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Stimulation of Proinsulin Biosynthesis by K^+ in Rat Pancreatic Islets

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Glucose-stimulated proinsulin biosynthesis is regulated mainly at the translational level. This study aims at investigating the possible role of the B-cell K^+ content in such a process. In order to increase the islet cells K^+ content, rat pancreatic islets exposed to a low D-glucose concentration (e.g., 2.5 m*M*) were incubated in the presence of 30 or 60 m*M* K^+, as distinct from a control extracellular K^+ concentration of 5 m*M*. Under these conditions, the K^+ content of the islets, as judged from the net uptake of $^{86}Rb^+$ over 60 min incubation, was increased to a level comparable to that otherwise found in the presence of 16.7 m*M* D-glucose. In the presence of 2.5–4.0 m*M* D-glucose, the rise in K^+ concentration from 5 to 30 and 60 m*M* caused a progressive increase in the incorporation of L-[4-^{3}H]phenylalanine into both all islet peptides and (pro)insulin. A preferential stimulation of proinsulin biosynthesis was only observed in islets incubated at 60 m*M* K^+ in the presence of 4.0 m*M* D-glucose. In relative terms, the K^+-induced increase in biosynthetic variables was less pronounced, however, than that otherwise evoked by a rise in D-glucose concentration from 2.5 to 4.0 m*M* to 5.6 or 16.7 m*M*. These findings may suggest that the effect of D-glucose to increase the K^+ content of islet cells represents one modality for coupling a rise in D-glucose concentration to stimulation of proinsulin biosynthesis.

Key Words: Rat pancreatic islets; proinsulin biosynthesis; potassium.

Introduction

Glucose-stimulated proinsulin biosynthesis is mainly regulated at the translational level *(1)*. A modest increase in preproinsulin gene transcription is only observed after long-term (>6 h) exposure of the B-cell to high glucose concentrations *(1)*. The upregulation of proinsulin biosynthesis at the translational level is very rapid, starting within about 20 min and reaching a maximal value by 60 min exposure of isolated pancreatic islets to high concentrations of D-glucose, without any change in preproinsulin mRNA levels *(2)*. The specific control mechanism of preproinsulin mRNA translation has remained largely elusive *(2)*. The major aim of the present study was to investigate the possible role of the B-cell K^+ content in such a process. The selection of this topic was motivated mainly by the following two considerations. First, the biosynthesis of proinsulin is severely impaired in K^+-deprived islets, in which the intracellular K^+ content is also markedly decreased *(3,4)*. Second, at normal extracellular K^+ concentration, D-glucose causes a time and concentration-related increase in islet K^+ content *(5,6)*.

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Results

$^{86}Rb^+$ Net Uptake

After 60 min incubation in the presence of 2.8 m*M* D-glucose and 5.0 m*M* K^+ , the net uptake of $^{86}Rb^+$ (8.9 μ*M*) averaged 513.5 ± 33.5 fmol/islet (n = 16). Assuming competition between $^{86}Rb^+$ and $^{39}K^+$, this would correspond to a K^+ content of 287 ± 19 pmol/islet. At the same K^+ concentration (5.0 m*M*), a rise in D-glucose concentration from 2.8 to 16.7 m*M* increased ($p < 0.001$) the net uptake of $^{86}Rb^+$ to 167.3 ± 15.6% (n = 16) of the mean value found within the same experiment(s) at the low hexose concentration (100.0 ± 6.1%; n = 16). As shown in Table 1 (Exp. 1), at 2.8 m*M* D-glucose but in the presence of only 1.0 m*M* K^+, the net uptake of $^{86}Rb^+$ was about three times higher ($p < 0.001$) than that measured within the same experiments in islets exposed to 5.0 m*M* K^+. When corrected for the K^+ concentration, however, the content of the islets incubated at 2.8 m*M* D-glucose and 1.0 m*M* K^+ would only represent 61.4 ± 7.4% (n = 15; $p < 0.001$) of that recorded, within the same experiments and at the same concentration of D-glucose, in the presence of 5.0 m*M* K^+. In the nominal absence of extracellular K^+ and in the presence of 2.8 m*M* D-glucose, the net uptake of $^{86}Rb^+$ averaged 169.8 ± 16.8% (n = 14;

Table 1
$^{86}Rb^+$ Net Uptake by Rat Pancreatic Islets

Exp. Nr.	D-glucose (m*M*)	K^+ (m*M*)	$^{86}Rb^+$ net uptake[a] (relative values)	*p* values (vs first line)
1.	2.8	5.0	100.0 ± 6.1 (16)	
	16.7	5.0	167.3 ± 15.6 (16)	<0.001
	2.8	1.0	307.0 ± 36.8 (16)	<0.001
	2.8	Nil	169.8 ± 16.8 (14)	<0.001
2.	2.5	5.0	100.0 ± 18.1 (18)	
	16.7	5.0	214.7 ± 33.6 (22)	<0.01
	2.5	30.0	39.2 ± 5.0 (23)	<0.001
	2.5	60.0	20.6 ± 3.1 (24)	<0.001

[a]Mean values (± SEM) are expressed relative to those found within the same experiment(s) in islets incubated for 60 min in the presence of 2.5 or 2.8 m*M* D-glucose and 5.0 m*M* K^+ (first line in each panel).

