Vol. 107, No. 1, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 16, 1982 Pages 350-356

PARADOXICAL ACTIVATION BY GLUCOSE OF QUININE-SENSITIVE POTASSIUM CHANNELS IN THE PANCREATIC B-CELL

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Received May 7, 1982

SUMMARY : A stepwise rise in extracellular glucose concentration from 8.3 to 16.7 mM paradoxically increases the outflow of ^{86}Rb from prelabelled pancreatic islets, as if the permeability to K^+ of the plasma membrane was suddenly and sustainedly increased. The mechanisms underlying this paradoxical response was investigated by exposing the islets to agents blocking either the Ca^{2+}-activated or voltage-sensitive K^+ channels. At concentrations exerting similar inhibitory effects upon the K^+ permeability of glucose-deprived islets, tetraethylammonium failed to affect, while quinine severely impaired the increase in ^{86}Rb efflux induced by the rise in glucose concentration. None of these drugs impeded the stimulation of Ca^{2+} influx evoked by the rise in glucose concentration. These findings suggest that glucose, in the 8.3-16.7 mM range, facilitates K^+ efflux from the pancreatic B-cell by stimulating a Ca^{2+}-sensitive modality of K^+ extrusion.

The stimulation by glucose of electrical activity in the pancreatic B-cell apparently depends on the ability of the sugar to decrease the plasma membrane permeability to K^+. Thus, a decrease in K^+ permeability with subsequent gating of voltage-sensitive Ca^{2+} channels is held responsible for the depolarizing and insulinotropic effect of glucose in the B-cell (1-6). Furthermore, it was proposed by several authors that, at high concentration, glucose enhances electrical activity and insulin release by preventing the activation by intracellular Ca^{2+} of a Ca^{2+}-sensitive modality of K^+ extrusion (7-9). Although the existence in islet cells of such a modality of K^+ extrusion is well documented (7, 10-14), the view that glucose, in high concentration, prevents the activation of the Ca^{2+}-sensitive K^+ permeability was recently challenged (13, 14). Indeed, an increase in the extracellular

0006-291X/82/130350-07$01.00/0

concentration of glucose from an intermediate to a high value, e.g. from 8.3 to 16.7 mM, does not decrease but, on the contrary, stimulates the efflux of ^{86}Rb (used as a tracer for K^+) from perifused islets (13, 14). This rise could reflect the stimulation and not the inhibition, by glucose of the Ca^{2+}-activated K^+ permeability, it being suppressed in the absence of extracellular Ca^{2+} (13, 14). Although the latter finding may appear conclusive, it is nevertheless conceivable that the increase in ^{86}Rb efflux induced by a rise in glucose concentration, from an intermediate to high value, reflects the stimulation by glucose of a voltage-sensitive, as distinct from Ca^{2+}-sensitive, modality of K^+ extrusion. The existence in the pancreatic B-cell of a voltage-dependent K^+ permeability was previously documented (12, 15), and an increase in electrical spiking activity can reasonably be suspected to be associated with stimulation of this voltage-sensitive process. Moreover, since the influx of Ca^{2+} into the B-cell through gated channels participates in the depolarizing effect of glucose (16-17), the absence of extracellular Ca^{2+} could conceivably prevent stimulation by glucose of a voltage-sensitive K^+ permeability. In order to decide whether a rise in glucose concentration, from an intermediate to high value, activates the Ca^{2+}-sensitive or voltage-sensitive K^+ conductance, we have investigated, in the present study, the influence of quinine and tetraethylammonium (TEA), respectively, upon the glucose-induced change in ^{86}Rb outflow. The selection of these drugs was motivated by the knowledge that quinine acts as a blocker of the Ca^{2+}-sensitive K^+ permeability, whereas TEA affects preferentially the voltage-sensitive K^+ permeability, whether in islets (10, 15) or other tissues (18-19).

MATERIALS AND METHODS

All experiments were performed with islets isolated by the collagenase technique from the pancreas of fed Wistar rats. The

media used for incubating, washing or perifusing the islets consisted of a Krebs-Ringer bicarbonate buffered solution supplemented with 0.5 % (w/v) dialyzed albumin (Fraction V, Sigma Chemical Company, St. Louis, Mo) and equilibrated against a mixture of O_2 (95 %) and CO_2 (5 %). The media also contained, as required, glucose, quinine sulfate (Sigma Chemical Company) and tetraethylammonium chloride (Merck, Schuckardt). In the presence of TEA, the osmolarity of the medium was kept constant by decreasing NaCl concentration.

