Biphasic Modulation of K^+ Permeability in Pancreatic Islets During Acute Stimulation with Glucose

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SUMMARY

The regulation of $^{86}Rb^+$ efflux (marker of K^+ permeability) during acute secretagogic stimulation with glucose was studied with cultured as well as freshly isolated pancreatic islets from rats and freshly isolated islets from mice. A perifusion system with minimal dead-space and "flow-through" characteristics conducive to abrupt, steep increases in ambient glucose was combined with multiple samplings of effluent to achieve high temporal resolution. Under these conditions, acute increases in perifusate glucose concentration from 4 to 16.7 mM or from 1 to 27.8 mM effected a biphasic change of the $^{86}Rb^+$ fractional efflux rate. A rapid reduction of $^{86}Rb^+$ efflux was interrupted by an evanescent increase in $^{86}Rb^+$ outflow, which appeared to be temporally coincident with the initiation of the first phase of stimulated insulin release. It is suggested that the glucose-induced biphasic oscillations in K^+ permeability may contribute to the well-known initial biphasic changes in β-cell membrane potential and insulin release during the inception of β-cell stimulus secretion coupling. DIABETES 32:820–824, September 1983.

Stimulation of β-cells with a high glucose concentration leads to an acute biphasic increase in insulin release.[1] This is paralleled by phasic changes in several β-cell processes, such as $^{45}Ca^{2+}$ efflux,[2] phosphate flush,[3] and depolarizing electrical activity.[4–6] The electrical activity is due at least in part to a reduction in K^+ permeability as judged by the acute reduction in the efflux of $^{42}K^+$ (or $^{86}Rb^+$ as a marker for K^+) from prelabeled islets during stimulation with glucose.[7–10] However, it is not clear from these reports whether acute transition from basal to maximal insulin secretion by glucose stimulation has additional effects on K^+ permeability that could contribute to the biphasicity of early, activated insulin release and the concomitant changes in islet membrane permeability. A recent examination of ionic events in islet stimulus-secretion coupling disclosed a minor and brief reascension, averaging 8%, from the initially depressed $^{86}Rb^+$ efflux during abrupt change of ambient glucose concentrations from zero to 16.7 mM in five perifusion experiments.[11] The present studies, which were underway when the above report[11] appeared, were designed to examine for biphasicity in early $^{86}Rb^+$ efflux with more certitude. A perifusion system was employed in which the change from basal to maximally stimulating concentrations of glucose could be effected precipitously (i.e., $t_{\frac{1}{2}}$ of 15–30 s vis-à-vis 60–70 s[11]) and effluents could be sampled frequently and from a smaller dead space than heretofore employed to facilitate dissection of efflux kinetics. To assess the universality of our findings, we also utilized a variety of islet preparations, i.e., islets that had been maintained in tissue culture to achieve stability and reproducibility of β-cell function, as well as islets that had been freshly isolated from two different species. A preliminary account of our results has been presented elsewhere.[12]

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MATERIALS AND METHODS

Pancreatic islets from female albino rats (Charles River Laboratory, Boston, Massachusetts) or female mice (lean littermates from the breeding of noninbred *ob/ob* mice at the Department of Histology and Cell Biology, University of Umeå, Sweden) were isolated with collagenase. For tissue culture, collagenase-isolated rat islets were maintained in RPMI 1640 (Gibco, Grand Island, New York) supplemented with 10% (vol/vol) fetal calf serum (Irvine Scientific Co., Irvine, California), 100 U/ml penicillin, 100 μg/ml streptomycin, and 11 mM D-glucose for 11 days as described elsewhere.[13,14]

Each experiment with freshly isolated rat or mouse islets or cultured rat islets was started with a stabilizing preincubation of 50–200 islets for 60 min at 37°C in 1 ml gassed

(95% O_2:5% CO_2) Krebs-Ringer buffer (pH 7.4) containing 10 mM 2-(N-hydroxyethylpiperazine-N′-yl)ethane-sulphonic acid (HEPES), 0.5 mg/ml bovine serum albumin, and 2.8 mM D-glucose (basal medium). The islets for studies of $^{86}Rb^+$ efflux were incubated for an additional 120 min in basal medium (200 μl) supplemented with 53 μM ^{86}RbCl essentially according to a previously described method.[8] Islets for insulin release experiments were incubated for an additional 90 min in basal medium (200 μl) containing 5.6 mM D-glucose but lacking ^{86}RbCl.