$p < 0.001$) of that found at the same hexose concentration in the presence of 5.0 m*M* K^+. Assuming that, under this experimental condition, the extracellular K^+ concentration would not exceed 0.1 m*M*, the islet K^+ content would only represent 3.4 ± 0.3% of that found in islets incubated for 60 min at 2.8 m*M* D-glucose in the presence of 5.0 m*M* K^+. The theoretical value of 0.1 m*M* was selected for this calculation because it slightly exceeds the value expected if all intracellular K^+ from 10 islets would be released in the incubation medium (50 μL), i.e., about 0.06 m*M*.

In a second series of experiments conducted in islets submitted to two washes after the 60 min incubation period, a rise in D-glucose concentration from 2.5 to 16.7 m*M* again increased ($p < 0.01$) the net uptake of $^{86}Rb^+$ to 214.7 ± 33.6% ($n = 22$) of that found, within the same experiment(s) and at the same K^+ concentration (5.0 m*M*), at the low hexose concentration (100.0 ± 18.1%; $n = 18$). The former percentage was not significantly different ($p > 0.25$) from that recorded in the first series of experiments (167.3 ± 15.6%), with an overall mean value of 194.8 ± 20.7% ($n = 38$). As shown in Table 1 (Exp. 2), in the presence of 2.5 m*M* D-glucose, the net uptake of $^{86}Rb^+$ decreased ($p < 0.001$), relative to the value found in islets exposed to 5.0 m*M* K^+, to 39.2 ± 5.0% ($n = 23$) in the presence of 30 m*M* K^+ and was further lowered ($p < 0.005$) to 20.6 ± 3.1% ($n = 24$) in the presence of 60 m*M* K^+. When corrected for the K^+ concentration, however, the net uptake of $^{86}Rb^+$, relative to that found in the presence of 5.0 m*M* K^+, was increased ($p < 0.001$) to 235.1 ± 29.8% ($n = 23$) at 30 m*M* K^+ and 246.7 ± 36.7% ($n = 24$) at 60 m*M* K^+. The latter two values were not significantly different from one another ($p > 0.8$).

Figure 1 illustrates the effect of increasing extracellular K^+ concentrations (0 to 60.0 m*M*) upon $^{86}Rb^+$ net uptake in these two series of experiments.

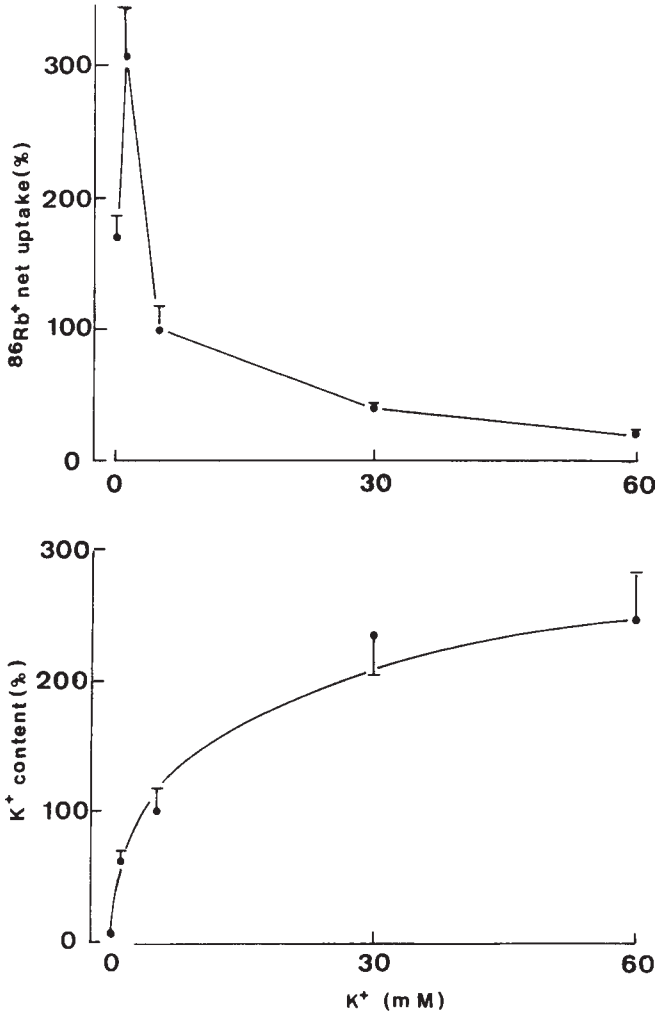

Fig. 1. Effect of increasing concentrations of extracellular K^+ upon $^{86}Rb^+$ net uptake by rat islets incubated in the presence of 2.5–2.8 m*M* D-glucose. Mean values (± SEM) refer to 14–24 individual determinations (Table 1) and are expressed relative to the mean value found within the same experiment(s) or 5.0 m*M* K^+. In the lower panel, the net uptake of $^{86}Rb^+$ was multiplied by the extracellular K^+ concentration in order to estimate the K^+ content of the islets.

