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The permissive effect of glucose, tolbutamide and high K^+ on arginine stimulation of insulin release in isolated mouse islets

M. P. Hermans[1], W. Schmeer[2] and J. C. Henquin[1,2]

[1] Unité de Diabétologie et Nutrition, University of Louvain, Faculty of Medicine, Brussels, Belgium, and
[2] I Physiologisches Institut, University of Saarland, Homburg/Saar, FRG

Summary. Mouse islets were used to study how glucose modulates arginine stimulation of insulin release. At 3 mmol/l glucose, arginine (20 mmol/l) decreased the resting membrane potential of B cells by about 10 mV, but did not evoke electrical activity. This depolarisation was accompanied by a slight but rapid acceleration of $^{86}Rb^+$ efflux and $^{45}Ca^{2+}$ influx. However, $^{45}Ca^{2+}$ efflux and insulin release increased only weakly and belatedly. When the membrane was depolarised by threshold (7 mmol/l) or stimulatory (10-15 mmol/l) concentrations of glucose, arginine rapidly induced or augmented electrical activity, markedly accelerated $^{86}Rb^+$ efflux, $^{45}Ca^{2+}$ influx and efflux, and triggered a strong and fast increase in insulin release. When glucose-induced depolarisation of the B-cell membrane was prevented by diazoxide, arginine lost all effects but those produced at low glucose. However, the delayed increase in release still exhibited some glucose-dependency. In contrast, depolarisation by tolbutamide, at low glucose, largely mimicked the permissive effect of high glucose. Depolarisation by high K^+ also amplified arginine stimulation of insulin release, but did not accelerate it as did glucose or tolbutamide. Omission of extracellular Ca^{2+} abolished the releasing effect of arginine under all conditions. The results thus show that the permissive action of glucose mainly results from its ability to depolarise the B-cell membrane. It enables the small depolarisation by arginine itself to activate Ca channels more rapidly and efficiently. Changes in the metabolic state of B cells may also contribute to this permissive action by increasing the efficacy of the initiating signal triggered by arginine.

Key words: Insulin release, arginine, glucose, tolbutamide, diazoxide, potassium, isolated islets, B cells, membrane potential, ionic fluxes.

Glucose not only stimulates insulin release, but also influences the B-cell response to other secretagogues. Since the observation of the synergistic effects of glucose and arginine [1], numerous in vivo [2-4] and in vitro studies [5-8] have established that the insulin secretory response to arginine increases with the concentration of glucose. In vitro experiments further demonstrated that glucose markedly accelerates arginine-induced insulin release. The cellular mechanisms of this permissive or modulatory action of glucose are still ill-defined. This is, however, an important issue not only for B-cell physiology, but also for B-cell pathology. Thus, recent observations suggest that an impairment of the modulation by glucose of arginine-induced insulin release is an early dysfunction of B cells in Type 2 (non-insulin-dependent) diabetic patients [9-11].

In the present study, performed with mouse islets, two approaches were followed to test the hypothesis that depolarisation of the B-cell membrane by glucose is essential for the sugar to modulate arginine-induced insulin release. First, we investigated whether suppression of the depolarising effect of glucose by diazoxide [12] alters the B-cell response to arginine. Second, we evaluated to what extent depolarisation by tolbutamide or high K^+ duplicates the permissive effect of glucose. Tolbutamide shares with glucose the property of depolarising the B-cell membrane by decreasing its K^+ permeability [12], whereas high K^+ depolarises by changing the equilibrium potential for K^+.

Materials and methods

All experiments were performed with islets of fed female NMRI mice (25-30 g), killed by decapitation. For electrophysiological experiments, a piece of pancreas was fixed in a small perifusion chamber, and the membrane potential of single B cells was continuously recorded with microelectrodes [13]. B cells were identified by the typical electrical activity that they display in the presence of 10-15 mmol/l glucose [14]. For all other experiments, islets were isolated after collagenase digestion of the pancreas. The techniques and