The method used for the measurement of ^{86}Rb and ^{45}Ca efflux from perifused islets has been described elsewhere (20, 21). Briefly, groups of 100 islets each were incubated for 60 min in the presence of 16.7 mM glucose and either ^{86}Rb (0.1-0.5 mM; 50 µCi/ml) or ^{45}Ca (1.12 mM; 200 µCi/ml). After incubation, the islets were washed three times and then placed in a perifusion chamber. The perifusate was delivered at a constant rate (1.0 ml/min) and the effluent continuously collected from the 31st to the 90th min, over successive periods of 1 min each, for the measurement of its radioactive content by scintillation counting. The efflux of ^{86}Rb and ^{45}Ca was expressed as a fractional outflow rate (FOR), which represents, at any given time, the ratio of radioactive efflux to islet content. The validity of ^{86}Rb as a tracer for ^{39}K has been previously assessed (22). All results are expressed as the mean (± SEM) together with the number of individual experiments (n). The increase in the rate of efflux above mean basal value was calculated as the integrated rate of ^{86}Rb or ^{45}Ca efflux observed during stimulation (45th to 68th min). The statistical significance of difference between mean experimental and control data was evaluated by the Student's t test or by analysis of variance.

RESULTS AND COMMENTS

At the respective concentration of 5 µM and 20 mM, quinine and TEA inhibit to the same extent the rate of ^{86}Rb efflux from islets perifused in the absence of glucose (6, 23). Hence, these concentrations were used to investigate the effect of the drugs upon the glucose-induced increase in the rate of ^{86}Rb efflux.

A sudden rise in the glucose concentration from 8.3 to 16.7 mM provoked a rapid, sustained and rapidly reversible increase in the rate of both ^{86}Rb and ^{45}Ca efflux from perifused islets (Fig. 1 and 2). As illustrated in the upper panel of Fig. 1, the increase in the rate of ^{86}Rb efflux induced by 16.7 mM glucose was not inhibited by 20 mM TEA, which was administered throughout the experiment. In contrast, 5 µM quinine, when present throughout the experiment, severely inhibited the stimulatory effect of 16.7 mM glucose upon the rate of ^{86}Rb efflux (Fig. 2, upper panel). Thus, as judged from the increase in the rate of ^{86}Rb efflux re-

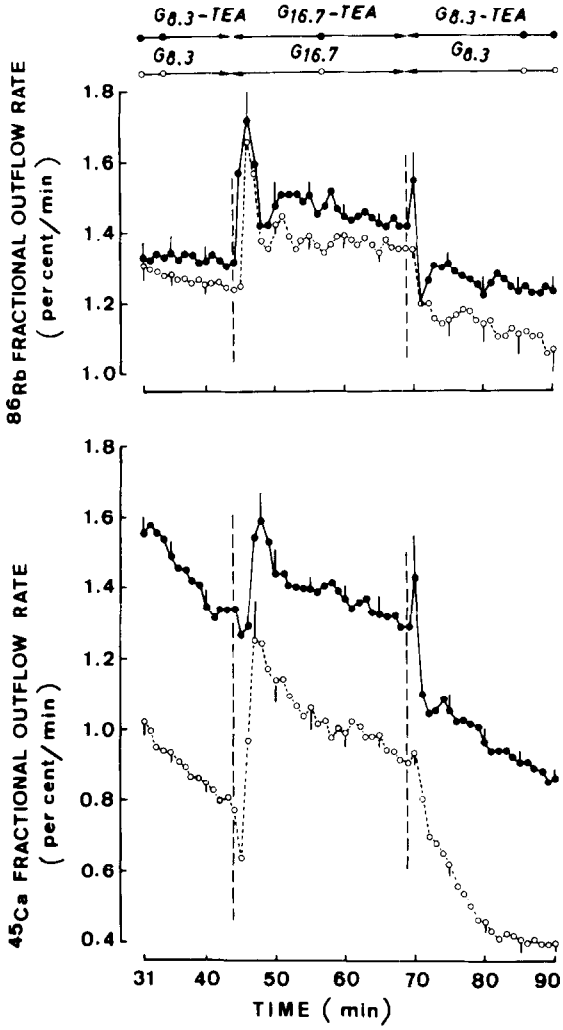

Fig. 1. Effect of a rise in glucose concentration from 8.3 to 16.7 mM upon the rate of ^{86}Rb efflux (upper panel) and ^{45}Ca efflux (lower panel) from islets perifused in the absence (o---o) or the presence of 20 mM TEA (●——●). Mean values (± S.E.M.) for ^{86}Rb and ^{45}Ca efflux are expressed as a fractional outflow rate and refer to at least 4 individual experiments in each case.

corded during the entire period of exposure to 16.7 mM glucose, the stimulatory effect of the sugar was reduced by 2.5 % ($P > 0.9$) and 89.3 % ($P < 0.025$) in the presence of 20 mM TEA and 5 µM quinine, respectively. These data clearly indicate that the increase in the rate of ^{86}Rb efflux induced by a rise in the glucose concentration within the 8.3-16.7 mM range reflects the stimulation of a Ca^{2+}-sensitive rather than a voltage-sensitive modality of K^+ extrusion.