Because the fractional outflow rate of $^{86}Rb^+$ from rat pancreatic islets is lower than that of $^{42}K^+$ *(6)*, a last series of experiments performed over 60 min incubation at 37°C was conducted in the presence of a final concentration of Rb^+ (10 μ*M* of $^{86}Rb^+$ and either 0, 50, or 110 μ*M* of $^{85}Rb^+$) always 500 times lower than that of $^{39}K^+$ (5.0, 30.0, or 60.0 m*M*). Under these experimental conditions, a rise in D-glucose concentration from 2.5 to 16.7 m*M* again significantly augmented ($p < 0.005$) $^{86}Rb^+$ net uptake by islets incubated in the presence of 5.0 m*M* K^+. Likewise, in the presence of 2.5 m*M* D-glucose, a rise in K^+ concentration from 5.0 m*M* to 30.0 and 60.0 m*M* progressively augmented ($p < 0.005$ or less) the K^+ content of the islets (as calculated from the radioactive content of the incubation medium and

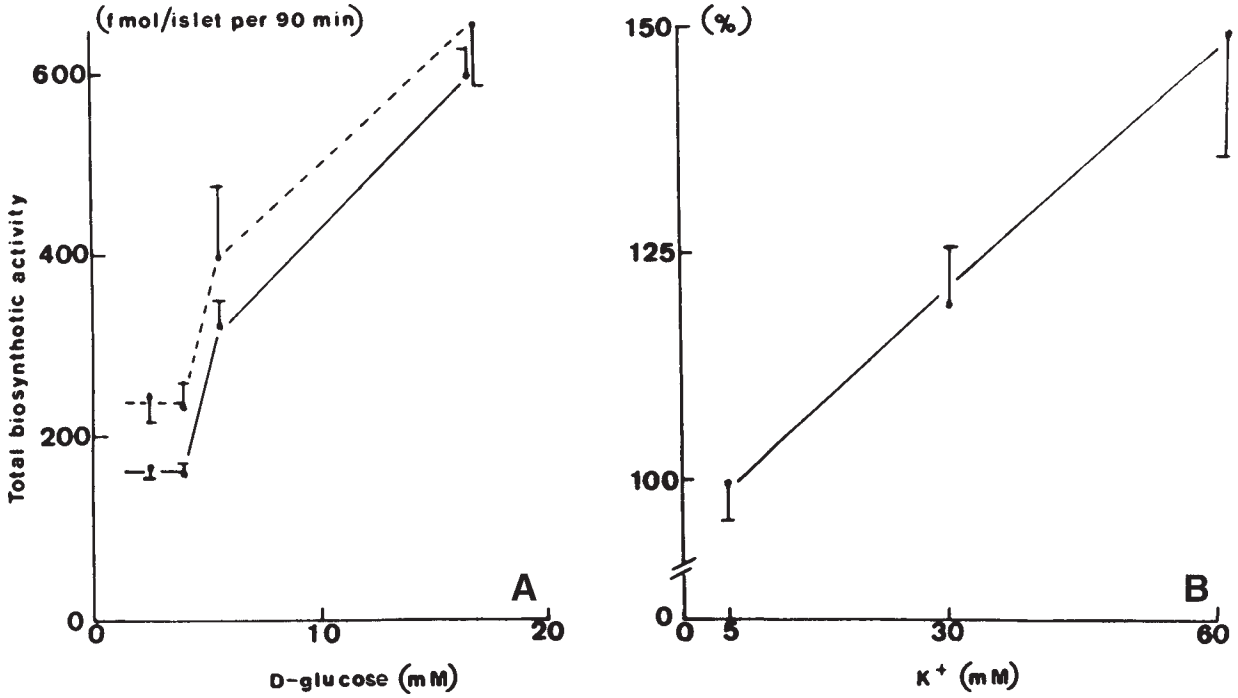

Fig. 2. (**A**) Effect of increasing concentrations of D-glucose upon total biosynthetic activity (absolute values) of islets incubated at either 5 m*M* K^+ (closed circles and solid line) or 60 m*M* K^+ (closed circles and dotted line); mean values (± SEM) refer to 21–33 individual observations at 2.5–4.0 m*M* D-glucose and 3–38 separate measurements at 5.6–16.7 m*M* D-glucose. (**B**) Effect of increasing concentrations of K^+ upon total biosynthetic activity (normalized values) of islets incubated in the presence of 2.5–4.0 m*M* D-glucose; mean values (± SEM) refer to 47–49 individual determinations.

its Rb^+/K^+ concentration ratio) from a control value of 100.0 ± 5.9% to 184.3 ± 20.3% and 293.6 ± 27.8% ($n = 20$ in all cases), respectively. Relative to the mean control value (D-glucose 2.5 m*M* and K^+ 5.0 m*M*) found within the same experiment(s), the results recorded in this last series of experiments at 16.7 m*M* D-glucose and 5.0 m*M* K^+ or at 2.5 m*M* D-glucose and either 30.0 or 60.0 m*M* K^+ were never significantly different ($p > 0.1$ or more) from those obtained in the two first series of experiments, the former results averaging 91.0 ± 5.8% ($n = 59$; $p > 0.3$) of the corresponding latter ones (100.0 ± 7.1%; $n = 85$).

