J. Physiol. (1981), **315**, *pp.* 143–156
With 12 text-figures
Printed in Great Britain

REGULATION OF ^{86}Rb OUTFLOW FROM PANCREATIC ISLETS: THE DUAL EFFECT OF NUTRIENT SECRETAGOGUES*

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(*Received 3 October 1980*)

SUMMARY

1. An increase in the concentration of extracellular D-glucose from zero to 1·7 mM or more (up to 16·7 mM) causes a rapid and sustained decrease in ^{86}Rb fractional outflow rate (FOR) from prelabelled and perifused pancreatic rat islets. The ^{86}Rb FOR also decreases when the concentration of D-glucose is raised from 1·7 mM or more (up to 5·6 mM) to higher values not exceeding 8·3 mM.

2. However, when the glucose concentration is raised from 8·3 mM (or 11·1 mM) to higher values, no decrease in ^{86}Rb FOR is observed and, instead, a transient increase in ^{86}Rb FOR now takes place.

3. Such a dual effect on ^{86}Rb FOR is also observed when α-ketoisocaproic acid is used as the nutrient secretagogue or when the latter keto acid is used in combination with D-glucose.

4. The transient increase in ^{86}Rb FOR evoked by D-glucose in islets already exposed to α-ketoisocaproate is abolished by mannoheptulose, suggesting that it depends on the integrity of glucose metabolism.

5. The transient increase in ^{86}Rb FOR evoked, under suitable experimental conditions, by D-glucose of α-ketoisocaproate is abolished in the absence of extracellular Ca^{2+} and mimicked by theophylline and tolbutamide, suggesting that it is attributable to an increase in cytosolic Ca^{2+} concentration. The latter view is supported by the fact that the increase in ^{86}Rb FOR coincides with an increase in ^{45}Ca FOR, provided that Ca^{2+} is not removed from the extracellular medium.

6. It is concluded that, in contrast to the situation found when the concentration of the nutrient secretagogue is increased from a non-insulinotropic to a higher value, the stimulation of Ca^{2+} entry into islet cells and the subsequent increase in insulin secretion evoked by D-glucose or α-ketoisocaproate when the concentration of these nutrients is increased from intermediate (8·3–10·0 mM) to higher values is not attributable to a decrease in K^+ conductance.

INTRODUCTION

Nutrients which stimulate insulin release in the pancreatic β-cell (e.g. D-glucose, L-leucine, α-ketoisocaproate) decrease the fractional outflow rate (FOR) of ^{42}K (or ^{86}Rb) from pancreatic islets (Sehlin & Täljedal, 1974; Boschero, Kawazu, Duncan &

* This paper is the fifth in a series.

0022-3751/81/5160-1240 $07.50

Malaisse, 1977; Henquin, 1978). This decrease in K^+ conductance is thought to represent an essential link in the secretory sequence between the site of glucose identification and that of insulin release. Indeed, the decrease in K^+ conductance is held responsible, in part at least, for a slow phase of β-cell membrane depolarization eventually leading to the gating of voltage-dependent Ca^{2+} channels (Meissner & Preissler, 1979).

The dose–action relationship characterizing the steady-state values of K^+ conductance (as judged from the ^{86}Rb FOR) at increasing concentrations of D-glucose displays a much lower apparent 'K_m' than that characterizing the corresponding steady-state values of insulin secretion rate, the K^+ conductance being little affected once the concentration of D-glucose exceeds approximately 8·3 mM (Henquin, 1978). This suggests that, when insulin release is affected by a change in D-glucose concentration from 8·3 mM to higher values, the actual increase in insulin output either depends on a minor fluctuation in K^+ conductance or is attributable to some other factor, independently of any change in K^+ conductance. The present study was undertaken to decide between these two mechanisms.

METHODS

The methods used to measure the fractional outflow rate (FOR) of ^{86}Rb (Carpinelli & Malaisse, 1980*a*), ^{45}Ca (Herchuelz, Sener & Malaisse, 1980*b*) and ^{32}P (Carpinelli & Malaisse, 1980*b*) and the release of insulin (Herchuelz & Malaisse, 1978) from perifused islets are described in detail in prior publications. Briefly, in most experiments groups of 100 islets each were prelabelled with ^{86}Rb and placed in a perifusion chamber (flow rate: 1 ml./min), the outflow of ^{86}Rb being expressed as an instantaneous FOR. In some experiments groups of 200 islets each were prelabelled with both ^{86}Rb and ^{45}Ca so that the FOR of both cations could be simultaneously monitored. In one series of experiments groups of 500 islets each were either prelabelled with both ^{86}Rb and ^{45}Ca or with ^{32}P. In this series of experiments the effluent medium was collected over successive periods of 1 min each (minutes 31–39 inclusive), 12 sec each (minutes 40–45 inclusive) and 18 sec each (minutes 46–49 inclusive). The concentration of D-glucose was also measured in the effluent, as described elsewhere (Malaisse, Carpinelli & Sener, 1981).

All results are expressed as the mean (±S.E. of mean) together with the number of individual observations (n). In all Figures, the vertical dashed line corresponds to the time at which the perifusate was derived from a different reservoir, no correction being made for the dead space of the perifusion system. Hence, the new medium reached the collecting vial about 60–90 sec later than the time shown by the vertical dotted line (see Fig. 12).

