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REGULATION OF CALCIUM FLUXES IN RAT PANCREATIC ISLETS *

QUININE MIMICS THE DUAL EFFECT OF GLUCOSE ON CALCIUM MOVEMENTS

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Summary

The effects of quinine and 9-aminoacridine, two blockers of potassium conductance in islet cells, on ^{45}Ca efflux and insulin release from perifused islets were investigated in order to elucidate the mechanisms by which glucose initially reduces ^{45}Ca efflux and later stimulates calcium inflow in islet cells. In the absence of glucose, 100 μM quinine stimulated ^{45}Ca net uptake, ^{45}Ca outflow rate and insulin release. Quinine also dramatically enhanced the cationic and the secretory response to intermediate concentrations of glucose, but had little effect on ^{45}Ca net uptake, ^{45}Ca fractional outflow rate and insulin release at a high glucose concentration (16.7 mM). The ability of quinine to stimulate ^{45}Ca efflux depended on the presence of extracellular calcium, suggesting that it reflects a stimulation of calcium entry in the islet cells. In the absence of extracellular calcium, quinine provoked a sustained decrease in ^{45}Ca efflux. Such an inhibitory effect was not additive to that of glucose, and was reduced at low extracellular Na^+ concentration. At a low concentration (5 μM), quinine, although reducing ^{86}Rb efflux from the islets to the same extent as a non-insulinotropic glucose concentration (4.4 mM), failed to inhibit ^{45}Ca efflux. In the presence of extracellular calcium, 9-aminoacridine produced an important but transient increase in ^{45}Ca outflow rate and insulin release from islets perifused in the absence of glucose. In the absence of extracellular calcium, 9-aminoacridine, however, failed to reduced ^{45}Ca efflux from peri-

* This paper is the IXth in a series.
Abbreviation: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*'-tetraacetic acid.

fused islets. It is concluded that quinine, by reducing K^+ conductance, reproduces the effect of glucose to activate voltage-sensitive calcium channels and to stimulate the entry of calcium into the B-cell. However, the glucose-induced inhibition of calcium outflow rate, which may also participate in the intracellular accumulation of calcium, does not appear to be mediated by changes in K^+ conductance.

Introduction

Electrophysiological and radioisotopic studies suggest that cationic movements across the pancreatic B-cell membrane are of paramount importance in the mechanisms by which glucose stimulates the release of insulin [1,2]. First, it was proposed that a glucose-induced decrease in membrane permeability to potassium might be responsible for the initial depolarization of the B-cell and, hence, for the gating of channels mediating the entry of calcium in islet cells [2,3]. The resulting increase in cytosolic calcium concentration is thought to trigger insulin release [1]. Second, at the same time when glucose reduces potassium efflux, the sugar also decreases calcium efflux from the islet cells [4,5]. The latter change was recently proposed to reflect a glucose-induced inhibition of Na-Ca countertransport, itself responsible for calcium extrusion from the islet cells [6]. The inhibition of calcium efflux may contribute to the glucose-induced accumulation of calcium in islet cells [4,6]. In a previous study, it was observed that a K^+-induced depolarization mimics the effect of glucose to stimulate calcium influx, but fails to reproduce the inhibitory effect of glucose upon calcium outflow [7]. In agreement with these observations, it was also found that tetraethylammonium, a selective blocker of K^+ permeability [8], acts in synergism with glucose to facilitate calcium entry into islet cells, but is unable to reproduce the effect of glucose to decrease calcium outflow from the islet cells [9]. Tetraethylammonium, however, is a weak inhibitor of K^+ conductance and does not stimulate insulin release in the absence of glucose [9,10]. The aim of the present study was to characterize further the inter-relationship between K^+ conductance and calcium movements in the process of glucose-induced insulin release. For this purpose, we have investigated the effects of quinine and 9-aminoacridine on ^{45}Ca efflux and insulin release from perifused rat pancreatic islets. Both drugs have been shown to reduce K^+ conductance in islet cells more efficiently than tetraethylammonium and to stimulate the release of insulin from these cells in the absence of glucose [11,12].

