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Stimulation of insulin secretion by glucose in the absence of diminished potassium ($^{86}Rb^+$) permeability

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Abstract—Two inhibitors of the nucleotide-sensitive K^+ (K_{ATP}) channel, tolbutamide and quinine, were utilized in order to assess the role of this channel in glucose-stimulated insulin release from perifused rat islets. In the absence of these drugs, the addition of 15 mM glucose elicited a marked biphasic stimulation of insulin secretion concomitant with a reduction in the rate of $^{86}Rb^+$ efflux. In the presence of either 500 μM tolbutamide or 100 μM quinine, a reduced rate of efflux of $^{86}Rb^+$ was observed together with an elevated rate of insulin release. Under such conditions, the addition of 15 mM glucose retained the ability to stimulate insulin secretion though this was associated with a marked increase in $^{86}Rb^+$ efflux. It is concluded that a net reduction in β-cell K^+ permeability is not an obligatory step in glucose-stimulated insulin release. Thus, glucose is likely to exert depolarizing actions on the β-cell in addition to the closure of K^+ channels.

The stimulation of insulin secretion by glucose and other nutrient secretagogues depends upon the metabolism of the nutrient within the β-cell [1] and the subsequent depolarization of the β-cell plasma membrane (for reviews, see Refs 2 and 3). It is currently believed that a rise in the cytosolic ATP/ADP ratio resulting in the closure of nucleotide-sensitive K^+ (K_{ATP}) channels provides the link between nutrient metabolism and plasma membrane depolarization [3, 4]. This channel also appears to be at least one of the sites of action of the hypoglycaemic sulphonylureas and quinine, both of which have been demonstrated to cause channel closure [5, 6].

We have utilized this particular action of these drugs in order to ascertain whether the insulinotropic effect of glucose persists under conditions of pharmacological inhibition of the K_{ATP} channel.

Materials and Methods

Islets were isolated by collagenase digestion from adult Wistar rats, and perifused at a rate of 1 mL/min with a physiological saline medium consisting of NaCl (110 mmol/L), KCl (5 mmol/L), $MgCl_2$ (1 mmol/L), $CaCl_2$ (1 mmol/L), $NaHCO_3$- (25 mmol/L) and HEPES (20 mmol/L), gassed with O_2/CO_2 (19:1 v/v). $^{86}Rb^+$ efflux and insulin release were measured in groups of 100 and 25 islets, respectively, as described elsewhere [7].

$^{86}Rb^+$ (4 mCi/mg) was obtained from the Radiochemical Centre (Amersham, U.K.). Tolbutamide and quinine were purchased from the Sigma Chemical Co. (Poole, U.K.). Analysis of statistical significance, utilizing the Student's *t*-test, was performed by comparing overall $^{86}Rb^+$ efflux or insulin secretory rates between the 30th and 35th min of perifusion with the corresponding rates between the 40th and 45th min.

Results and Discussion

The addition of 15 mM glucose to perifused rat islets resulted in a rapid reduction in the rate of efflux of $^{86}Rb^+$ from preloaded islets (Fig. 1A; $P < 0.001$), confirming previous reports [8, 9]. It is currently believed that this effect represents a reduction in plasma membrane K^+ permeability resulting from closure of K_{ATP} channels [3, 4] and accounts for the depolarization of the β-cell plasma membrane observed in response to the sugar [10]. This depolarization is thought to result in the opening of voltage-sensitive calcium channels [11] and calcium entry into the cell which triggers insulin release [12]. The accompanying biphasic stimulation of insulin release by glucose ($P < 0.001$) is shown in Fig. 2A.