RESULTS

Dose–action relationship for the response to D-*glucose*

The mean value for ^{86}Rb FOR reached in each individual experiment after 17–21 min exposure to various concentrations of D-glucose was used to characterize the steady-state effect of the sugar upon ^{86}Rb FOR. As illustrated in Fig. 1, D-glucose decreased ^{86}Rb FOR in a dose-dependent and sigmoidal fashion, with the steepest part of the curve corresponding to the 2·8–4·4 mM range of D-glucose concentrations. The steady-state value of ^{86}Rb FOR was little affected once the concentration of D-glucose exceeded 8·3 mM.

The pattern illustrated in Fig. 1 was in good agreement with dynamic data obtained when D-glucose, in concentrations ranging from 1·7 to 16·7 mM, was administered to islets previously deprived of glucose (Fig. 2). In these experiments the ^{86}Rb FOR

before introduction of glucose (minutes 17–21) averaged 5·37 ± 0·22 per cent/min (n = 16). The magnitude of the paired decrease in ^{86}Rb FOR, taken as the difference between the mean values recorded from minutes 17–21 and 33–37, respectively, was positively correlated with the concentration of D-glucose used to stimulate the islets from the minute 22 onwards (r = 0·775, n = 16, $P < 0·001$). However, relative

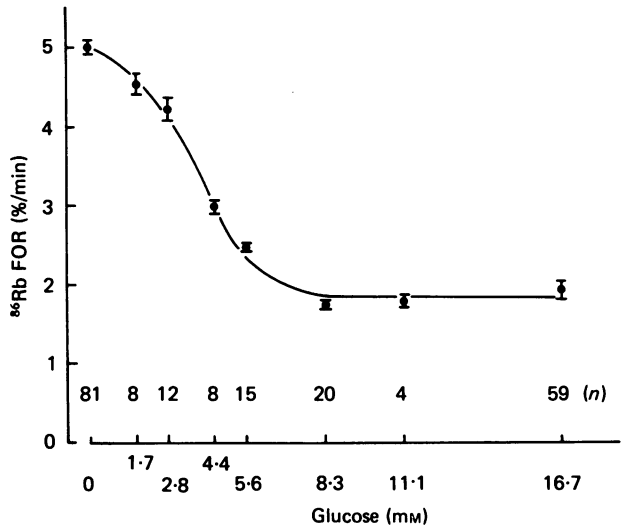

Fig. 1. Dose–action relationship for the steady-state values of ^{86}Rb FOR (minutes 17–21) as a function of D-glucose concentration. Mean values (±S.E. of mean) are shown, together with the number of individual determinations (n) at each concentration of D-glucose.

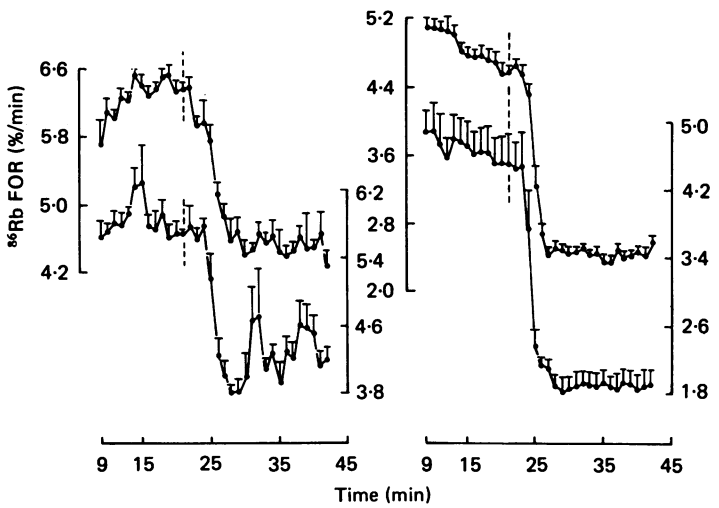

Fig. 2. Effect of an increase in D-glucose concentration from zero to 1·7 mM (upper left curve), 2·8 mM (lower left curve), 4·4 mM (upper right curve) or 16·7 mM (lower right curve) upon ^{86}Rb FOR. Mean values (± S.E. of mean) refer to four experiments in each case. The vertical dashed lines correspond to the time at which the new medium reached the perifusion chamber. The ordinate axis is drawn on the left for the upper curves and the right for the lower curves.

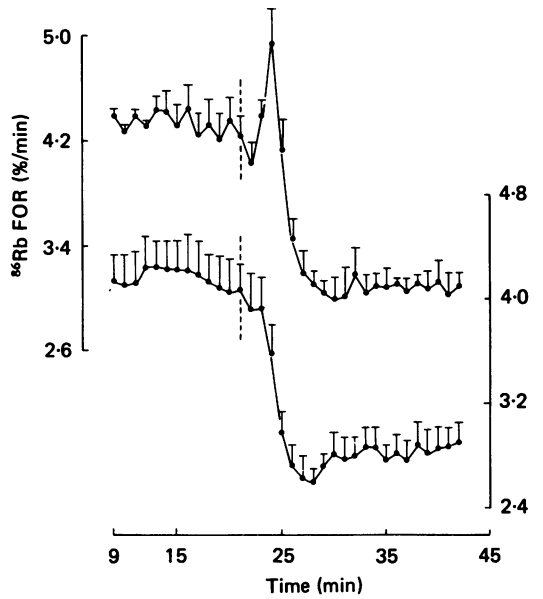

Fig. 3. Effect of an increase in D-glucose concentration from zero to 1·7 mM (upper curve) or 2·8 mM (lower curve) upon ^{86}Rb FOR from islets exposed throughout perifusion to L-glutamine (10·0 mM). Same presentation as in Fig. 2.