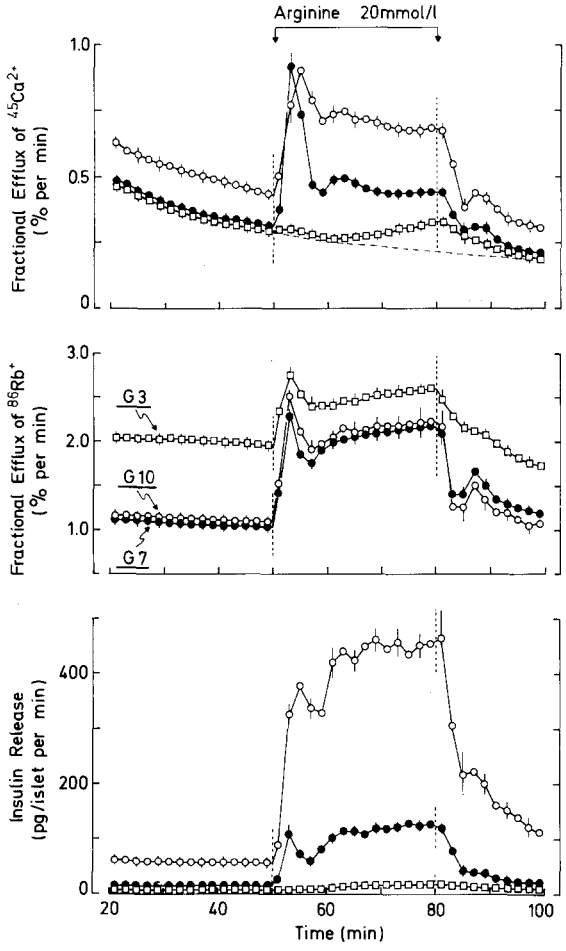

Fig. 1. Effects of arginine on $^{45}Ca^{2+}$ efflux, $^{86}Rb^{+}$ efflux and insulin release from mouse islets perifused with a medium containing various concentrations of glucose (G): 3 mmol/l (□), 7 mmol/l (●) or 10 mmol/l (○). Arginine was added to all solutions between 50 and 80 minutes. The dashed line in the upper panel shows control experiments with 3 mmol/l glucose alone. Values are means ± SEM for 4 experiments

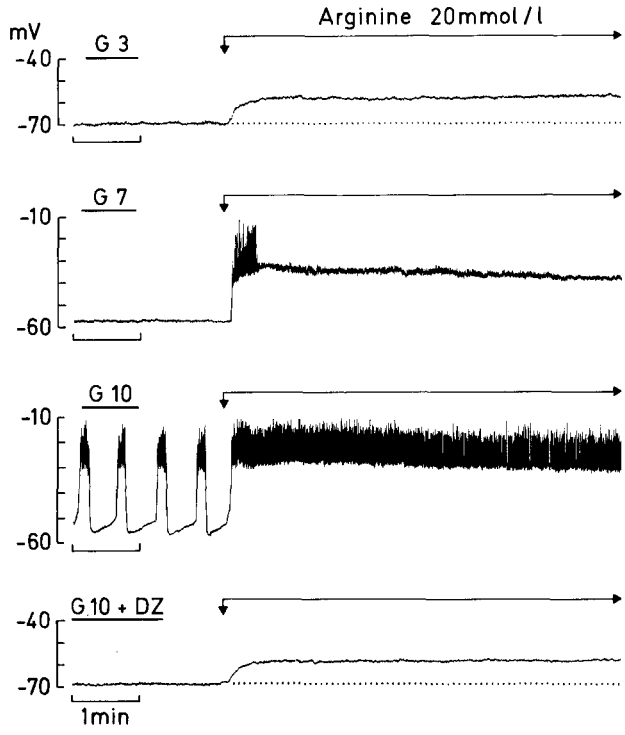

Fig. 2. Effects of arginine on the membrane potential of mouse B cells perifused with a medium containing the indicated concentration of glucose (G, mmol/l). In the experiment shown by the lower panel, the medium was supplemented by 0.1 mmol/l diazoxide (DZ). Arginine was added as indicated by the arrows. The stippled line in the upper and lower records is drawn at the level of the resting potential. These records were obtained in different mice and are representative of results obtained in 4–5 experiments

the dynamic system of perifusion used to monitor the efflux of $^{45}Ca^{2+}$ or $^{86}Rb^{+}$ (used as tracer for K^{+}) from preloaded islets have been described in detail [15]. Batches of 30 islets ($^{86}Rb^{+}$ efflux) or 40 islets ($^{45}Ca^{2+}$ efflux) were placed in parallel chambers and perifused at a flow rate of 1.35 ml/min. During the experiments of $^{86}Rb^{+}$ efflux, a portion of each effluent fraction was drawn for measurement of immunoreactive insulin, with rat insulin as standard (Novo Research Institute, Bagsvaerd, Denmark). $^{45}Ca^{2+}$ uptake by islet cells was measured as described previously [16], using [6,6′-^{3}H]sucrose as marker of the extracellular space.