In the light of these findings, most of the experiments dealing with the effect of the extracellular K^+ concentration upon islet biosynthetic activity were conducted at low D-glucose concentration (2.5–4.0 m*M*) and at increasing concentrations of K^+ (5.0, 30.0, and 60.0 m*M*).

Total Biosynthetic Activity

As judged from the incorporation of L-[4-^{3}H]phenylalanine (0.4 μ*M*) into TCA-precipitable material, the biosynthetic activity averaged, at normal K^+ concentration (5 m*M*) 168.6 ± 15.1 ($n = 33$) and 155.2 ± 14.4 ($n = 22$) fmol/islet per 90 min in the presence of 2.5 and 4.0 m*M* D-glucose, respectively, these two mean values not being significantly different from one another ($p > 0.5$). At the same K^+ concentration, however, the total biosynthetic activity was increased ($p < 0.001$) to 319.2 ± 31.2 ($n = 8$) and 596.4 ± 29.6 ($n = 38$) fmol/islet per 90 min in the presence of 5.6 and 16.7 m*M* D-glucose, respectively (Fig. 2A).

In the presence of 2.5 m*M* D-glucose, the mean total biosynthetic activity was increased to 211.4 ± 18.7 ($n = 27$; $p < 0.1$) and 246.9 ± 30.9 ($n = 26$; $p < 0.02$) fmol/islet per 90 min at K^+ concentrations of 30 and 60 m*M*, respectively. Relative to the corresponding mean value found within the same experiment at 5 m*M* K^+ (100.0 ± 8.0%; $n = 27$), these activities averaged 117.5 ± 9.9% ($n = 27$) and 141.0 ± 19.1% ($n = 26$) at 30 and 60 m*M* K^+, respectively.

A comparable situation was observed in the presence of 4.0 m*M* D-glucose. Thus, relative to the corresponding mean value found within the same experiment(s) at 5 m*M* K^+ (100.0 ± 5.1%; $n = 22$), the total biosynthetic activity averaged 120.4 ± 9.4% ($n = 22$) and 160.6 ± 19.6% ($n = 21$) at 30 and 60 m*M* K^+, respectively. These two percentages were not significantly different ($p > 0.4$ or more) from those recorded in the presence of 2.5 m*M* D-glucose. As illustrated in Fig. 2B, in the range between 2.5 and 4.0 m*M* D-glucose, the total biosynthetic activity progressively increased from 100.0 ± 4.9% at K^+ 5 m*M* ($n = 49$) to 118.8 ± 6.8% ($n = 49$; $p < 0.03$) at K^+ 30 m*M* and 149.8 ± 13.7% ($n = 47$; $p < 0.001$) at K^+ 60 m*M*.

In the presence of 5.6 m*M* D-glucose, the results recorded at 30 and 60 m*M* K^+ averaged 127.3 ± 15.8% ($n = 16$) of

the mean corresponding values found within the same experiments at 5 m*M* K^+ (100.0 ± 9.7%; *n* = 8). The increase in mean total biosynthetic activity attributable to the rise in K^+ concentration failed, however, to achieve statistical significance ($p > 0.1$) in the islets exposed to 5.6 m*M* D-glucose.

In the presence of 16.7 m*M* D-glucose, the results were not affected significantly by a rise in K^+ concentration to either 30 or 60 m*M* ($p > 0.2$), averaging at these high K^+ concentrations 100.3 ± 15.3% (*n* = 6) of the mean corresponding value found within the same experiment at 5 m*M* K^+ (100.0 ± 17.3%; *n* = 3). Thus, in relative terms, the effect of high K^+ concentrations upon total biosynthetic activity progressively decreased and eventually faded out as the concentration of D-glucose was raised from non-stimulating levels (2.5–4.0 m*M*) to higher values (5.6 and 16.7 m*M*).

Incidentally, in these experiments, the radioactive content of the TCA precipitates averaged 94.1 ± 0.8% (*n* = 33) and 97.8 ± 0.3% (*n* = 38) of the total islet radioactive content in islets incubated at 5 m*M* K^+ in the presence of 2.5 and 16.7 m*M* D-glucose, respectively. Under the other 10 experimental conditions investigated in this study, such a percentage yielded mean values in between the two extreme values mentioned above, which were indeed significantly different from one another ($p < 0.001$).

