extracellular potassium during the whole experiment upon IRI secretion induced by 300 mg glucose/100 ml. Perfusion medium contained either a normal sodium concentration ($\bullet$ — $\bullet$) or 24 mM sodium ($\circ$ — $\circ$). Each point represents the mean $\pm$ SEM of 4 experiments

ports [5, 6, 12]. The few studies, which have been so far concerned with the effect of a K^+ -free medium on IRI release, reported a stimulation at both low and high glucose concentrations during incubation of adult [2] and fetal [3] pancreatic pieces.

The present experiments demonstrate that the ab-

reintroduction of a normal K^+ concentration (Fig. 3). This lends support to the idea that IRI releasing mechanisms are affected by alterations of the intracellular concentrations of cations secondary to the omission of extracellular K^+ , rather than by the absence of K^+ in the medium as such. Nevertheless, restoration of the glucose effect upon correction of K^+ concentration indicated that the absence of the cation for 55 min did not irreversibly damage the releasing function of the beta cell.

It is well known that the absence of extracellular K^+ decreases the activity of the membrane ATP-ase responsible for the uphill transmembrane transport of Na^+ and K^+ [13, 14]. The existence of such Na^+/K^+ pump in islet cells was suggested by studies of Howell and Taylor [15] who showed an inhibition of glucose-induced $^{42}K^+$ uptake by ouabain. The similarity of the modifications in glucose-induced secretion that we observed in the absence of extracellular K^+ and in the presence of ouabain [16] suggests that these changes result from a blockade of the Na^+/K^+ pump. It seems reasonable to assume that this blockade leads to a progressive accumulation of Na^+ and a concomitant depletion of K^+ in beta cells as in other cells.

The augmented intracellular Na^+ concentration cannot account for the progressive inhibition of the second phase of glucose-evoked secretion observed in the absence of K^+ . This inhibition is indeed more pronounced when the cellular accumulation of Na^+ is

prevented, by decreasing to 24 mM the concentration of the cation in the perfusate, a condition which does not affect the release in the presence of 6 mM K^+ [5]. By contrast, the diminution in intracellular K^+ , due to the reduced activity of the Na^+/K^+ pump, may well explain our findings. Thus, this cellular K^+ loss is facilitated when choline (125 mM) partially replaces Na^+ in the medium [17, 18] and may be regarded as responsible for the increased inhibition of the second phase noted in this situation (Figs. 1 and 4).

On the contrary, the more rapid and augmented early secretory phase of the response to glucose after 25 min of K^+ deprivation (Fig. 3) might be brought about by a cellular enrichment in Na^+ [2, 5]. However, the first phase also appears to be K^+ -dependent. Indeed, its rate of onset and its magnitude are reduced

Table 3. *Effect of potassium deprivation on glucose oxidation by isolated islets*

Time of incubation	Glucose oxidized (pmol/islet)		P
	6 mM K^+	0 mM K^+	
60 min	22.4 ± 1.8 (6)	20.5 ± 3.1 (7)	NS
120 min	41.2 ± 4.8 (7)	26.0 ± 3.1 (7)	< 0.05
60 min ^a	18.5 ± 1.7 (12)	6.4 ± 1.3 (12)	< 0.001

Incubations were carried out in a medium containing 300 mg glucose/100 ml and the indicated concentration of K^+ .

Results are means ± SEM of the number of determinations indicated in parentheses. Statistical significance (P) refers to the comparison between the control (6 mM K^+) and the experimental group (0 mM K^+). NS = not significant.

^a Islets perfused for 60 min with a medium containing 6 or 0 mM K^+ and no glucose, before incubation.

below control values if K^+ has been absent and Na^+ lowered to 24 mM since the beginning of the equilibration period (Fig. 4). As the same decrease in Na^+ concentration does not alter the effect of glucose in the presence of 6 mM K^+ [5], the inhibition and the retardation of the early phase noted when the partial substitution with choline occurs in the absence of K^+ seem to be best explained by the facilitation of cellular K^+ depletion.

There is no doubt that Na^+ plays an important role in the regulation of insulin release as underlined by the abolition of the insulinotropic effect of glucose when the cation is totally substituted by choline as well in a medium deprived of K^+ (Fig. 2) as in perfusates containing 6 mM [5] or 24 mM K^+ [6]. However, the modulation of glucose-stimulated insulin secretion by changes in intracellular Na^+ appear to be dependent on the cellular K^+ content.

Our results showed a clear inhibition of glucose oxidation by the islets after removal of K^+ from the medium³. This inhibitory effect was not detectable,

³ A similar observation was made in islets of *ob/ob* mice by J. Sehlin and I.-B. Täljedal (personal communication)

however, after 60 min of incubation unless the islets had been previously perfused for 1 h in a K^+ -free medium. The more pronounced and earlier decrease in $^{14}CO_2$ production noted under this condition might be accounted for by a facilitation of cellular K^+ depletion in a system where the bathing medium is constantly renewed. Other experimental conditions blocking the Na^+/K^+ pump also inhibit the metabolic activity of islets of Langerhans. Thus, ouabain has been reported to diminish both glucose oxidation [19] and glycolysis [19, 20]. This diminution of the metabolism is indeed expected from the reduced need for energy secondary to blockade of the Na^+/K^+ pump [21]. It is known however that different metabolic steps are affected by lowering K^+ concentration [22]; some of them might be involved in the process leading to IRI release. Such metabolic changes are in accordance with the inhibition of IRI secretion noted after K^+ removal.

In conclusion, the present data have provided new evidence that changes in intracellular Na^+ and K^+ concentrations influence both phases of glucose-induced secretion. It is suggested that accumulation of Na^+ in the beta cells facilitates IRI release, but that K^+ depletion depresses both phases of secretion, possibly by altering glucose metabolism. The changes in IRI release noted in the absence of extracellular K^+ appear to be the result of these antagonistic actions.

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