The perifusion medium used was a bicarbonate-buffered solution, pH 7.4, that was supplemented with bovine serum albumin (1 mg/ml), except for electrophysiological recordings. Ca^{2+}-free solutions were prepared by replacing $CaCl_2$ by $MgCl_2$. When the concentration of KCl was increased, that of NaCl was decreased accordingly. As albumin binds sulphonylureas, tolbutamide was used at a lower concentration (25 μmol/l instead of 100 μmol/l) in most electrophysiological experiments (made without albumin) than in other experiments.

Diazoxide and tolbutamide were gifts from Schering Corp. (Bloomfield, NJ, USA) and Hoechst A.G. (Frankfurt, FRG) respectively. All other reagents were from Merck A.G. (Darmstadt, FRG). Radiochemicals were obtained from the Radiochemical Center (Amersham, Bucks, UK).

Presentation of data

Electrophysiological experiments are illustrated by recordings which are representative of the indicated number of experiments, performed with different mice, or are presented as means (± SEM). All other data are presented as means (± SEM) for a certain number of experiments (different islet preparations), or for a certain number of batches of islets (at least three separate experiments).

Results

Effects of arginine in the presence of various concentrations of glucose

Addition of 20 mmol/l arginine to a medium containing a non-stimulatory concentration of glucose (3 mmol/l) caused a rapid acceleration of $^{86}Rb^{+}$ efflux, and a delayed, small and monotonic increase in $^{45}Ca^{2+}$ efflux and insulin release (Fig. 1). After 30 min of stimulation with arginine, insulin secretion was approximately doubled (15 ± 2 vs 8 ± 1 pg/islet per min; $n = 4$). The resting membrane potential of B cells averaged −68.8 ± 1.4 mV ($n = 5$) in the presence of 3 mmol/l glucose. Arginine caused a rapid and sustained depolarisation (Fig. 2) by 10.4 ± 0.8 mV, but did not evoke electrical activity. Only the membrane poten-

Table 1. Effects of arginine on $^{45}Ca^{2+}$ uptake by mouse islets (pmol/islet. 5 min)

Experimental conditions (mmol/l)	Controls	+Arginine (20 mmol/l)	
		0–5 min	30–35 min
Glucose 3	2.04 ± 0.10	4.25 ± 0.21	4.76 ± 0.22
Glucose 7	2.93 ± 0.16[a]	9.83 ± 0.41[a]	7.09 ± 0.27[a]
Glucose 10	5.78 ± 0.19[a]	11.47 ± 0.34[a]	9.20 ± 0.32[a]
Glucose 10 + Diazoxide 0.1	2.29 ± 0.12	4.62 ± 0.21	5.45 ± 0.27

Batches of ten islets were incubated in 100 µl medium of the indicated composition, layered on silicone oil. All incubations lasted 35 min, but the tracers were added for the last 5 min only. When the effect of arginine was tested, the amino acid was added with the tracers to measure the initial effect (0–5 min), or was present from the start of the incubation to measure the steady state effect (30–35 min). The incubation was terminated by centrifuging the islets through the oil. Values are means ± SEM for 15 batches of islets. [a] $p < 0.001$ vs 3 mmol/l glucose (t test)

Table 2. Effects of diazoxide on arginine-induced insulin release by mouse islets perifused with different concentrations of glucose

Glucose (mmol/l)	Insulin release (ng/islet. 30 min)	
	Without diazoxide	With diazoxide
3	0.08 ± 0.01	0.08 ± 0.01
7	2.63 ± 0.24	0.19 ± 0.01
10	10.0 ± 0.51	0.49 ± 0.09
15	15.4 ± 1.47	1.32 ± 0.24

All experiments were similar to those illustrated by Figure 3. The concentration of glucose was the same during the whole experiment, and arginine (20 mmol/l) was added between 50 and 80 min. When tested, diazoxide (0.1 mmol/l) was added from 40 min onwards. Total insulin release induced by arginine was calculated after subtraction of the basal rate measured immediately prior to addition of arginine. Values are means ± SEM for 4–5 experiments