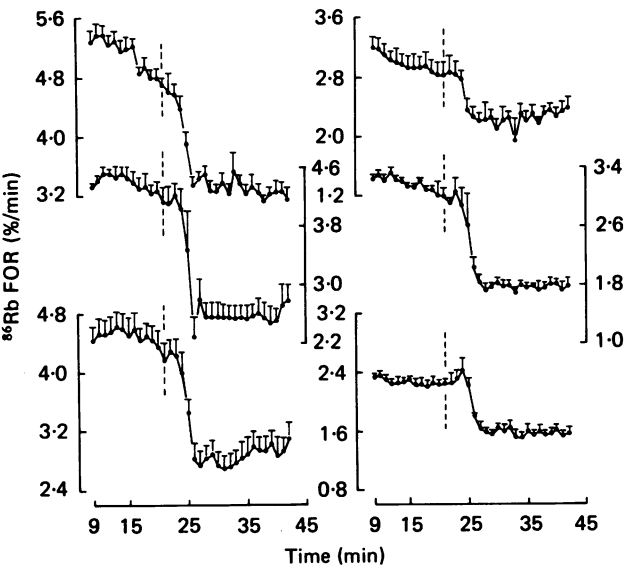

Fig. 4. Effect of an increase in D-glucose concentration from 1·7 to 2·8 mM (upper left curve), 1·7 to 4·4 mM (middle left curve), 2·8 to 4·4 mM (lower left curve), 4·4 to 5·6 mM (upper right curve), 4·4 to 8·3 mM (middle right curve) and 5·6 to 8·3 mM (lower right curve) upon ^{86}Rb FOR. Same presentation as in Fig. 2.

to the increment in sugar concentration, the inhibition of ^{86}Rb FOR was most marked in the low range of D-glucose concentrations. Thus, for each millimolar increase in glucose concentration, the fall in ^{86}Rb FOR averaged $0{\cdot}74 \pm 0{\cdot}10$ %/min per mM ($n = 12$) when the concentration of D-glucose was increased from zero to either 1·7, 2·8 or 4·4 mM, as distinct ($P < 0{\cdot}005$) from only $0{\cdot}16 \pm 0{\cdot}01$ %/min per mM ($n = 4$) when the concentration of D-glucose was raised from zero to 16·7 mM.

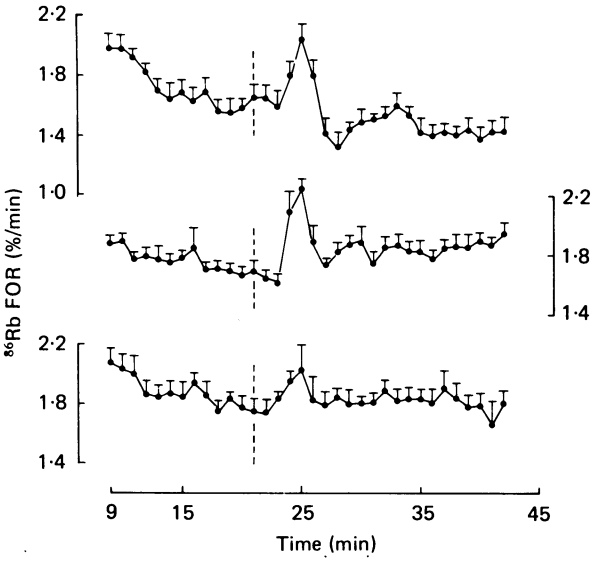

Fig. 5. Effect of an increase in D-glucose concentration from 8·3 to 11·1 mM (upper curve), 8·3 to 16·7 mM (middle curve) and 11·1 to 16·7 mM (lower curve) upon ^{86}Rb FOR. Same presentation as in Fig. 2.

As illustrated in Fig. 3, the inhibitory effect of D-glucose in low concentrations (1·7–2·8 mM) upon ^{86}Rb FOR was not abolished when the perifusate circulated throughout the experiment contained L-glutamine (10 mM), a nutrient readily utilized by the islets to cover part of their basal energy expenditure (Malaisse, Sener, Carpinelli, Anjaneyulu, Lebrun, Herchuelz & Christophe, 1980). In these experiments the initial ^{86}Rb FOR (minutes 17–21) averaged $4{\cdot}20 \pm 0{\cdot}14$ %/min and the glucose-induced decrease in ^{86}Rb FOR (minutes 17–21 *versus* minutes 33–37) amounted to $0{\cdot}54 \pm 0{\cdot}09$ %/min per mM ($n = 8$ in both cases). Thus, both the initial value for ^{86}Rb FOR and the sugar-induced decrease in ^{86}Rb FOR were lower in the presence than absence of L-glutamine.

The dose-related effect of D-glucose upon ^{86}Rb FOR was further investigated by provoking less ample variations in the sugar concentration (Figs. 4 and 5). As judged from the paired difference in mean values recorded, in each experiment, from minutes 17–21 and 33–37, respectively, the inhibitory effect of D-glucose was again less marked at high than at low concentrations of the sugar. Indeed, for each millimolar increase in glucose concentrations, the paired difference averaged $0{\cdot}91 \pm 0{\cdot}07$ ($n = 20$), $0{\cdot}40 \pm 0{\cdot}08$ ($n = 8$) and $-0{\cdot}01 \pm 0{\cdot}01$ ($n = 12$) %/min per mM at glucose concentrations ranging from zero to 4·4 mM, 4·4 to 8·3 mM, and 8·3 to 16·7 mM, respectively. In this series of experiments, however, a qualitative change in the response to glucose also

became evident. As long as the glucose concentration was increased to values inferior or equal to 8·3 mM, a rapid and sustained decrease in ^{86}Rb FOR was observed (Fig. 4). When the glucose concentration was raised from 8·3 (or 11·1 mM) to higher values, no significant fall in ^{86}Rb FOR could be detected any more and, instead, a transient increase in ^{86}Rb FOR was now always observed (Fig. 5). The peak value in ^{86}Rb FOR was always reached at minute 25 of perfusion, i.e. 4 min after the new medium was introduced in the perifusion chamber.