Tritiated Islet Peptides

After separation by gel chromatography (Fig. 3), the total radioactive content of all islet peptides yielded, in essence, the same information as that derived from the measurement of the TCA-precipitable material. Thus, at 5 m*M* K^+, it was not significantly different ($p > 0.5$) in islets exposed to either 2.5 m*M* D-glucose (97.9 ± 14.6 fmol/islet; *n* = 5) or 4.0 m*M* D-glucose (112.3 ± 18.0 fmol/islet; *n* = 4), but increased ($p < 0.005$ or less) to 240.5 ± 3.5 (*n* = 2) and 424.2 ± 45.6 (*n* = 6) fmol/islet in the presence of 5.6 and 16.7 m*M* D-glucose, respectively. At 5 m*M* K^+, the paired ratio in the total radioactive content of islet peptides found in the presence of 16.7 m*M*/2.5 m*M* D-glucose averaged 4.12 ± 0.63 (geometric mean; *n* = 5; $p < 0.001$ vs 1), a value not significantly different ($p > 0.3$) from that derived from the mean radioactive content of the TCA-precipitable material in the same five experiments (i.e., 3.48 ± 0.28; $p < 0.001$ vs 1), with a correlation coefficient of 0.873 ($p < 0.06$) between the two series of measurements.

Likewise, in the presence of 2.5-4.0 m*M* D-glucose, the tritiated islet peptides separated by gel chromatography averaged, in the islets exposed to 30 and 60 m*M* K^+ respectively, 125.3 ± 9.2% (*n* = 8; $p < 0.02$ vs 1) and 153.3 ± 22.2% (*n* = 8; $p < 0.025$ vs 1) of the paired value found at 5 m*M* K^+, these percentages being comparable ($p > 0.5$ or more) to those illustrated in Fig. 2B.

Finally, in the presence of 5.6 m*M* D-glucose, the tritiated islet peptides found in islets exposed to 30 or 60 m*M* K^+ averaged 129.3 ± 14.9% (*n* = 4) of the paired value recorded at 5 m*M* K^+, once again in fair agreement with the measurements of TCA-precipitable material (127.3 ± 15.8%).

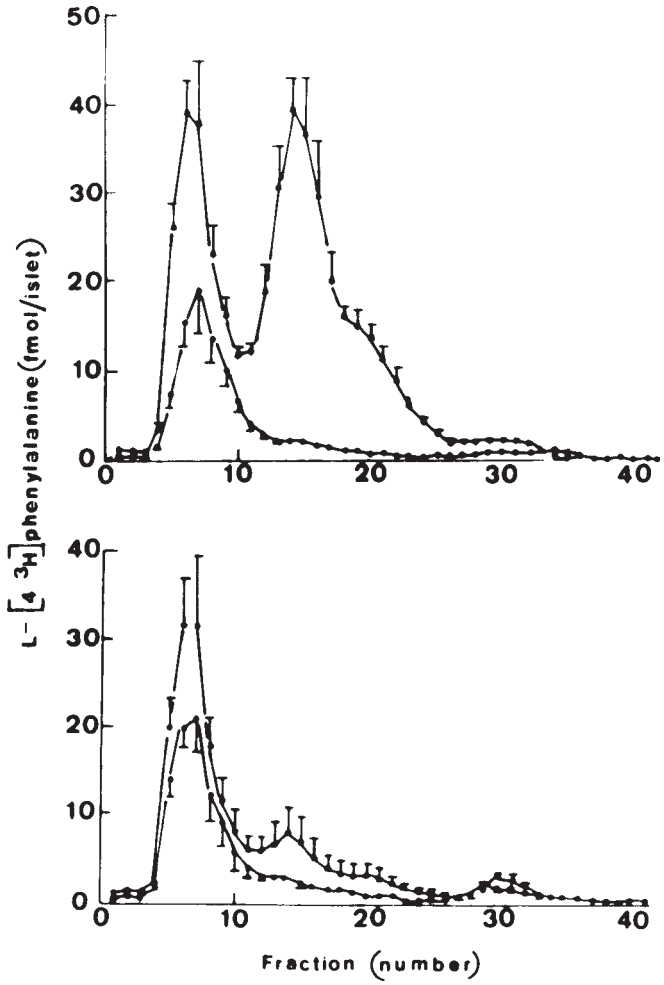

Fig. 3. Elution profile of tritiated material separated by gel chromatography. The upper panel refers to islets exposed to either 2.5 m*M* D-glucose (*n* = 5) or 16.7 m*M* D-glucose (*n* = 6) at 5 m*M* K^+. The lower panel refers to islets exposed to 4.0 m*M* D-glucose at either 5 m*M* K^+ (*n* = 4) or 60 m*M* K^+ (*n* = 4). The SEM is shown only when it exceeded the size of the mean point.

Tritiated (Pro)Insulin

The effects of changes in D-glucose and/or K^+ concentrations on the tritiation of proinsulin and insulin was comparable to that found in the case of all islet peptides.