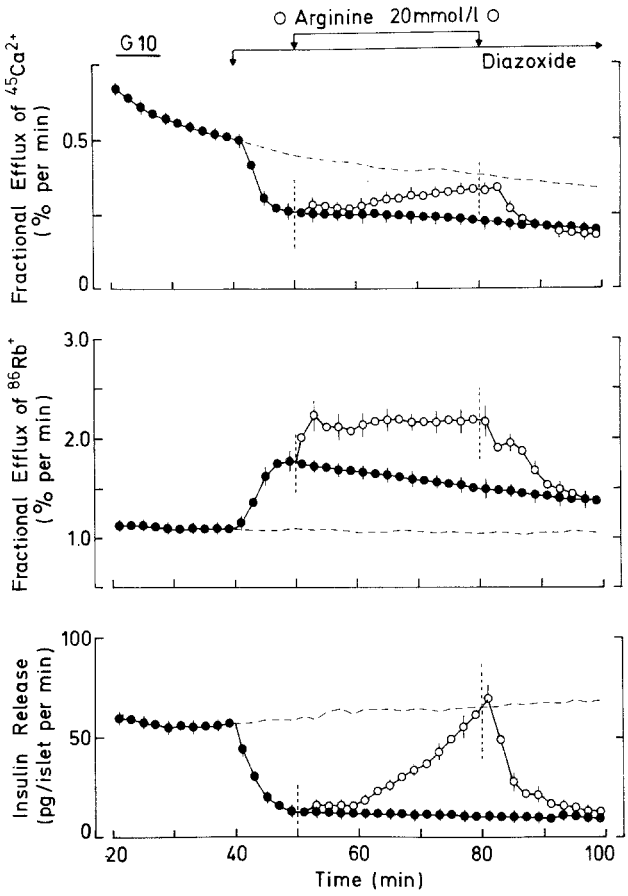

Fig. 3. Diazoxide modification of the effects of arginine on $^{45}Ca^{2+}$ efflux, $^{86}Rb^{+}$ efflux, and insulin release from mouse islets perifused with a medium containing 10 mmol/l glucose (G10). Diazoxide (0.1 mmol/l) was added to all solutions from 40 min onwards. Arginine was added to test solutions (O) between 50 and 80 minutes. Control experiments without diazoxide and arginine are shown by the dashed lines. Values are means ± SEM for 4–5 experiments

tial noise (small fluctuations of the membrane potential) was more pronounced than in the absence of arginine. Under similar conditions, Ca^{2+} influx (estimated by the 5-min $^{45}Ca^{2+}$ uptake) was doubled by arginine (Table 1).

As shown in Figure 1, 7 mmol/l glucose decreased the rate of $^{86}Rb^{+}$ efflux, did not significantly affect $^{45}Ca^{2+}$ efflux and marginally increased insulin release (13 ± 2 vs 8 ± 1 pg/islet per min; $n = 4$). Addition of arginine was followed by a rapid, biphasic and reversible increase in $^{86}Rb^{+}$ efflux, $^{45}Ca^{2+}$ efflux and insulin release (Fig. 1). At this threshold concentration of glucose, the B-cell membrane was slightly depolarised (−58.8 ± 1.7 mV) but weak electrical activity (slow waves with spikes) was present in only one of four cells. Arginine caused a rapid and sustained depolarisation and induced electrical activity (Fig. 2). This activity consisted first in large spikes, then in rapid fluctuations of the membrane potential, and eventually (three of four cells) in small slow waves appearing after 10–15 min (not shown). $^{45}Ca^{2+}$ uptake was strongly stimulated by arginine, the increase being larger during the first 5 min than after 30 min of stimulation (Table 1).

In the presence of a stimulatory concentration of glucose (10 mmol/l), the rates of $^{45}Ca^{2+}$ efflux and insulin release were higher than at 7 mmol/l glucose, whereas the rate of $^{86}Rb^{+}$ efflux was not significantly different (Fig. 1). Subsequent addition of arginine brought about a large biphasic increase in $^{86}Rb^{+}$ efflux, $^{45}Ca^{2+}$ efflux and insulin release (Fig. 1). The typical electrical activity induced by 10 mmol/l glucose was rapidly suppressed by arginine, that caused persistent depolarisation at the plateau level, and continuous spike activity (Fig. 2.). $^{45}Ca^{2+}$ uptake was augmented by 100 and 60% during the first and last 5 min of arginine stimulation respectively (Table 1).