Materials and Methods

All experiments were performed with islets isolated by the collagenase technique [13] from the pancreas of fed albino rats.

Incubation, washing and perifusion media. The media used for incubating, washing or perifusing the islets consisted of a Krebs-Ringer bicarbonate-buffered solution supplemented with 0.5% (w/v) dialyzed albumin (Fraction V; Sigma Chemical Co., St. Louis, MO) and equilibrated against a mixture of

O_2 (95%) and CO_2 (5%). Some media contained no $CaCl_2$ and were enriched with 0.5 mM EGTA. In some experiments, NaCl (115 mM) was replaced by sucrose (220 mM) in order to maintain close to normal osmotic pressure. The media also contained, when required, glucose.

Experimental procedure

Insulin release and ^{45}Ca efflux from perifused islets. The method used for the measurement of ^{45}Ca efflux and insulin release from perifused islets is described in detail elsewhere [14]. Briefly, groups of 100 islets were incubated for 60 min in the presence of ^{45}Ca (1.12 mM; 0.2 mCi/ml) and glucose 16.7 mM. After incubation, the islets were washed three times and eventually placed in a perifusion chamber. The perifusate was delivered at a constant rate (1.0 ml/min). From the 31st to the 90th minute the effluent was continuously collected over successive 1 min periods. An aliquot of the effluent (0.4 ml) was used for scintillation counting while the remainder was stored at – 20°C for insulin assay. At the end of the perifusion, the radioactive content of the islets was also determined. The efflux of ^{45}Ca (cpm per min) was expressed as a fractional outflow rate (% of instantaneous islet content per min).

^{86}Rb efflux from perifused islets. The method used for the measurement of ^{86}Rb efflux from perifused islets is detailed in a previous publication [15]. Briefly, groups of 100 islets were incubated in the presence of ^{86}Rb (0.3—0.6 mM; 100 μCi/ml) and glucose (16.7 mM). After incubation, the islets were washed three times and then placed in a perifusion system similar to that used for the measurement of ^{45}Ca efflux. From the 7th to the 40th minute, the effluent was continuously collected over successive 1 min periods and examined for its radioactive content by Cerenkov counting. The efflux of ^{86}Rb efflux is also expressed as a fractional outflow rate. The validity of ^{86}Rb as a tracer for the study of $^{39}K^+$ handling in the islets has been assessed elsewhere [15].

Insulin release by incubated islets. Groups of eight islets were incubated for 90 min in 1.0 ml of incubation medium, the release of insulin being measured as described elsewhere [16].

^{45}Ca net uptake. The method used for the measurement of ^{45}Ca net uptake by the islets is described in detail elsewhere [17]. Briefly, groups of 100 islets were incubated for 90 min in the presence of ^{45}Ca. They were then washed four times and then transferred in subgroups of eight islets to counting vials.

^{86}Rb and ^{22}Na uptake. Groups of 10 islets were placed in polythene microcentrifuge tubes and incubated for 30 min at 37°C in 0.05 ml of a non-radioactive medium containing sucrose (1.0 mM). A second aliquot (0.05 ml) of medium enriched with [^{3}H]sucrose and ^{86}Rb or ^{22}Na was then added to the tubes. After incubation for 90 min the islets were separated from the surrounding medium by centrifugation through a layer of di(*n*-butyl)phthalate. The net uptake of ^{86}Rb or ^{22}Na was corrected for contamination by extracellular radioactivity as described elsewhere [15,18].

Oxidation of nutrients by isolated islets. For the measurement of glucose oxidation, groups of 15 islets were incubated for 120 min in small glass tubes placed in the counting vials. The incubation media (0.06 ml) contained [U-^{14}C]glucose (3—10 μCi/ml). As described elsewhere [19], at the end of the

incubation, 0.1 ml HCl (0.2 M) and 0.2 ml hyamine hydroxide were injected through the rubber stopper in the glass tube containing the incubation medium and the counting vial, respectively. The method used to measure $^{14}CO_2$ output from islets prelabelled with [U-^{14}C]palmitate is described in detail elsewhere [20].