At 5 m*M* K^+, it averaged 21.5 ± 2.3 (*n* = 5) and 24.4 ± 2.2 (*n* = 4) fmol/islet in the presence of 2.5 and 4.0 m*M* D-glucose, respectively, and was increased ($p < 0.001$) to 111.4 ± 7.2 (*n* = 2) and 265.6 ± 33.5 (*n* = 6) fmol/islet in the presence of 5.6 and 16.7 m*M* D-glucose (Fig. 4).

In the presence of 2.5–4.0 m*M* D-glucose, the total amount of tritiated proinsulin and insulin progressively increased from 26.5 ± 2.5 fmol/islet (*n* = 9) at K^+ 5 m*M* to 34.1 ± 3.2

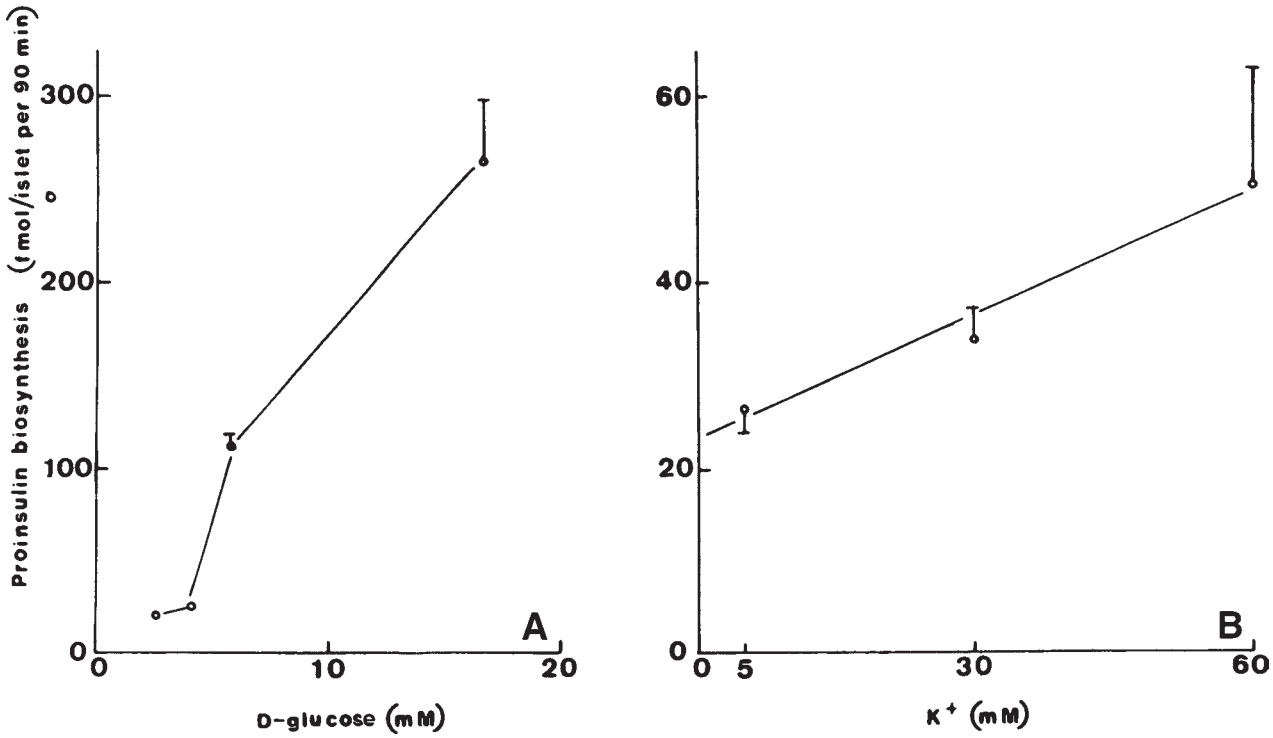

Fig. 4. (**A**) Effect of increasing concentrations of D-glucose upon the synthesis of (pro)insulin by islets incubated at 5 m*M* K^+; mean values (± SEM) refer to two to six individual observations. (**B**) Effect of increasing concentrations of K^+ upon the synthesis of (pro)insulin by islets exposed to 2.5–4.0 m*M* D-glucose; mean values (± SEM) refer to eight to nine individual determinations.

($n = 8$) and 50.3 ± 12.6 ($n = 8$) fmol/islet as the K^+ concentration was raised from 5 to 30 and 60 m*M* (Fig. 4). The coefficient of correlation between the two variables amounted to 0.4411 ($n = 25$; $p < 0.05$). Relative to the paired control value found at 5 m*M* K^+, the readings averaged 121.8 ± 11.0% and 155.9 ± 33.4% (geometric means; $n = 8$ in both cases) at 30 and 60 m*M* K^+. The increase in tritiated proinsulin biosynthesis due to the rise in K^+ concentration was thus highly significant ($p < 0.02$).