At 15 mmol/l glucose, the control rate of insulin release was 3-fold higher than at 10 mmol/l glucose, and the effect of arginine approximately 50% larger (Table 2).

Control experiments without extracellular Ca^{2+} were performed at 10 mmol/l glucose. Under these conditions, arginine still increased $^{86}Rb^{+}$ efflux, but

did not stimulate $^{45}Ca^{2+}$efflux or insulin release (not shown).

Modifications of the effects of arginine by diazoxide

Diazoxide is known to increase K^+ permeability of the B-cell membrane [12]. Its addition to a medium containing 10 mmol/l glucose accelerated $^{86}Rb^+$ efflux from islet cells, hyperpolarised the membrane (-66.3 ± 1.7 mV; $n = 4$) and abolished electrical activity in B cells, inhibited $^{45}Ca^{2+}$ influx and efflux, and suppressed insulin release (Figs. 2 and 3; Table 1). However, diazoxide did not prevent arginine from depolarising the B-cell membrane (by 9.5 ± 0.9 mV), from accelerating $^{86}Rb^+$ efflux, and from stimulating $^{45}Ca^{2+}$ influx and efflux (Figs. 2 and 3; Table 1). These ionic and electrical effects of arginine in the presence of 10 mmol/l glucose and 0.1 mmol/l diazoxide were qualitatively and quantitatively similar to those produced by the amino acid in the presence of 3 mmol/l glucose alone. Arginine also increased insulin release under these conditions, but this secretory response consistently displayed a progressive and monotonic time-course (Fig. 3), and was considerably smaller than in the absence of diazoxide, except at 3 mmol/l glucose (Table 2). A clear increase with the concentration of glucose was still evident, however. This residual secretory response to arginine in the presence of 10 mmol/l glucose and 0.1 mmol/l diazoxide was completely prevented by omission of extracellular Ca^{2+} (not shown).

Effects of arginine in the presence of tolbutamide

In the presence of 3 mmol/l glucose, tolbutamide (100 μmol/l) decreased $^{86}Rb^+$ efflux, accelerated $^{45}Ca^{2+}$ efflux, and slightly stimulated insulin release (Fig. 4). Subsequent addition of arginine was followed by a prompt increase in $^{86}Rb^+$ efflux, $^{45}Ca^{2+}$ efflux, and insulin release. These stimulatory effects of the amino acid were larger and, except for $^{86}Rb^+$ efflux, much faster than in the presence of 3 mmol/l glucose alone (compare Fig. 4 with Fig. 1). Omission of extracellular Ca^{2+} abolished the changes in $^{45}Ca^{2+}$ efflux and insulin release brought about by tolbutamide alone or in combination with arginine, but did not prevent the changes in $^{86}Rb^+$ efflux (not shown).

At the concentration of 25 μmol/l and in the presence of 3 mmol/l glucose, tolbutamide rapidly depolarised the B-cell membrane and induced continuous spike activity for a few minutes (Fig. 5). Thereafter, the membrane potential started to oscillate in slow waves with bursts of spikes superimposed on the plateau. Addition of arginine caused a fast depolarisation to the plateau potential where large spikes appeared, soon followed by smaller fluctuations of the membrane potential (Fig. 5). When tolbutamide was used at a concentration of 100 μmol/l, the B-cell membrane was persistently depolarised, but arginine increased the frequency of the spikes (not shown).