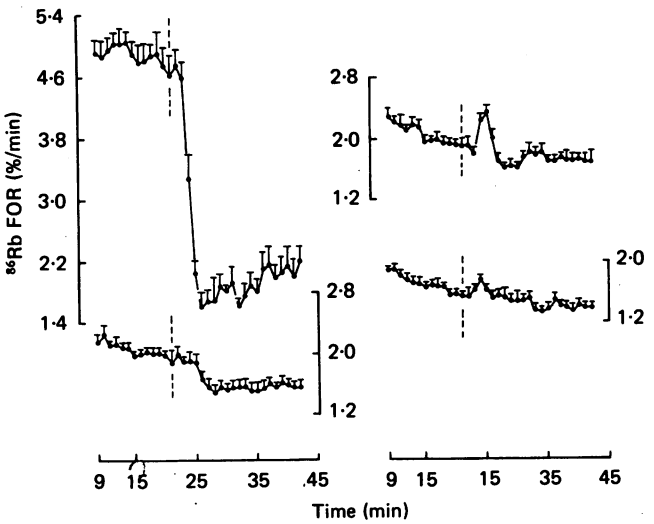

Fig. 6. Effect of an increase in α-ketoisocaproate concentration from zero to 5 mM (upper left curve), 5 to 10 mM (lower left curve) and 10 to 20 mM (right curves). The perifusate contained no glucose, and, in one case (lower right curve), was deprived of extracellular Ca^{2+} and enriched with EGTA (0·5 mM). Same presentation as in Fig. 2.

Dose–action relationship for the response to other nutrients

The dual effect of D-glucose was not an exceptional feature. Indeed, when α-ketoisocaproate was used instead of D-glucose as the nutrient secretagogue, step-wise increases in the concentration of α-ketoisocaproate up to 10·0 mM inhibited ^{86}Rb FOR, whereas a transient increase in ^{86}Rb FOR (peaking at the minute 25) was observed when the concentration of α-ketoisocaproate was increased from 10·0 to 20·0 mM (Fig. 6).

Likewise, L-glutamine (10·0 mM) inhibited ^{86}Rb FOR in the absence of glucose, but caused a transient increase in ^{86}Rb FOR when the islets were already exposed to D-glucose at 5·6 or 8·3 mM. In the latter case, this transient increase was followed by a modest secondary rise in ^{86}Rb FOR (Fig. 7).

A transient increase in ^{86}Rb FOR was also observed when α-ketoisocaproate at 10·0 mM was administered to islets already exposed to D-glucose at 8·3 mM or, inversely, when D-glucose at 8·3 mM was administered to islets already exposed to α-ketoisocaproate at 10·0 mM (Fig. 8). The latter situation provided the opportunity for investigating whether the transient increase in ^{86}Rb FOR evoked by D-glucose depends on the integrity of its metabolism, as is claimed to be the case for the inhibitory action of D-glucose upon ^{86}Rb FOR (Boschero & Malaisse, 1979). For this

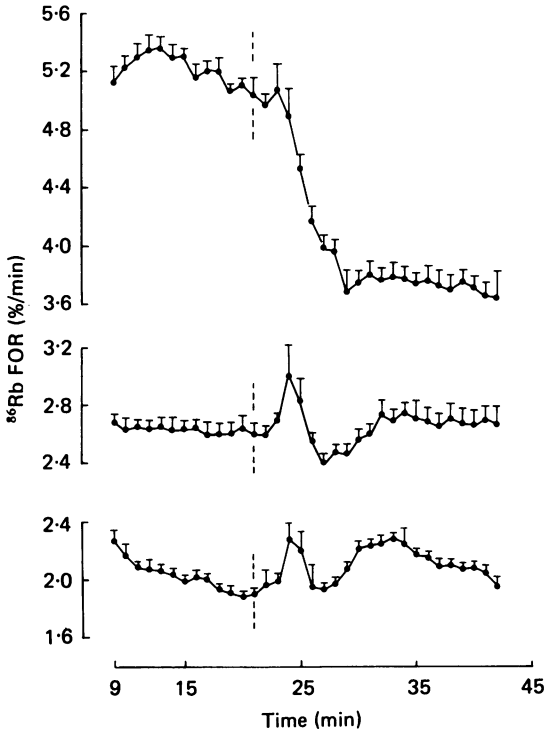

Fig. 7. Effect of an increase in L-glutamine concentration from zero to 10 mM upon ^{86}Rb FOR from islets deprived of glucose (upper curve; $n = 4$) or exposed throughout perifusion to D-glucose at 5·6 mM (middle curve; $n = 8$) or 8·3 mM (lower curve; $n = 4$). Same presentation as in Fig. 2.