Perifusion of islets in the presence of supramaximal concentrations of either tolbutamide (500 μM; Fig. 1B) or quinine (100 μM; Fig. 2C) resulted in a reduced fractional outflow rate of $^{86}Rb^+$ ($P < 0.001$ in both cases), presumably reflecting inhibition of K_{ATP} channels by these compounds [5, 6]. In the presence of either drug, the subsequent addition of 15 mM glucose caused a pronounced increase in the rate of efflux of $^{86}Rb^+$ (Fig. 1B and C; $P < 0.002$ and 0.01, respectively). In contrast to a previous report [13], this increase was not preceded by a transient reduction in $^{86}Rb^+$ outflow rate in the presence of quinine in response to glucose. The underlying mechanisms to this apparent increase in net K^+ permeability are uncertain, although this effect is reminiscent of the enhanced rate of $^{86}Rb^+$ efflux observed by Carpinelli and Malaisse [14] upon raising the glucose concentration from 8.3 mM to higher concentrations. These authors proposed that increased cytosolic [Ca^{2+}] may be responsible for this glucose-stimulated increase in K^+ permeability. An increase in

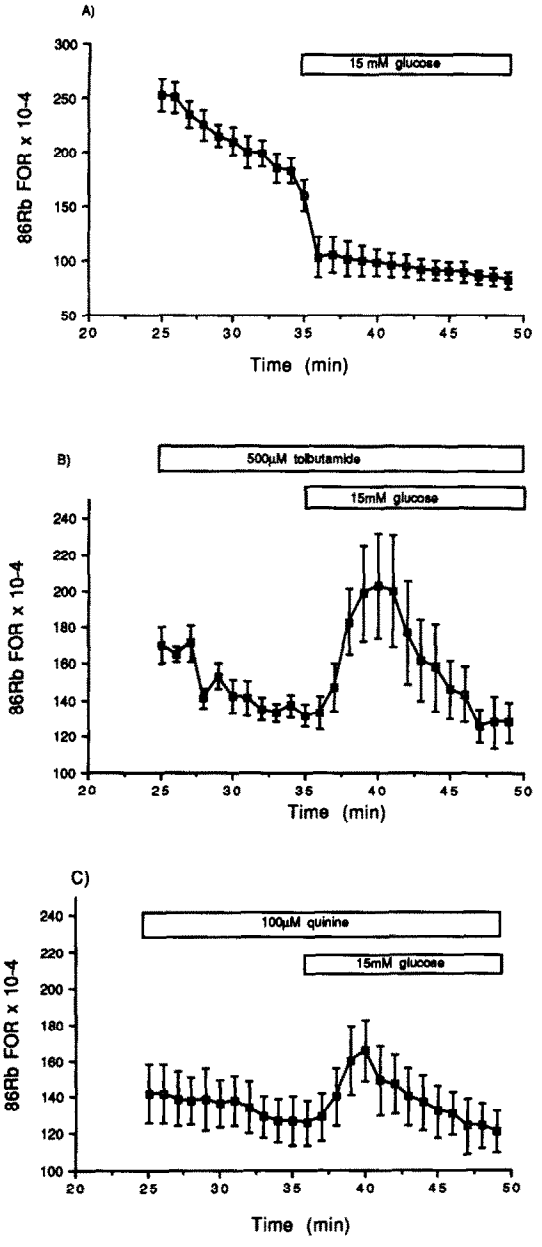

Fig. 1. Efflux of $^{86}Rb^+$ (FOR, fractional outflow rate) from preloaded, perifused rat pancreatic islets. Effects of 15 mM glucose alone (A), in the presence of 500 μM tolbutamide (B) or 100 μM quinine (C). Glucose was added to the perifusion medium at the 35th min. Each point is the mean ± SEM of four separate determinations.