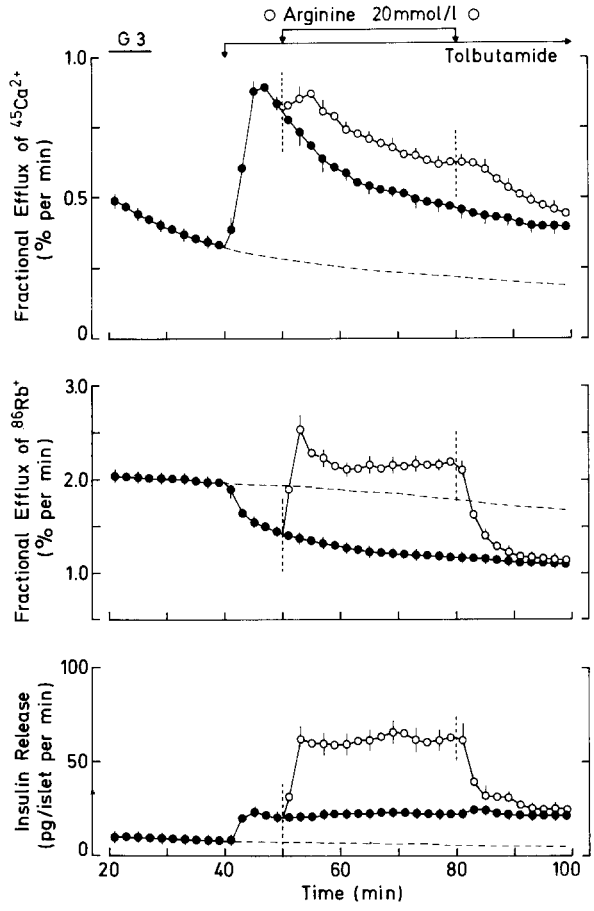

Fig. 4. Effects of tolbutamide and arginine on $^{45}Ca^{2+}$ efflux, $^{86}Rb^+$ efflux, and insulin release from mouse islets perifused with a medium containing 3 mmol/l glucose (G3). Tolbutamide (100 μmol/l) was added to all solutions from 40 min onwards. Arginine was added to test solutions (○) between 50 and 80 minutes. Control experiments without tolbutamide and arginine are shown by the dashed lines. Values are means ± SEM for 4–5 experiments

Effects of arginine in the presence of high K^+

Raising the concentration of K^+ to 14 mmol/l in a medium containing 3 mmol/l glucose markedly accelerated $^{86}Rb^+$ and $^{45}Ca^{2+}$ efflux, but stimulated insulin release only slightly (Fig. 6). Subsequent addition of arginine further increased $^{45}Ca^{2+}$ efflux, $^{86}Rb^+$ efflux, and insulin release. However, only the stimulation of $^{86}Rb^+$ efflux was rapid. The acceleration of $^{45}Ca^{2+}$ efflux was delayed and the increase in insulin release was progressive (Fig. 6). These two changes, unlike all other effects of arginine, were not well reversible upon withdrawal of the amino acid. Omission of extracellular Ca^{2+} abolished the changes in $^{45}Ca^{2+}$ efflux and insulin release brought about by high K^+ and arginine, but did not prevent the changes in $^{86}Rb^+$ efflux (not shown).

High K^+ depolarised the B-cell membrane by an average of 19.3 ± 0.6 mV, and arginine caused a further

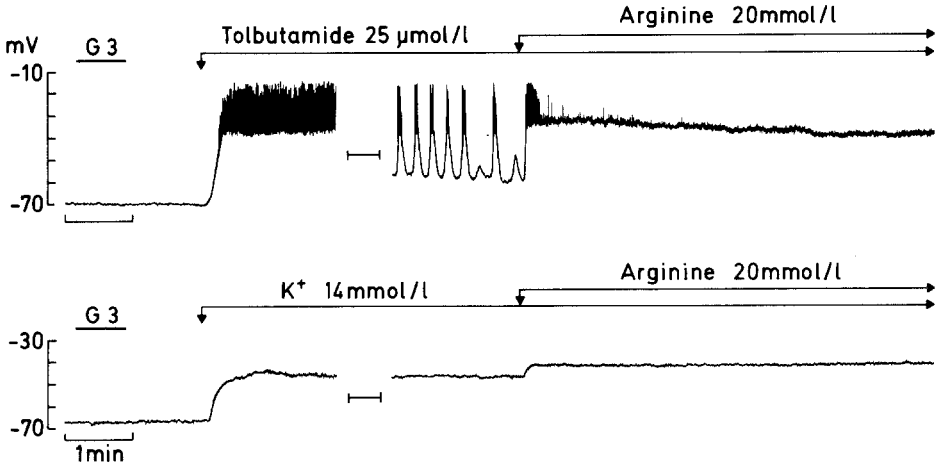

Fig. 5. Effects of tolbutamide and arginine (upper panel) or of high K^+ and arginine (lower panel) on the membrane potential of B cells perifused with a medium containing 3 mmol/l glucose (G3). The interruption in the two records corresponds to 6 min, and arginine was added to the medium 10 min after tolbutamide or high K^+. These records were obtained in different mice and are representative of results obtained in 5 and 6 experiments