Thereafter, islets were removed from the incubation vials, washed twice in about 5 ml nonradioactive basal medium, and placed on a polycarbonate filter (5-μm pore size; Nucleopore Corp., Pleasanton, California) in a small perifusion chamber (13-mm Pop-Top Holder; Nucleopore Corp.) attached to a peristaltic pump. The chambers had a total "dead space" of 305 μl; $t_{\frac{1}{2}}$ for washout of the perifusion chamber and efflux tubing was between 15 and 30 s at a perifusion rate of 1 ml/min. The whole apparatus was kept at 37°C in a thermoregulated closed infant incubator and all manipulations and collections were performed with gloves introduced into the incubator through insulated apertures without disrupting thermal equilibrium.

Perifusion with basal medium containing nonstimulatory concentrations of glucose (1–4 mM as shown in Figures 1–3) was conducted for 15 min for the studies of $^{86}Rb^+$ efflux or 30 min for the studies of insulin release. Collections of effluent were secured at 60-s intervals for the last 5 min. Acute stimulation was then effected by switching to perifusion media containing 16.7 or 27.8 mM glucose. The vertical lines in Figures 1–3 correspond to the time at which the new medium reached the perifusion chamber, as determined previously by a variety of calibration experiments. The change was achieved by transferring from one temperature-equilibrated reservoir to another with a three-way valve (LV-3; Pharmacia Fine Chemicals, Uppsala, Sweden). Following transfer, collections were secured at 15-s intervals in the experiments with cultured rat islets or freshly isolated mouse islets, and at 20-s intervals for the experiments with freshly isolated rat islets. All collections were secured manually.

$^{86}Rb^+$ radioactivity in the effluent fractions and remaining in the islets at the end of perifusion was measured in a liquid scintillation spectrometer. Fractional efflux is defined as the fraction of $^{86}Rb^+$ leaving the islets during a certain period of time in relation to the cellular content of $^{86}Rb^+$ at the start of that period. Insulin was measured by radioimmunoassay using rat insulin as a standard and polyethylene glycol to precipitate antibody-bound insulin.[15] Commercially available chemicals of analytical grade were used for the preparation of reagents in all experiments.

RESULTS

Kinetics of the perifusion system at the standard flow rates of 1 ml/min are depicted in the lower portion of the left-hand panel of Figure 1. After acute increases in the glucose content of the perifusate from 4 to 16.7 mM, 58 ± 2% of the maximum glucose concentration was present in the effluent within 30 s—i.e., in the second posttransfer collection—and 87 ± 4% was demonstrable 15 s later, in the third collection (N = three separate experiments). The concurrent changes in rates of $^{86}Rb^+$ efflux from prelabeled rat islets, which had been stabilized previously by tissue culture,[13,14] are depicted