Insulin biosynthesis. Protein and insulin biosynthesis by the islets was estimated by using a technique described in a previous publication [21]. Briefly, after incubation for 90 min in the presence of L-[^{3}H]leucine (4.2 μCi/ml), groups of 60 islets were repeatedly washed, transferred in groups of 10 in a phosphate buffer, and sonicated. Protein synthesis was estimated after trichloroacetic acid precipitation of an aliquot of the homogenate. Newly synthesized insulin was characterized after reaction of the homogenate with an excess of guinea-pig anti-insulin serum. In each set of experiments, insulin biosynthesis was also expressed in per cent of total protein synthesis.

Lactate dehydrogenase activity in islets and incubation medium. The method used for the measurement of lactate dehydrogenase has been described in detail elsewhere [22].

Calculations and statistics. All results are expressed as the mean (±S.E.) together with the number of individual determinations or experiments (n). The statistical significance of differences between mean experimental and control data was assessed by use of Student's t-test.

Results

Effect of quinine upon ^{45}Ca efflux and insulin release from islets perifused in the absence of glucose. In the absence of glucose, quinine provoked a modest but sustained increase in insulin release (Fig. 1, lower panel). The effect of quinine was neither immediate nor rapidly reversible. Thus, a significant increase ($P < 0.05$) in insulin release above basal value (39th to 44th minute) was only observed 6 min after exposure of the islets to quinine, while the removal of the drug from the perifusate produced a transient increase which was not followed by any immediate reduction in insulin output. Quinine (100 μM) induced a dual modification of ^{45}Ca efflux: a small and short-lived initial fall was followed by a marked but transient increase in ^{45}Ca efflux (Fig. 1, upper panel). The secondary increase in ^{45}Ca efflux occurred concomitantly with insulin release and reached during the 55th to 57th minute of perifusion a maximal value averaging 190.7 ± 3.7% ($P < 0.001$) of the mean value found during the 5 min period preceding the addition of quinine to the perifusate. The efflux then rapidly declined. Removal of the drug produced first a transient and modest increase followed by a further decline in the ^{45}Ca fractional outflow rate.

When the same experiment was carried out in the absence of extracellular Ca^{2+} and in the presence of EGTA, quinine only induced a monophasic reduction in the ^{45}Ca fractional outflow rate (Fig. 2, upper panel). Thus, the mean slope of the regression line characterizing the changes in ^{45}Ca fractional outflow rate as a function of time was steeper in the presence (—0.022 ± 0.004%/min^2; minute 44—57) than in the absence (—0.006 ± 0.001%/min^2; minute 31—44) of quinine ($P < 0.01$). The inhibitory effect of quinine on the ^{45}Ca fractional