In the presence of 5.6 m*M* D-glucose, however, the tritiation of proinsulin and insulin was no more affected significantly ($p > 0.7$) by the rise in K^+ concentration from 5 to either 30 or 60 m*M*.

Preferential Stimulation of Proinsulin Biosynthesis

At K^+ 5 m*M*, the paired ratio between tritiated (pro)insulin and all ^{3}H-labeled peptides increased ($p < 0.01$ or less) from 23.6 ± 3.6% ($n = 5$) and 22.6 ± 1.9% ($n = 4$) in islets exposed to 2.5 and 4.0 m*M* D-glucose, respectively, to 46.3 ± 2.3% ($n = 2$) and 62.3 ± 2.1% ($n = 6$) in islets exposed to 5.6 and 16.7 m*M* D-glucose. This documents a preferential stimulation by D-glucose of proinsulin biosynthesis relative to that of other islet peptides.

As a rule, such a paired ratio between tritiated (pro)insulin and total islet peptides was not significantly affected by changes in extracellular K^+ concentration. The sole indication of a preferential stimulation of proinsulin biosynthesis as a result of the rise in K^+ concentration was recorded in the islets exposed to 60 m*M* K^+ in the presence of 4.0 m*M* D-glucose. Indeed, in this case, the individual ratios between tritiated (pro)insulin and the total amount of ^{3}H-labeled peptides represented 140.7 ± 18.4% ($p < 0.05$) of the paired values found within the same experiments at lower K^+ concentrations, which averaged 20.7 ± 1.3% ($n = 8$).

Proinsulin Conversion to Insulin

The paired ratio between tritiated insulin and the total amount of ^{3}H-labeled proinsulin and insulin averaged, at 5 m*M* K^+, 21.0 ± 2.3% ($n = 5$), 24.8 ± 3.5% ($n = 5$), 19.3 ± 2.5% ($n = 2$), and 23.6 ± 1.6% ($n = 6$) in the presence of 2.5, 4.0, 5.6, and 16.7 m*M* D-glucose, respectively, none of these values being significantly different from one another. It was also little affected by a rise in K^+ concentration, averaging at 30 and 60 m*M* K^+, respectively, 25.0 ± 3.5% ($n = 4$) and 20.8 ± 1.6% ($n = 4$) in the presence of 2.5 m*M* D-glucose and 24.3 ± 2.6% ($n = 4$) and 26.0 ± 0.9% ($n = 4$) in the presence of 4.0 m*M* D-glucose. At the most, there was a trend for the increase in either D-glucose concentration from 2.5 to 16.7 m*M* (at K^+ 5 m*M*) or K^+ concentration from 5 to 30 and 60 m*M* (at D-glucose 2.5 or 4.0 m*M*) to increase the fractional conversion of tritiated proinsulin to ^{3}H-labeled insulin. Thus, pooling all available data, such a fractional conversion averaged at high D-glucose or K^+ concentrations, 111.5 ± 5.3% (geometric mean; $n = 21$; $p < 0.05$ vs 1) of the paired value found within the same experiment(s) at low D-glucose (2.5 m*M*) or K^+ (5 m*M*) levels.

Discussion

As indicated in the introduction, the major aim of the present experiments was to explore the possible role of a rise in intracellular K^+ content as a coupling factor in the stimulation of proinsulin biosynthesis by D-glucose.

The procedure here used to increase islet cell K^+ content consisted in raising the extracellular K^+ concentration from 5 to 30 and 60 m*M*. Although the effect of such a rise in K^+ concentration upon the efflux of $^{86}Rb^+$ from prelabeled islets had already been investigated in a prior study *(7)*, no information was available, to our knowledge, on its effect upon the K^+ content of the islet cells. The present results clearly indicate that a rise in extracellular K^+ concentration from 5 to 30 and 60 m*M* indeed increased the cell content in K^+ to a level comparable to that otherwise caused by a rise in D-glucose concentration, from 2.5–2.8 m*M* to 16.7 m*M*, at normal extracellular K^+ concentration (5 m*M*). The present results also unambiguously document that at low concentrations of D-glucose (2.5 and 4.0 m*M*), below or close to the threshold concentration of the hexose required to stimulate biosynthetic activity in the islets, a rise in extracellular K^+ concentration and, hence, an increase in K^+ cell content, coincides with stimulation of total biosynthetic activity, as well as the synthesis of proinsulin. This effect progressively faded out at higher concentrations of D-glucose (5.6 and 16.7 m*M*), when the biosynthetic activity of the islet was already stimulated by the hexose at normal extracellular K^+ concentration (5 m*M*).

The K^+-induced stimulation of biosynthetic activity is unlikely to result from an increase in L-[4-^{3}H]phenylalanine uptake by the islet cells. Thus, as judged from the data obtained after gel chromatography, the islet content in this tritiated amino acid averaged, in the islets exposed to 2.5–4.0 m*M* D-glucose, 11.0 ± 2.9 fmol/islet at K^+ 5 m*M* (n = 9), 10.5 ± 1.1 fmol/islet at K^+ 30 m*M* (n = 8), and 11.4 ± 1.0 fmol/islet at K^+ 60 m*M* (n = 8). None of these mean values were significantly different from one another ($p > 0.5$ or more).