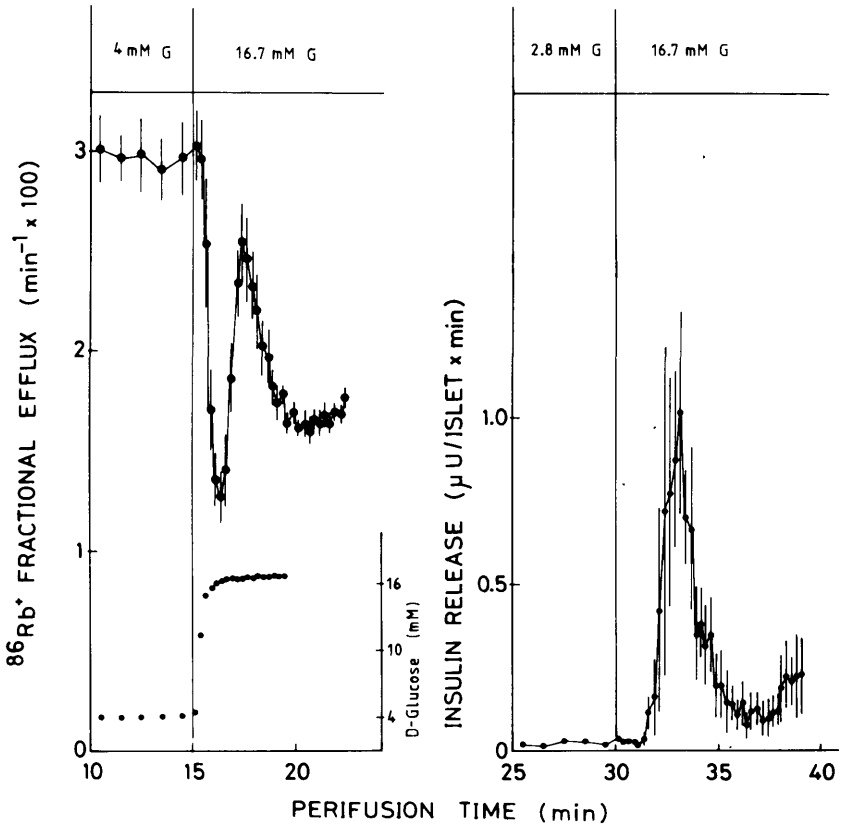

FIGURE 1. Phasic changes in $^{86}Rb^+$ efflux and insulin release from prelabeled cultured rat islets during acute stimulation with glucose: the upper part of the left panel shows $^{86}Rb^+$ fractional efflux rates (mean ± SEM; N = 4) when the glucose concentration in the perifusate was raised acutely from 4 to 16.7 mM. The vertical line corresponds to the time at which the new medium reached the perifusion chamber. Changes in the glucose content of the effluent under these conditions are shown in the lower left panel (mean ± SEM; N = 3). The panel to the right summarizes insulin release from cultured islets when glucose was changed from 2.8 to 16.7 mM (mean ± SEM; bars when larger than width of symbol; N = 3). For both sets of experiments, effluents were collected at 60-s intervals for 5 min before increasing ambient glucose and at 15-s intervals thereafter.

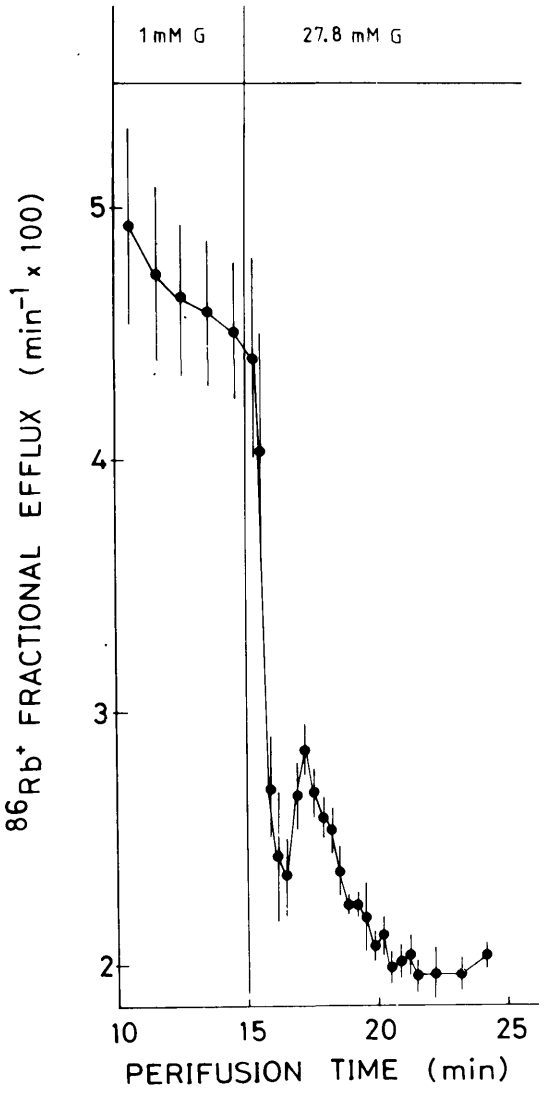

FIGURE 2. Phasic changes in $^{86}Rb^+$ efflux from prelabeled freshly isolated rat islets during acute stimulation with glucose: means ± SEM values for rates of $^{86}Rb^+$ efflux in four separate experiments are depicted. Effluents were collected every 60 s before increasing ambient glucose and at 20-s intervals for the first 7 min thereafter.

in the upper portion of the left panel of Figure 1. A rapid reduction in $^{86}Rb^+$ efflux was observed within 30–45 s after the transfer to higher glucose concentration and the decrement progressed to nadir levels at 90 s, that is, in the sixth posttransfer collection. Thereafter, a transitory increase in the rates of $^{86}Rb^+$ efflux supervened, which peaked at 150 s. Subsequent rates of $^{86}Rb^+$ outflow declined again and finally stabilized at plateau levels from about the 20th collection onward (N = 4; Figure 1).