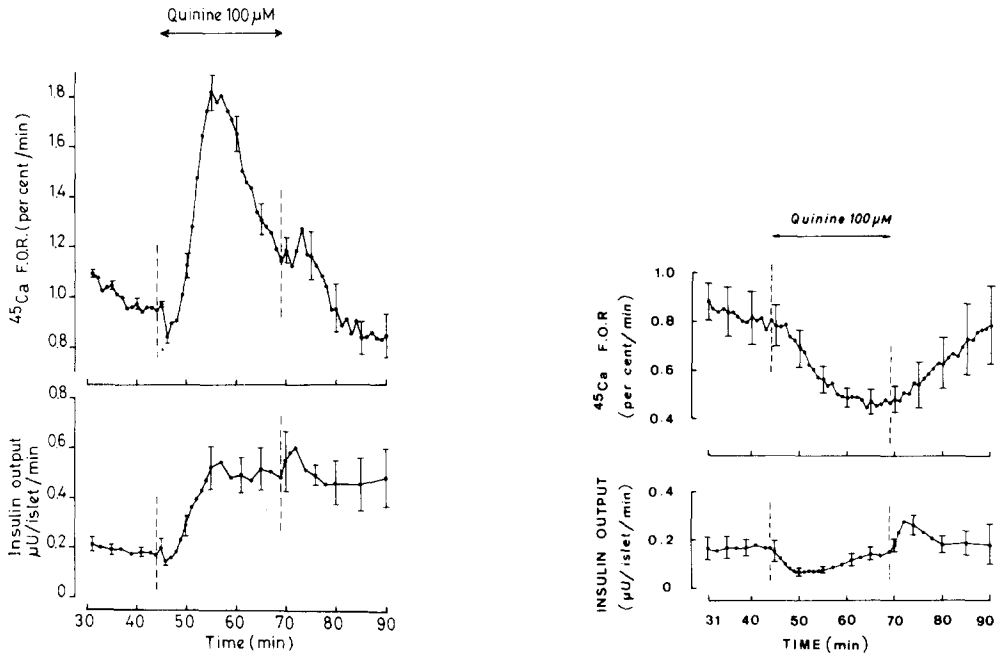

Fig. 1. Effect of quinine (100 μM) upon ^{45}Ca efflux (upper panel) and insulin release (lower panel) from perifused islets. Basal medium contained calcium (1 mM) and was deficient in glucose. Mean values (±S.E.) for ^{45}Ca efflux are expressed as a fractional outflow rate (F.O.R.) (see Materials and Methods) and refer to four individual experiments. Mean values (±S.E.) for insulin output are expressed in μU/islet per min and refer to the same four individual experiments.

Fig. 2. Effect of quinine (100 μM) upon ^{45}Ca efflux (upper panel) and insulin release (lower panel) from islets perifused in the absence of glucose and extracellular calcium. Mean values (±S.E.) for ^{45}Ca efflux and insulin release are expressed as in Fig. 1 and refer to four individual experiments.

outflow rate appeared as a slowly developing and reversible phenomenon. In these experiments, quinine only provoked minor changes in insulin output, which occurred at a close-to-basal rate in the Ca^{2+}-deficient media (Fig. 2, lower panel).

Effect of glucose on ^{45}Ca efflux and insulin release from islets perifused in the presence of quinine. After 39—44 min exposure of the islets to a medium containing quinine (100 μM) and calcium (1 mM) but deficient in glucose, the rate of insulin release averaged 0.17 ± 0.05 μU/islet per min, a value comparable ($P > 0.5$) to that observed at the same time in the absence of quinine (compare lower panels of Figs. 1 and 3). Addition of glucose (16.7 mM) produced an increase in insulin release which was only partially and lately reversible. After 40—44 min of perifusion in the presence of quinine, the ^{45}Ca fractional outflow rate averaged 0.81 ± 0.09% per min, a value comparable to that observed in the absence of quinine (Fig. 3, upper panel). In the presence of the drug, glucose (16.7 mM) produced an initial fall and a secondary rise in ^{45}Ca efflux. Thus, about 3 min after the addition of quinine, a significant decrease ($P < 0.025$) in ^{45}Ca efflux occurred relative to the mean control value found within the same experiment between the 40th and 44th