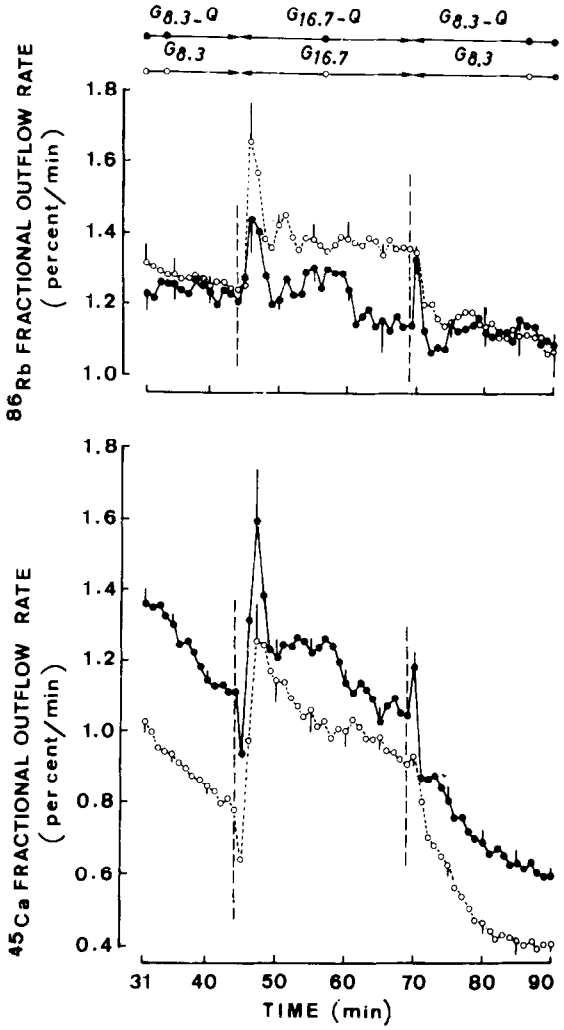

Fig. 2. Effect of a rise in glucose concentration from 8.3 to 16.7 mM upon the rate of ^{86}Rb efflux (upper panel) and ^{45}Ca efflux (lower panel) from islets perifused in the absence (o---o) or the presence of 5 μM quinine (●——●). Mean values (± S.E.M.) for ^{86}Rb and ^{45}Ca efflux are expressed as a fractional outflow rate and refer to at least 4 individual experiments in each case.

Such a view is further strenghtened by the observation that 16.7 mM glucose induced a similar increase in Ca^{2+} influx whether the islets were perifused in the presence of TEA or quinine. Indeed, the rise in glucose concentration from 8.3 to 16.7 mM provoked an increase in ^{45}Ca efflux which was of similar magnitude ($P > 0.5$) whether the islets were perifused in the presence of quinine or TEA, respectively (Figures 1 and 2 lower panels). The

glucose-induced increase in the rate of ^{45}Ca efflux from perifused islets is known to reflect stimulation of Ca^{2+} influx into the islet cells and to correspond to a process of Ca-Ca exchange in which influent ^{40}Ca displaces ^{45}Ca from intracellular binding sites (24). Incidentally, both TEA and quinine increased the basal rate of ^{45}Ca efflux observed in the presence of 8.3 mM glucose (min 40-44; $P < 0.001$ in both cases). This is in good agreement with previous observations (6, 23) that TEA and quinine are able to act synergistically with glucose to stimulate the entry of Ca^{2+} into the islet cells and, by doing so, to increase ^{45}Ca efflux.

In conclusion, the present data establish that a rise in the glucose concentration from an intermediate to a high value stimulates, rather than inactivates, the Ca^{2+} - or quinine-sensitive modality of K^+ extrusion. In our opinion, this novel information is essential to elucidate the mechanism by which glucose, in high concentration, suppresses the burst pattern of bioelectrical activity, causing continuous spiking and sustained insulin release (25).

ACKNOWLEDGEMENTS

This work was supported in parts by grants from the Belgian Foundation for Scientific Research. P. Lebrun is a Research Fellow of the Belgian National Foundation for Scientific Research. The authors are indebted to J. De Clerck, M. Herman and G. Sterckx for skilful technical assistance and to C. Demesmaeker for secretarial help.

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