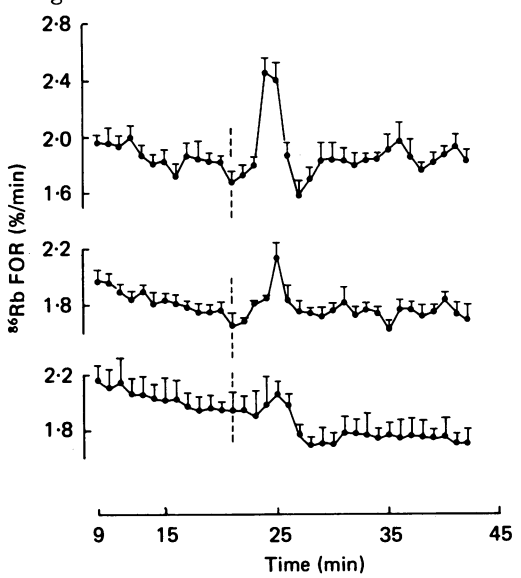

Fig. 8. Effect of an increase in α-ketoisocaproate concentration from zero to 10 mM upon ^{86}Rb FOR from islets exposed throughout perifusion to D-glucose at 8·3 mM (upper curve). Effect of an increase in D-glucose concentration from zero to 8·3 mM upon ^{86}Rb FOR from islets exposed throughout perifusion to α-ketoisocaproate at 10 mM (middle and lower curves). In the lower curve D-mannoheptulose (14 mM) was introduced together with D-glucose. Same presentation as in Fig. 2.

purpose, islets stimulated with α-ketoisocaproate (10·0 mM) throughout the perifusion period were suddenly exposed to the combination of D-glucose (8·3 mM) and D-mannoheptulose (14·0 mM), the latter heptose being known to inhibit insulin release induced by D-glucose but not induced by α-ketoisocaproate (Malaisse, Lea & Malaisse-Lagae, 1968; Hutton, Sener, Herchuelz, Atwater, Kawazu, Boschero, Somers, Devis & Malaisse, 1980). Under these conditions the glucose-induced transient increase in ^{86}Rb FOR was much less marked than in the absence of mannoheptulose (Fig. 8). Thus, as judged from the paired difference in ^{86}Rb FOR (minute 25 minus minute 21), the glucose-induced increase in ^{86}Rb FOR averaged 0·48 ± 0·10 and 0·11 ± 0·14 %/min in the absence and presence of D-mannoheptulose, respectively, the latter value failing to achieve statistical significance (Table 1).

TABLE 1. Conditions in which a transient increase in ^{86}Rb FOR was observed

Minute 0 to 21	Minute 22 onwards	Minute 25 minus 21 minute
D-glucose (8·3)	D-glucose (11·1)	0·37 ± 0·04* (4)
D-glucose (8·3)	D-glucose (16·6)	0·56 ± 0·05* (4)
D-glucose (11·1)	D-glucose (16·7)	0·30 ± 0·07** (4)
α-KIC (10·0)	α-KIC (20·0)	0·44 ± 0·14* (4)
α-KIC (10·0)†	α-KIC (10·0)†	0·16 ± 0·07 (4)
D-glucose (8·3)	D-glucose (8·3) + α-KIC (10·0)	0·73 ± 0·16** (4)
α-KIC (10·0)	α-KIC (10·0) + D-glucose (8·3)	0·48 ± 0·10** (4)
α-KIC (10·0)	α-KIC (10·0) + D-glucose (8·3) + MH (14·0)	0·11 ± 0·14 (4)
D-glucose (8·3)	D-glucose (8·3) + theophylline (1·4)	0·53 ± 0·06* (4)
Minute 0 to 21	**Minute 22 onwards**	**Minute 24 minus 21 minute**
D-glucose (5·6)	D-glucose (5·6) + L-glutamine (10·0)	0·50 ± 0·22 (8)
D-glucose (8·3)	D-glucose (8·3) + L-glutamine (10·0)	0·38 ± 0·12*** (4)
Minute 0 to 44	**Minute 45 onwards**	**Minute 48 minus 44 minute**
D-glucose (8·3)	D-glucose (16·7)	0·44 ± 0·05* (4)
D-glucose (8·3)†	D-glucose (16·7)†	−0·10 ± 0·03 (4)
D-glucose (8·3)	D-glucose (8·3) + tolbutamide (0·4)	1·51 ± 0·26* (6)

The nutrients (α-KIC: α-ketoisocaproate; MH: D-mannoheptulose) and drugs concentrations expressed in mM) administered during the initial (minute 0 to minute 21 or 44) and late (minute 22 or 45 onwards) periods of perifusion are shown together with the paired difference in ^{86}Rb FOR (last column) just before 3–4 min after introducing the new medium into the perifusion chamber. Mean values (± S.E.M.) for such a paired difference are shown together with the number of individual observations (in parentheses) and the statistical significance of the observed change in ^{86}RB FOR (*, $P < 0·005$; **, $P < 0·025$; ***, $P < 0·05$). †, Perifusion media containing no Ca^{2+} and enriched with EGTA (0·5 mM).

Participation of Ca^{2+} in the nutrient-induced transient increase in ^{86}Rb FOR

In the last part of this study we investigated whether the transient increase in ^{86}Rb FOR evoked by D-glucose or α-ketoisocaproate may be somehow related to changes in the handling of Ca^{2+} by the islet cells. For this purpose we first examined the influence of extracellular Ca^{2+} upon the response to D-glucose or α-ketoisocaproate.

In the control experiments performed at normal extracellular Ca^{2+} concentration, the transient increase in ^{86}Rb FOR provoked by a rise in D-glucose concentration from 8·3 to 16·7 mM was associated with a biphasic increase in both ^{45}Ca FOR and insuiin

release (Fig. 9). When the glucose concentration was brought back to 8·3 mM, the rate of insulin release decreased towards its initial value. The ^{86}Rb FOR was transiently decreased when the glucose concentration was returned to its initial value.