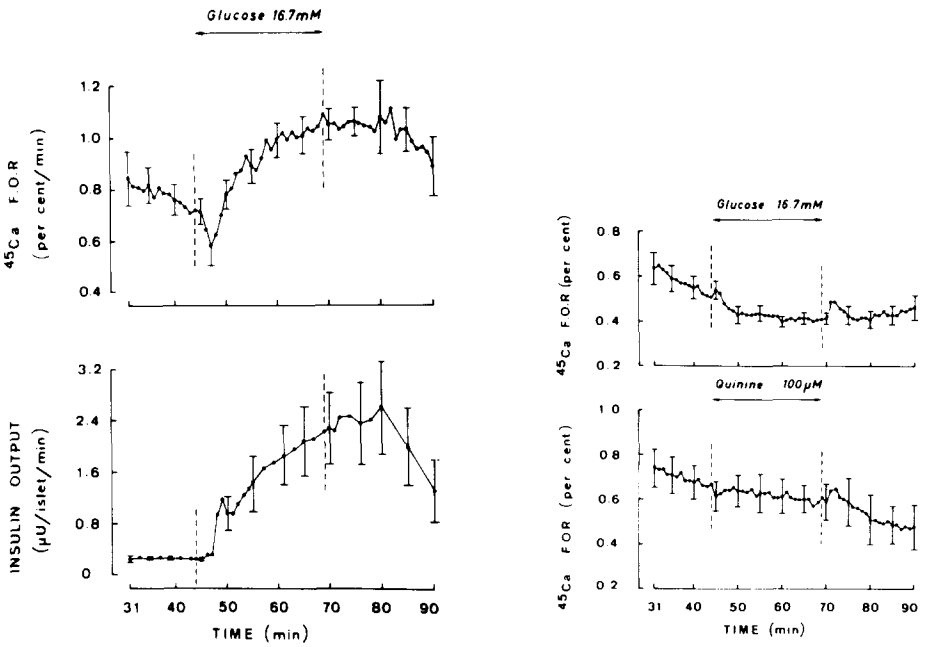

Fig. 3. Effect of a rise in the glucose concentration from 0 to 16.7 mM upon ^{45}Ca efflux (upper panel) and insulin release (lower panel) from islets perifused in the presence of quinine (100 μM). Basal medium contained calcium (1 mM). Mean values (±S.E.) for ^{45}Ca efflux and insulin release are expressed as in Fig. 1 and refer to six individual experiments.

Fig. 4. (Upper panel) Effect of a rise in the glucose concentration from 0 to 16.7 mM on ^{45}Ca efflux from islets perifused in the presence of quinine (100 μM). (Lower panel) Effect of quinine (100 μM) on ^{45}Ca efflux from islets perifused in the presence of glucose (16.7 mM). Under both experimental conditions, basal media were deficient in calcium. Mean values (±S.E.) for ^{45}Ca efflux are expressed as in Fig. 1 and refer to four or ten individual experiments.

minute of perifusion. As in the case of insulin release, the late increase in ^{45}Ca fractional outflow rate was not immediately reversible.

After prolonged exposure (40—44 min) of the islets to quinine (100 μM) in a medium deficient in calcium and glucose, the fractional outflow rate of ^{45}Ca averaged 0.52 ± 0.041% per min, a value significantly lower ($P < 0.025$) than that observed at the same time in the absence of quinine (compare upper panels of Figs. 2 and 4). Glucose (16.7 mM) had little effect upon ^{45}Ca fractional outflow rate in the Ca^{2+}deficient islets exposed to quinine. At most, glucose provoked a slight decrease in ^{45}Ca fractional outflow rate and, conversely, a slight but sustained increase in ^{45}Ca efflux was seen upon removal of the sugar from the perifusate.

Effect of quinine (100 μM) upon ^{45}Ca efflux and insulin release from islets perifused in the presence of glucose. In the presence of glucose (16.7 mM) and Ca^{2+} (1 mM), the rate of insulin release as measured during the 39th to 44th minute of perifusion averaged 2.1 ± 0.2 μU/islet per min (Fig. 5, lower panel). Quinine (100 μM) produced an immediate and sustained, but not rapidly reversible, potentiation of glucose-induced insulin release. Under the latter experimental conditions, quinine (100 μM) induced a modest and transient