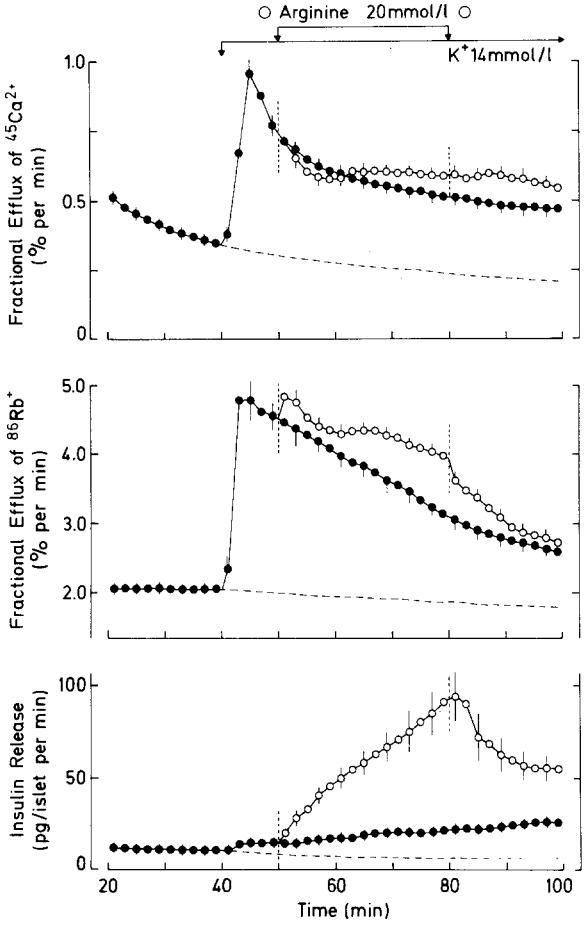

Fig. 6. Effects of high K^+ and arginine on $^{45}Ca^{2+}$ efflux, $^{86}Rb^+$ efflux, and insulin release from mouse islets perifused with a medium containing 3 mmol/l glucose. In all solutions, K^+ concentration was raised from 4.8 to 14 mmol/l from 40 min onwards. Arginine was added to test solutions (O) between 50 and 80 minutes. Control experiments with normal K^+ and without arginine are shown by the dashed lines. Values are means ± SEM for 4–5 experiments

depolarisation by 5.2 ± 0.4 mV ($n = 6$). No electrical activity occurred under these conditions (Fig. 5).

Diazoxide (100 µmol/l) did not impair arginine-induced insulin release in the presence of 14 mmol/l K^+ (1.85 ± 0.35 vs 1.33 ± 0.26 ng/islet. 30 min; $n = 4$).

Discussion

The prominent observations of the present study are that hyperpolarisation of the B-cell membrane by diazoxide prevents the permissive effect of glucose on arginine-induced insulin release, whereas depolarisation by tolbutamide or high K^+, at low glucose, partially mimicks that permissive effect.

Arginine enters mouse islet cells through a transport system that is specific for cationic amino acids and that is not affected by glucose or diazoxide [17]. The amino acid is practically not metabolised [18], and does not appear to have short-term effects on B-cell metabolism [19–21]. Our previous studies [22–24], recently supported by patch clamp experiments [21], have led us to suggest that arginine, and other cationic amino acids, depolarise the B-cell membrane because of their transport in a positively charged form, and that this depolarisation activates an influx of Ca^{2+} which eventually triggers insulin release. Diazoxide, on the other hand, hyperpolarises the B-cell membrane by increasing its permeability to K^+ [12, 25], without affecting glucose metabolism at the concentration used here [26]. At first glance it is thus surprising that diazoxide antagonized arginine-induced insulin release, since its mechanism of action should make it a specific inhibitor [27] of the secretory response to agents which depolarise the B-cell membrane by decreasing its permeability to K^+ (e.g. glucose, leucine, tolbutamide). However, the inhibition observed here does not result from an interference of diazoxide with the genuine effect of arginine, but from a suppression of the permissive effect of glucose. Thus, the changes in ionic fluxes, membrane potential and insulin release brought about by arginine in the presence of 10 mmol/l glucose and diazoxide were similar to those induced at 3 mmol/l glucose. This interpretation is fully supported by the observation that diazoxide did not impair the stimulation of insulin release by arginine in the presence of high K^+ (which depolarises not by decreasing K^+ permeability of the B-cell membrane, but simply by shifting the equilibrium potential for K^+).