In the absence of extracellular Ca^{2+} and presence of the Ca-chelator EGTA (0·5 mM), the transient increase in ^{86}Rb FOR was virtually abolished, together with a suppression of the glucose-induced rise in ^{45}Ca FOR (Fig. 10). Likewise, when the concentration of α-ketoisocaproate was raised from 10·0 to 20·0 mM, and when the perifusate administered throughout the experiment was deprived of Ca^{2+} and enriched with EGTA (0·5 mM), the increase in ^{86}Rb FOR (minute 25 minus minute

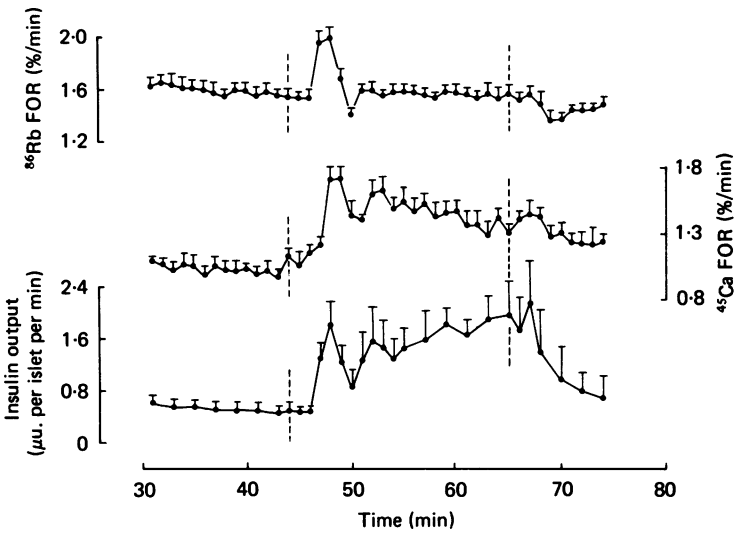

Fig. 9. Effect of an increase in D-glucose concentration from 8·3 to 16·7 mM (minute 44) and the reverse change (minute 65) upon ^{86}Rb FOR (upper curve; $n = 6$), ^{45}Ca FOR (middle curve; $n = 6$) and insulin output (lower curve; $n = 2$). Same presentation as in Fig. 2.

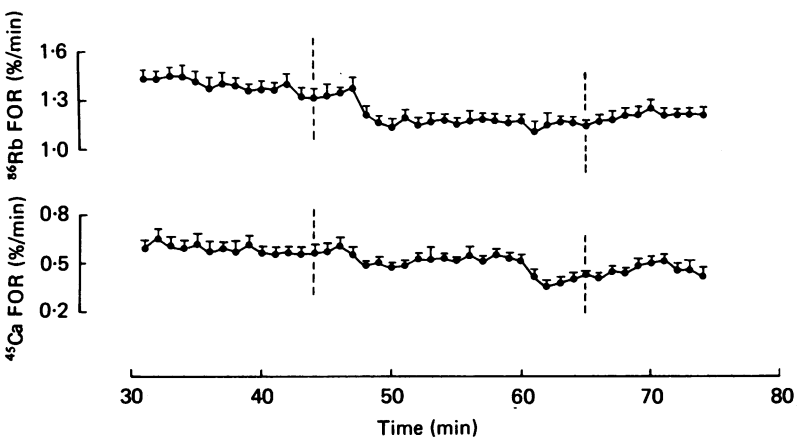

Fig. 10. Effect of an increase in D-glucose concentration from 8·3 to 16·7 mM (minute 44) and the reverse change (minute 65) upon ^{86}Rb FOR (upper curve; $n = 4$) and ^{45}Ca FOR (lower curve; $n = 4$) in islets exposed throughout perifusion to media containing EGTA (0·5 mM) but no Ca^{2+}. Same presentation as in Fig. 2.

21) failed to achieve statistical significance, amounting to no more than $0{\cdot}16 \pm 0{\cdot}17$ %/min compared with a control value of $0{\cdot}44 \pm 0{\cdot}13$ %/min ($P < 0{\cdot}05$) at normal extracellular Ca^{2+} concentration (Fig. 6, Table 1).

The idea that Ca^{2+} participates in the nutrient-induced increase in ^{86}Rb FOR led us to explore the effect of theophylline and tolbutamide upon ^{86}Rb FOR in islets exposed to glucose at 8·3 mM throughout the perifusion period. These drugs are thought to favour Ca^{2+} accumulation in the cytosol of the β-cell, theophylline by causing an intracellular redistribution of Ca^{2+} (Brisson, Malaisse-Lagae & Malaisse, 1972) and tolbutamide by facilitating Ca^{2+} entry into the islet cells (Kawazu, Sener, Couturier & Malaisse, 1980). Both theophylline and tolbutamide mimicked the effects of an increase in D-glucose concentration from 8·3 mM to higher values, in that they both caused a transient increase in ^{86}Rb FOR (Fig. 11).