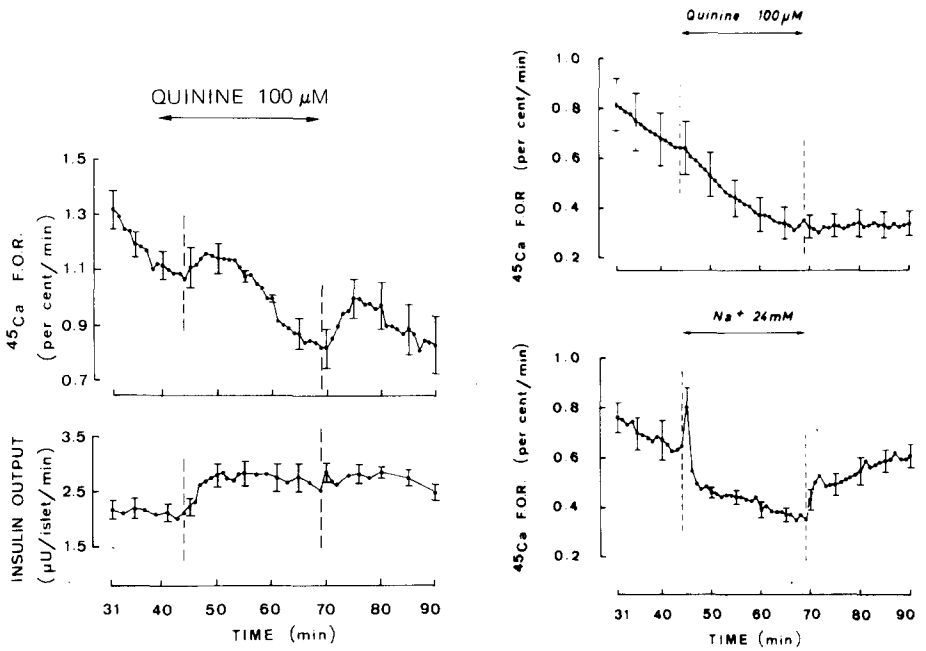

Fig. 5. Effect of quinine (100 μM) upon ^{45}Ca efflux (upper panel) and insulin release (lower panel) from islets perifused in the presence of glucose (16.7 mM). Basal medium contained calcium (1 mM). Mean values (±S.E.) for ^{45}Ca efflux and insulin release are expressed as in Fig. 1 and refer to 10 individual experiments.

Fig. 6. (Upper panel) Effect of quinine (100 μM) on ^{45}Ca efflux from islets perifused at low extracellular Na concentration (24 mM). (Lower panel) Effect of a low extracellular Na concentration (24 mM) on ^{45}Ca efflux from islets perifused in the presence of quinine (100 μM). Under both experimental conditions, basal media were deficient in calcium and glucose. Mean values (±S.E.) for ^{45}Ca efflux and insulin release are expressed as in Fig. 1 and refer to four to six individual experiments.

increase in ^{45}Ca fractional outflow rate. When the drug was removed from the perifusate, a sustained increase in ^{45}Ca fractional outflow rate was noticed. In the absence of extracellular calcium, quinine failed to affect ^{45}Ca fractional outflow rate from islets perifused in the presence of 16.7 mM glucose (Fig. 4, lower panel).

Effect of quinine (100 μM) upon ^{45}Ca efflux from islets perifused at low extracellular sodium concentration. When the islets were perifused in the absence of extracellular Ca^{2+} and at low extracellular Na^+ concentration (24 mM), the ^{45}Ca fractional outflow rate, as recorded during the 40th to 44th minute of perifusion, was slightly lower than at normal extracellular Na^+ concentration (compare upper panels of Figs. 2 and 6). Under the former experimental condition, quinine did not exert any obvious effect upon ^{45}Ca fractional outflow rate (Fig. 6, upper panel). Thus, the mean slope of the regression lines characterizing the changes in ^{45}Ca fractional outflow rate as a function of time averaged $-0.014 \pm 0.001\%/min^2$ in the absence (minute 31—44) and $-0.019 \pm 0.03\%/min^2$ in the presence (minute 44—57) of quinine ($P > 0.1$).