It is well established that depolarisation of the B-cell membrane by glucose requires metabolic degradation of the sugar [for reviews: 28, 29]. By using diazoxide, it is possible to dissociate the metabolic changes from the membrane potential changes brought about by glucose. The obvious conclusion that emerges from the study is that the metabolic changes are not sufficient, whereas the depolarisation is necessary for glucose to exert its permissive action on arginine-induced insulin release. One could thus expect that the B-cell response to arginine would also be amplified when the B-cell membrane is depolarised by agents which, unlike glucose, do not serve as fuels and do not accelerate cellular metabolism. This prediction was indeed verified with tolbutamide and high K^+, the effect of glucose being more closely duplicated by the sulphonylurea, likely because they similarly affect the electrical properties of the B-cell membrane [12, 25, 28, 30]. Taken together, our observations therefore indicate that the depolarisation is necessary and sufficient, whereas changes in the metabolic state of B cells are neither sufficient nor necessary.

This does not imply, however, that the metabolic state of B cells has no influence on the secretory response to arginine. Thus, the amplitude, but not the rapidity, of this response still increased somewhat with the concentration of glucose, when the membrane potential was clamped at a hyperpolarised level by diazoxide. Furthermore, the insulin response to arginine was more than 2-fold larger in the presence of 7 mmol/l glucose, than in the presence of 3 mmol/l glucose and tolbutamide, in spite of a similar electrical activity in B cells. It thus seems that the fuel function of glucose somehow amplifies the response to the initiating signal of arginine.

Under all experimental conditions tested, arginine rapidly depolarised the B-cell membrane and accelerated $^{86}Rb^+$ efflux from islet cells. At low glucose or in the presence of high glucose and diazoxide, the depolarisation was similar to that produced by a threshold concentration of the sugar. Although slow waves or spikes never occurred, $^{45}Ca^{2+}$ influx was stimulated. This does not necessarily mean that arginine activates voltage-independent Ca channels [31]. Depolarisation by high K^+ also failed to evoke electrical activity. It is likely that the small fluctuations in membrane potential (noise) seen during arginine stimulation, and well studied during K^+-stimulation [32] are the electrical counterpart of the increase in Ca^{2+} influx. One should indeed keep in mind that slow waves and spikes occur only when a decrease in K^+ permeability contributes to the depolarisation of the B-cell membrane. Another puzzling observation is the discrepancy between the rapid increase in $^{45}Ca^{2+}$ influx and only delayed acceleration of $^{45}Ca^{2+}$ efflux, that arginine causes at low glucose or in the presence of high glucose and diazoxide. This acceleration of $^{45}Ca^{2+}$ efflux and the simultaneous small increase in insulin release could coincide with an overstepping of B cells ability to sequester entering Ca^{2+}. Alternatively, arginine could produce still unidentified, time-dependent, changes in B cells. The present study was not designed to answer these questions.

In contrast, when the B-cell membrane was depolarised by glucose or tolbutamide, arginine rapidly triggered spike activity, stimulated $^{45}Ca^{2+}$ influx, accelerated $^{45}Ca^{2+}$ efflux and induced insulin release. Comparison of the time-course and magnitude of all these changes at 7 or 10 mmol/l glucose shows that they correlate rather well. It is thus clear that under these conditions, arginine caused activation of voltage-dependent Ca channels.

In conclusion, two mechanisms appear to underlie the permissive action of glucose on arginine-stimulation of insulin release. The essential mechanism is the depolarisation of the B-cell membrane: it permits the small depolarising action of arginine to activate Ca channels more effectively. The greater influx of Ca^{2+} that ensues stimulates release more rapidly and efficiently. Another action of glucose, the nature of which is still unclear, increases the efficacy of the initiating signal triggered by arginine. The permissive action of non-metabolised secretagogues would only involve the first mechanism. These conclusions may also help to interpret observations made in vivo. Thus, it has been reported that, like glucose, tolbutamide can potentiate the insulin response to intravenous arginine in normal subjects [33, 34]. However, the potentiating effect of tolbutamide is less pronounced than that of a glucose concentration achieving a similar stimulation of B cells, and is greatly enhanced if glucose is also infused to counteract the hypoglycaemia otherwise produced by the sulphonylurea [10, 34].

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Dr. J. C. Henquin
Unité de Diabétologie et Nutrition
UCL 54.74
Avenue Hippocrate 54
B-1200 Brussels
Belgium