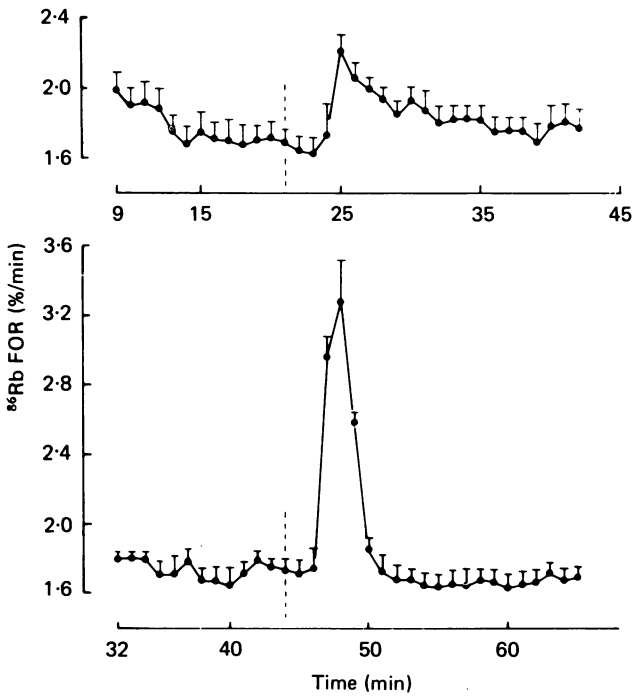

Fig. 11. Effect of theophylline (1·4 mM; upper curve) and tolbutamide (0·4 mM; lower curve), administered from the time shown by the vertical dashed line, upon ^{86}Rb FOR from islets exposed throughout perifusion to D-glucose at 8·3 mM. Same presentation as in Fig. 2.

In order to document further the existence of a cause-to-effect link between Ca^{2+} accumulation in the islet cells and the transient increase in ^{86}Rb FOR, the experiments illustrated in Fig. 9 were repeated, but the effluent was now collected over successive periods of 12 sec each (instead of 60 sec) so that the time course for the glucose-induced increase in ^{86}Rb and ^{45}Ca FOR could be established with greater accuracy. In these experiments we also measured the glucose concentration of the effluent and the ^{32}P FOR from the islets. The time courses for the glucose-induced increases in ^{86}Rb and ^{45}Ca FOR, respectively, were virtually superimposable (Fig.

12). Indeed, examination of individual data indicated that the first sample in which the ^{86}Rb FOR and ^{45}Ca FOR, respectively, exceeded by more than 2 S.D. the mean value recorded for the last twelve samples in which the concentration of glucose did not significantly differ from its initial value, was collected 123 ± 10 sec and 136 ± 14 sec ($n = 4$ in both cases) after switching from one perifusion medium (D-glucose at

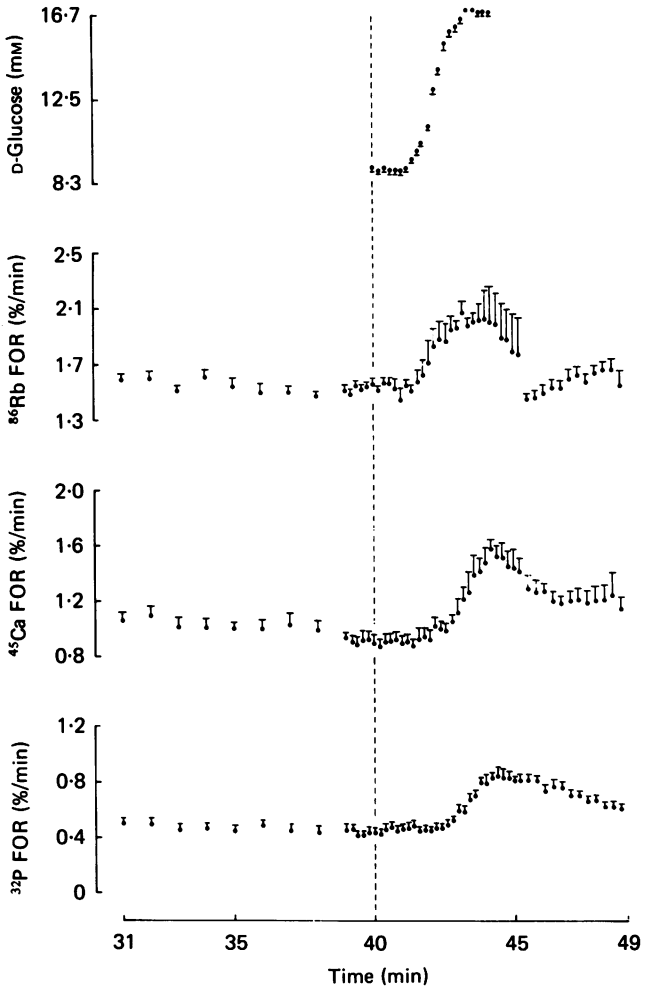

Fig. 12. Effect of an increase in D-glucose concentration from 8·3 to 16·7 mM upon the concentration of D-glucose in the effluent, ^{86}Rb FOR, ^{45}Ca FOR and ^{32}P FOR. Same presentation as in Fig. 2.

8·3 mM) to another (D-glucose at 16·7 mM). The latter times are not significantly different from one another. Likewise, the curves for ^{86}Rb and ^{45}Ca FOR reached their maximal value at the same time, i.e. about 4 min after the switch in perifusion medium. The glucose-induced increase in ^{32}P FOR, however, was initiated somewhat later than the increase in ^{86}Rb FOR. Thus, the first sample in which the ^{32}P FOR exceeded by more than 2 S.D. the mean value recorded over the twelve same

successive samples was collected 162 ± 14 sec ($n = 4$) after the switch in perifusion medium, i.e. about 40 sec later ($P < 0.07$) than the time at which an increase in ^{86}Rb FOR had been first observed.

DISCUSSION

The present work confirms that D-glucose and α-ketoisocaproate cause a dose-related and sustained decrease in K^+ conductance in pancreatic islet cells as judged from the FOR of ^{86}Rb (Boschero *et al.* 1977; Henquin, 1978; Hutton *et al.* 1980). In addition our work reveals that these nutrients provoke a transient increase in ^{86}Rb FOR, when their concentration is increased from intermediate (e.g. 8·3 mM-D-glucose or 10·0 mM-α-ketoisocaproate) to higher values.