Effect of a low extracellular concentration of sodium upon ^{45}Ca efflux

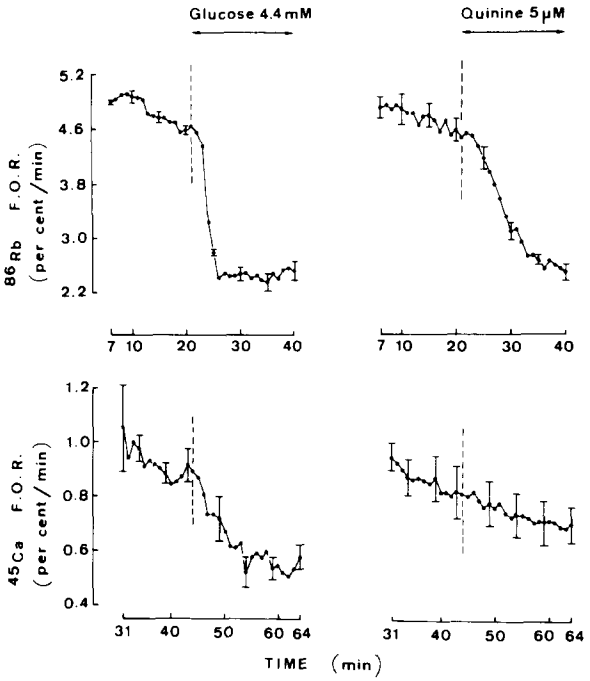

Fig. 7. Effect of 4.4 mM glucose (left panels) and quinine 5 μM (right panels) on ^{86}Rb efflux (upper panels) and ^{45}Ca efflux (lower panels) from islets perifused in the presence of extracellular calcium. Mean values (±S.E.) for ^{86}Rb and ^{45}Ca efflux are expressed as a fractional outflow rate (F.O.R.) and refer to three to four individual experiments.

from islets perifused in the absence of extracellular calcium and in the presence of quinine. In the absence of extracellular calcium but in the presence of quinine, the ^{45}Ca fractional outflow rate as recorded during the 40th to 44th minute of perifusion was slightly lower than in the absence of quinine (compare Fig. 6, lower panel, with Fig. 2, upper panel). Lowering the extracellular Na^+ concentration from 139 to 24 mM further reduced ^{45}Ca fractional outflow rate. The latter effect was rapidly reversible upon return to a normal extracelular Na^+ concentration.

Effect of glucose (4.4 mM) and quinine (5 μM) upon ^{86}Rb and ^{45}Ca efflux. At a concentration of 5 μM, quinine produced a sustained decrease in ^{86}Rb efflux (Fig. 7, upper right-hand panel). The magnitude of such a decrease was comparable to that induced by 4.4 mM glucose (Fig. 7, upper left-hand panel). Indeed, the mean value of efflux recorded during the 14th to 19th minute of perifusion in the presence of quinine was not significantly different from that observed at the same time in the presence of 4.4 mM glucose ($P > 0.5$). At the same low concentration (5 μM), quinine failed to affect ^{45}Ca efflux from islets perifused in the presence of extracellular calcium (1 mM; Fig. 7, lower right-hand panel), whilst glucose (4.4 mM) provoked an immediate and sustained decrease in ^{45}Ca efflux (Fig. 7, lower left-hand panel).

Effect of 9-aminoacridine upon ^{45}Ca efflux and insulin release. In the presence of extracellular calcium (1 mM), 0.5 mM 9-aminoacridine produced an important but transient increase in insulin release from islet perifused in the