It is also most unlikely that the increase in biosynthetic activity due to a rise in K^+ concentration is attributable to an increase in D-glucose catabolism. Thus, a rise in K^+ concentration from 5 to 30 or 60 m*M* fails to affect significantly the generation of ^{3}HOH from D-[5-^{3}H]glucose and that of $^{14}CO_2$ and ^{14}C-labeled acidic metabolites or amino acids from D-[U-^{14}C]glucose in rat pancreatic islets *(8)*. A higher K^+ concentration (90 m*M*) may even affect adversely the catabolism of D-glucose in the islets, whether at 2.8, 8.3, or 16.7 m*M* D-glucose *(8,9)*.

Likewise, the depolarization of the plasma membrane caused by high extracellular K^+ concentrations and the resulting gating of voltage-sensitive Ca^{2+} channels appears as an unlikely explanation for the stimulation of islet biosynthetic activity. Such an activity is indeed little affected by various procedures designed to alter the influx of Ca^{2+} into the islet cells *(10,11)*.

Finally, the stimulation of proinsulin biosynthesis at high concentrations of extracellular K^+ cannot be blamed on the concomitant decrease in extracellular Na^+ concentration. Indeed, it was previously shown that substitution of Na^+ by Li^+ or choline impairs rat islet biosynthetic activity *(10)*.

When the concentration of D-glucose (4.0 m*M*) was close to the threshold value for stimulation of biosynthetic activity at normal K^+ concentration and when the latter concentration was raised to a high value (60 m*M*), a preferential stimulation of proinsulin biosynthesis, relative to that of other islet peptides, as otherwise occurring at increasing concentrations of D-glucose, was also observed.

It should be duly emphasized, however, that, in such a respect, as well as in terms of the relative magnitude of the increase in either total biosynthetic activity or proinsulin synthesis, the affect of a rise in K^+ concentration was much more modest than that of a rise in D-glucose concentration.

Nevertheless, these two procedures apparently also shared in common a modest increase in the fractional conversion of proinsulin to insulin. We feel authorized, therefore, to propose that the well-known effect of D-glucose to increase the K^+ content of pancreatic endocrine cells represents one modality for coupling a rise in extracellular D-glucose concentration to the stimulation of proinsulin biosynthesis. This proposal is mot meant, by any means, to deny the possible participation of other second messengers in such a coupling process.

Materials and Methods

The experiments were approved by the Commission d'Ethique et du Bien-Etre Animal of our Faculty.

Pancreatic islets were isolated by the collagenase procedure *(12)* from fed Wistar rats.

For measuring $^{86}Rb^+$ net uptake, groups of 10 islets each were incubated for 60 min at 37°C in 50 µL of a Hepes- and bicarbonate-buffered medium *(13)* containing bovine serum albumin (5 mg/mL), $^{86}Rb^+$ (9–10 µ*M*; 2–5 µCi/mL), and D-glucose and K^+ at the concentrations indicated in the text. This medium was equilibrated against a mixture of O_2/CO_2 (19/1, v/v). The incubation time was selected to allow the ^{86}Rb content of the islets to reach a steady-state value *(6)*. After incubation, the islets were separated from the incubation medium by centrifugation of the latter medium through an oil layer *(14)*. In one series of experiments, the islets were submitted to two washes with an iced medium identical to that used for incubation, except for the absence of $^{86}Rb^+$, prior to the centrifugation procedure described above. Alterations in the K^+ extracellular concentration were achieved by equimolar substitution of NaCl by KCl (or vice versa) in order to maintain in all cases the same osmolarity.

For assessing biosynthetic activity, groups of eight islets each were incubated for 90 min at 37°C in 1.0 mL of the same medium as mentioned above, except for the absence

of $^{86}Rb^{+}$ and presence of 0.4 μ*M* L-[4-^{3}H]phenylalanine (10 μCi/mL). After incubation, the islets were washed twice with 1.0 mL of an iced medium containing 1.0 m*M* unlabeled L-phenylalanine and then sonicated (thrice 15 s) in 0.8 mL of acetic acid (2 *M*). Aliquot parts (25 μL each) of the islet homogenates were examined for their content in trichloroacetic acid-precipitable and soluble radioactive material. For separation of tritiated islet peptides by gel chromatography, equal aliquot parts from all available islet homogenates obtained after incubation at a given D-glucose and K^{+} concentration in the same experiment were pooled together. An aliquot part (500 μL) of these pooled homogenates was mixed with 25 μL of a 20% (w/v) solution of bovine serum albumin and then placed on a Bio-Gel P-30 column (Bio-Rad, Richmond, CA), using 2 *M* acetic acid as eluent *(15)*.

All results are presented as mean values (± SEM) together with the number of separate observations (*n*). The statistical significance of differences between mean values was assessed by use of Student's *t*-test.

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