A decrease in K^+ conductance was observed at concentrations of D-glucose which are well below those required to stimulate insulin release (Fig. 2 and 4). This is not meant to deny that such a decrease represents an essential step in the stimulus–secretion coupling of nutrient-induced insulin release. Incidentally, the experiments performed in islets first exposed to D-glucose in the 2·8–4·4 mM range clearly indicate that the inhibitory effect of the sugar upon ^{86}Rb FOR is not attributable to a prior depletion of the ATP content of the islet cells. Indeed, in this range of D-glucose concentrations the ATP content of the islets is not different from that found at higher concentrations of the sugar (Malaisse, Hutton, Kawazu, Herchuelz, Valverde & Sener, 1979). The mechanism by which nutrients cause a decrease in K^+ conductance is considered in detail elsewhere, emphasis being currently given to the possible role of NAD(P)H in such a process (Boschero & Malaisse, 1979; Henquin, 1980). The finding that L-glutamine, like D-glucose, decreases ^{86}Rb FOR is compatible with such a view, since this amino acid may indeed invoke a more reduced state in cytosolic redox couples (Malaisse *et al.* 1980).

The transient increase in K^+ conductance evoked by D-glucose or α-ketoisocaproate when the islets were already exposed to a concentration of these nutrients sufficient to stimulate insulin release is a new finding. This transient increase is apparently dependent on the integrity of nutrient metabolism. Indeed, in islets already exposed to α-ketoisocaproate, the administration of D-glucose caused a lesser increase in ^{86}Rb FOR in the presence than absence of mannoheptulose (Fig. 8). The finding that L-glutamine, in the presence of D-glucose at 5·6 or 8·3 mM, transiently increased ^{86}Rb FOR also supports the metabolic hypothesis. Indeed, L-glutamine is readily metabolized in islet cells (Malaisse *et al.* 1980). It could be argued, however, that L-glutamine diminishes D-glucose oxidation (Malaisse *et al.* 1980), so that the amino acid could increase ^{86}Rb FOR by antagonizing the inhibitory effect of D-glucose upon K^+ conductance.

Three series of observations suggest that the transient increase in K^+ conductance may be linked to an increase in the cytosolic concentration of Ca^{2+} with subsequent activation of a Ca-dependent modality of K^+ extrusion. First, the nutrient-induced transient increase in ^{86}Rb FOR was abolished in the absence of extracellular Ca^{2+}, in which case the nutrient-induced stimulation of a process of ^{40}Ca–^{45}Ca exchange (Herchuelz, Couturier & Malaisse, 1980*a*) was also suppressed (Fig. 10).

Secondly, both theophylline and tolbutamide mimicked the effect of D-glucose (11·1 or 16·7 mM) to augment ^{86}Rb FOR in islets already exposed to D-glucose at 8·3 mM.

Under the present experimental conditions theophylline and tolbutamide are both thought to increase the cytosolic concentration of Ca^{2+} in islet cells, theophylline by causing an intracellular redistribution of Ca^{2+} (Brisson *et al.* 1972) and tolbutamide by facilitating Ca^{2+} entry in the islet cells (Kawazu *et al.* 1980). The transient increase in ^{86}Rb FOR evoked by tolbutamide, like that evoked by D-glucose, is suppressed in the absence of extracellular Ca^{2+} (data not shown).

Thirdly, the time course for the glucose-induced transient increase in ^{86}Rb FOR virtually coincided with that of the initial increase in ^{45}Ca FOR. As the latter increase is secondary to facilitated ^{40}Ca inflow (Herchuelz *et al.* 1980*a*), it may well occur somewhat later than the increase in ^{40}Ca influx (Malaisse *et al.* 1981). The chronology of cationic events thus appears adequate to postulate a cause-to-effect link between facilitated ^{40}Ca inflow and activation of the Ca-dependent modality of K (or Rb) extrusion from the islet cells. Incidentally, our data suggest that the glucose-induced release of inorganic phosphate occurs too late to play a causative role in the transient augmentation of ^{86}Rb FOR (Fig. 12). Theoretically, the release of inorganic phosphate could cause cell depolarization and, by doing so, increase ^{86}Rb FOR (Boschero & Malaisse, 1979).

Our data clearly indicate that the stimulation of both ^{40}Ca inflow and insulin release due to an increase in D-glucose concentration from 8·3 to 16·7 mM is not attributable to any decrease in K^+ conductance. This is in good agreement with bioelectrical data indicating that, in this range of concentration, D-glucose does not affect the threshold potential, the plateau potential and the maximum repolarization potential in β-cells but, instead, increases the duration of the phases of activity, the burst pattern being eventually replaced by a continuous spike activity (Meissner & Preissler, 1979). The latter phenomenon is currently attributed to the fact that D-glucose in high concentrations somehow prevents the activation of either an electrogenic Na–K pump (Meissner & Preissler, 1980) or the Ca-dependent K^+ permeability (Atwater, Dawson, Ribalet & Rojas, 1979). At the first glance, the latter hypothesis would appear incompatible with the present observation that an increase in D-glucose concentration from 8·3 to 16·7 mM causes activation of the Ca-dependent K^+ channel. Therefore, the present findings call for further studies on the mechanism by which D-glucose in concentrations exceeding 8·3 mM affects Ca handling, bioelectrical activity and insulin release in the pancreatic β-cell.

This work was supported in part by grants from the Belgian Foundation for Scientific Medical Research. A.R.C. is a Research Fellow of the São Paulo State Research Foundation.

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