The kinetics of insulin release from cultured islets, as assessed in separate experiments (N = 3) but under similar conditions, are depicted in the right panel of Figure 1. Basal rates of insulin release were not significantly increased during the first six posttransfer collections—that is, for the first 90 s after abruptly increasing glucose in the perifusate from 2.8 to 16.7 mM. The first phase of stimulated insulin release was detectable in the seventh collection and peaked in the 13th collection, that is, after 195 s of perifusion with 16.7 mM glucose. The descending limb of the first phase of stimulated insulin release reached nadir values in the 26th collection and was then succeeded by the manifest onset of the second phase (Figure 1).

Freshly isolated rat and mouse islets were employed to document that the oscillations in $^{86}Rb^+$ efflux were not unique for cultured islets. Figure 2 depicts the changes in rates of $^{86}Rb^+$ efflux from prelabeled freshly isolated rat islets in four perifusion experiments in which ambient glucose was abruptly changed from 1 to 27.8 mM. The transfer effected a rapid decline to nadir values at 100 s, which was followed by a transient reascension of $^{86}Rb^+$ efflux rates to peak values at 140 s. Figure 3 summarizes the patterns of $^{86}Rb^+$ efflux, which we observed in four separate experiments with freshly isolated mouse islets. Here, also, an abrupt increase in the glucose content of the perifusate from 4 to 16.7 mM elicited a rapid reduction in $^{86}Rb^+$ efflux (nadir at 60 s), which was followed by a secondary, transient increase (peak at 105 s; Figure 3). Since paired experiments with insulin collections were not secured in the studies with freshly isolated islets, direct correlations with kinetics of insulin release cannot be made.

DISCUSSION

Glucose-induced reductions of $^{86}Rb^+$ efflux (K^+ permeability) are known to be more sensitive to ambient glucose than insulin release, and maximal reductions are attained with lesser concentrations of glucose than are required for maximal stimulation of insulin secretion.[10,16] To avoid complications of kinetic analysis due to such differences in dose-response properties, our experimental system was designed to effect the transition from basal to maximally stimulatory concentrations of glucose for both processes as rapidly as possible. As shown in Figure 1, the "flow-through" and dead-space characteristics of our present perifusion system enabled us to fulfill this objective; effluent concentrations of glucose approximating inflow levels were achieved within approximately 45 s. Under these conditions and in confirmation and extension of earlier findings,[7–10] acute exposure of prelabeled cultured rat islets to maximally secretagogic amounts of glucose caused a prompt reduction in rates of $^{86}Rb^+$ outflow. However, whereas the attenuation of $^{86}Rb^+$ outflow became manifest within 45 s, and achieved nadir levels within 90 s, the anticipated increases in insulin release could not be documented until a total of 105 s had elapsed and peak values for first-phase-stimulated insulin release did not occur until the 13th collection at 195 s. By that time, the obtunded rates of $^{86}Rb^+$ efflux had undergone a transitory reascension, which was followed by a second phase of diminution.

The main reason why Rb^+ phasicity was so clearly shown in these experiments (and only suggestively[11] or not at all in previous work by ourselves and others) is probably to be found in the design of the perifusion system. It should be noted that we observed the initial oscillations of $^{86}Rb^+$ efflux from cultured rat islets in the first 10 fractions collection at 15-s intervals (Figure 1). Obviously, to be able to detect these evanescent oscillations during acute perturbations of stimulus-secretion coupling, the resolving power of the perifusion system must be abetted by rapid changes in the glucose concentration around the islets as well as frequent sampling.