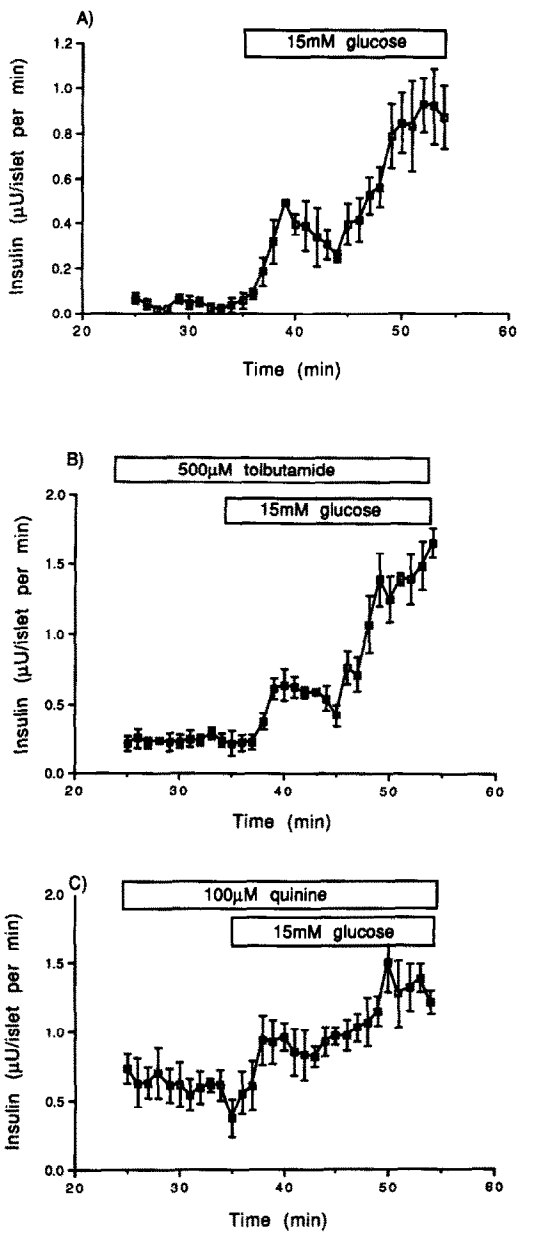

Fig. 2. Secretion of insulin from perifused rat pancreatic islets. Effects of 15 mM glucose alone (A), in the presence of 500 μM tolbutamide (B) or 100 μM quinine (C). Glucose was added to the perifusion medium at the 35th min. Each point is the mean ± SEM of four separate determinations.

$^{86}Rb^+$ efflux rate could arise from the opening of Ca^{2+}- or voltage-sensitive K^+ channels [3]. The use of specific inhibitors of these different channel types may help to clarify this point.

The insulin output from perifused islets in the absence of glucose was found to be enhanced in the presence of tolbutamide and more strikingly so in the presence of quinine (Fig. 2B and C; $P < 0.001$ in both cases). This probably reflected the depolarizing actions of these drugs. However, more noteworthy, the addition of glucose in the presence of these drugs again resulted in a marked, biphasic stimulation of insulin release (Fig. 2B and C; $P < 0.001$ in both cases), despite the accompanying increases in $^{86}Rb^+$ outflow. Whilst the measurement of $^{86}Rb^+$ efflux from perifused islets clearly does not permit the study of the activities of individual K^+ channel types, the present findings suggest that glucose can stimulate insulin release with an accompanying increase in net K^+ permeability. This is not to deny that the depolarization of the β-cell observed upon addition of glucose to a previously glucose-

free medium involves a reduction in K^+ permeability due to the closure of K_{ATP} channels. However, such a reduction in K^+ permeability may not be an obligatory step in the stimulation of the β-cell by raising the glucose concentration within the range effective for stimulating insulin release. Thus, it is likely that glucose can exert an additional action upon the β-cell, such a mechanism may be important in modulating the β-cell membrane potential and hence electrical and secretory activity above threshold glucose concentrations (approx. 5 mM), where little further change in K_{ATP} channel activity is observed [15]. This conclusion contrasts with that of a previous study [16] which suggested that glucose could regulate β-cell activity at higher concentrations via the modulation of K_{ATP} channel activity. This suggestion was based on the ability of tolbutamide to further stimulate β-cell electrical activity in the presence of 10 mM glucose. However, such a conclusion requires the assumption that tolbutamide stimulates the β-cell solely via the closure of K_{ATP} channels. There is increasing evidence that sulphonylureas may influence cellular function via a number of actions [17–20].

In conclusion, glucose causes a marked, biphasic stimulation of insulin release under conditions where K_{ATP} channel activity is suppressed by tolbutamide or by quinine and where glucose results in an apparent increase in net K^+ permeability. Glucose is therefore likely to stimulate the β-cell by an additional mechanism to reduced K_{ATP} channel activity.

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