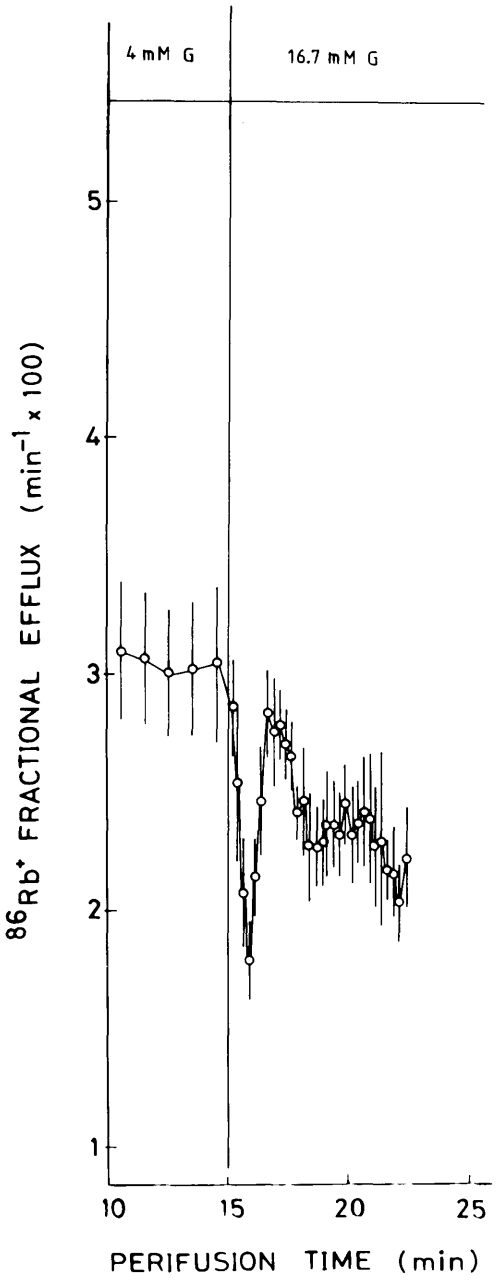

FIGURE 3. Phasic changes in $^{86}Rb^+$ from prelabeled freshly isolated mouse islets during acute stimulation with glucose: means ± SEM values for rates of $^{86}Rb^+$ efflux in four separate experiments are depicted. Effluents were collected every 60 s before increasing ambient glucose and at 15-s intervals thereafter.

The small dead-space and rapid "flow-through" properties of our system favored the former. Therefore, our finding of similar biphasic $^{86}Rb^+$ efflux when we also examined freshly isolated rat islets and freshly isolated mouse islets with this system renders it likely that the phenomenon is a general property of early stimulus-secretion coupling in normally functioning islets. It remains for future work to define its precise functional relationship to the triggering of activated insulin release, although it is tempting to suggest that the $^{86}Rb^+$ excursions may mirror some of the alterations in electrical potential[4-6] that occur at that time. Cyclic variations of K^+ conductance as measured with microelectrodes have been suggested as a possible explanation for the rhythmic oscillations of the membrane potential in β-cells in the presence of elevated glucose concentrations under steady-state conditions.[17,18] However, it is not known whether this regular burst pattern and the initial oscillation of the membrane potential following acute stimulation with glucose represent exactly the same set of ionic events.

In analyzing the actions of nutrient secretogogue along these lines, Carpinelli and Malaisse[19] reported that elevations of the glucose concentration from 8 to 16.7 mM during islet perifusion led to a monophasic transient increase of the $^{86}Rb^+$ efflux, reminiscent of the secondary peak that we found. However, stimulation of resting β-cells (0–4 mM glucose) with increased glucose concentration (1.7–16.7 mM glucose) produced only a monophasic reduction of the $^{86}Rb^+$ efflux. At present, the reason for this discrepancy between our results and those of Carpinelli and Malaisse[19] is not well understood, although differences in the characteristics of their perifusion system may have been contributory. Approximately 4 min was required for the glucose content in the effluents from their perifusion to progress from 8.3 to 16.7 mM (Figure 12 in ref. 19) so that resolution may have been compromised even with the 12-s collections that were secured in some of their experiments.[19] Moreover, interpretative complexities may have arisen from their attempts to isolate finite segments of the dose-response curve,[19] since both phases of the $^{86}Rb^+$ efflux pattern would contribute variably during the integrated sequence of full stimulus-secretion coupling. For example, their attempts to define the true lag for onset of "phosphate flush"[3] on the basis of the change in the efflux of ^{32}P from prelabeled islets during a change of perifusate glucose from 8.3 to 16.7 mM glucose[19] may have been complicated by the failure to appreciate that the K_m for phosphate flush is slightly less than 8.3 mM glucose and the V_{max} is approximately 11.1 mM.[20]

Using energy-dispersive x-ray analysis of fixed and extensively washed β-cells prepared for scanning electron microscopy, Kalkhoff and Siegesmund[21] found a biphasic change of the residual K^+ content of isolated rat islet β-cells when glucose concentration in the perifusion medium was acutely increased from 2.8 to 16.7 mM. Direct comparisons between their results and ours cannot be made because they focused on the K^+ content of single β-cells, whereas we have monitored $^{86}Rb^+$ transits in the whole islet. As yet, the contribution of β-cell K^+ to total islet K^+ has not been established. However, if islet $^{86}Rb^+$ patterns truly reflect the changes in the flux of the K^+ in the 80% of the islet volume (60–70% of islet cell population) that consists of β-cells,[22] our observations would suggest that the initial rise in β-cell K^+ described by Kalkhoff and Siegesmund[21] may be due to a retardation of outflow, whereas the subsequent decrement in total islet K^+ may be due to an augmented efflux.

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