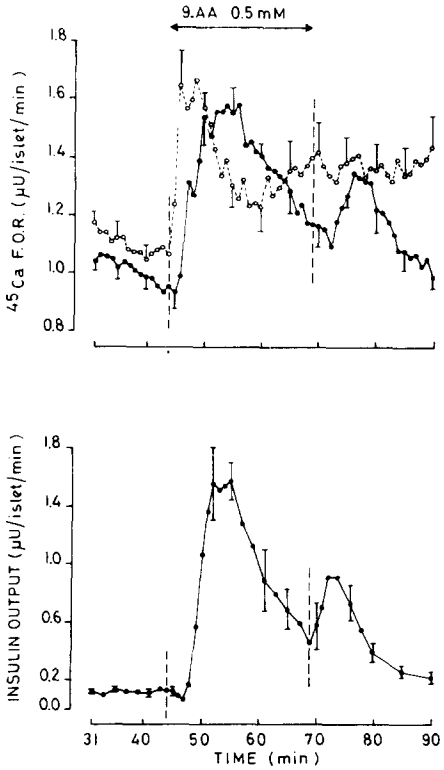

Fig. 8. Effect of 9-aminoacridine upon ^{45}Ca efflux (upper panel) and insulin release (lower panel) from islets perifused either in the presence (closed circles) or the absence (open circles) of extracellular calcium. Basal media contained no glucose. Mean values (±S.E.) for ^{45}Ca efflux and insulin release are expressed as in Fig. 1 and refer to four to eight individual experiments. 9-AA, 9-aminoacridine.

absence of glucose (Fig. 8). The secretory response was not immediate, a significant increase in the rate of insulin above basal value (minute 39—44) being only observed after 5 min exposure to 9-aminoacridine ($P < 0.025$). Removal of the drug was followed by a transient increase in insulin output. The changes in ^{45}Ca efflux induced by 9-aminoacridine were similar to those in insulin release. Indeed, addition of 9-aminoacridine produced a somewhat delayed, important but transient increase in ^{45}Ca fractional outflow rate. Similarly, an increase in ^{45}Ca efflux was observed on removal of the drug from the perifusate.

When the islets were perifused in the absence of extracellular calcium, 9-aminoacridine, in contrast to quinine, still provoked an important increase in ^{45}Ca efflux. The latter increase occurred more rapidly than in the presence of extracellular calcium and was not rapidly reversible (Fig. 8).

Effect of quinine upon insulin release and other variables in incubated islets. Quinine failed to affect the integrity of the islet cells, as judged from the release of lactate dehydrogenase in the incubation medium. When groups of 20 islets were incubated for 90 min in 50 μl of a medium containing glucose (8.3 mM) and increasing concentrations of quinine, the enzyme activity measured in the incubation medium averaged 0.10 ± 0.02, 0.12 ± 0.01, 0.12 ±

TABLE I

EFFECT OF QUININE AND TETRAETHYLAMMONIUM ON GLUCOSE-INDUCED INSULIN RELEASE BY INCUBATED ISLETS

Insulin release is expressed in μU/islets per 90 min.

Glucose (mM)	Control	Quinine (0.1 mM)	Quinine (0.1 mM) + tetraethylammonium (10 mM)
0	26.2 ± 3.0 (28)	27.8 ± 2.0 (18)	15.9 ± 2.6 (10)
5.6	37.7 ± 5.1 (29)	113.7 ± 5.5 (20)	120.7 ± 2.9 (10)
8.3	102.0 ± 8.9 (29)	206.9 ± 7.1 (31)	209.5 ± 3.2 (10)
11.1	224.3 ± 6.0 (29)	268.9 ± 10.8 (29)	264.9 ± 7.6 (10)
16.7	292.4 ± 11.8 (18)	300.1 ± 10.6 (18)	295.4 ± 15.1 (8)

TABLE II

EFFECT OF QUININE UPON DIFFERENT PARAMETERS OF ISLET FUNCTION

The net uptake of [U-^{14}C]palmitate occurred during a 120 min preincubation performed in the absence of quinine. The output of $^{14}CO_2$ from the prelabelled islets was measured over the ensuing 120 min of incubation performed in the absence or presence of quinine. TCA, trichloroacetic acid. IRI, immunoreactive insulin.

Parameter	Glucose (mM)	Quinine (mM)		
		0	0.1	
[U-^{14}C]Palmitate uptake (pmol/islet per 120 min)	8.3	1.60 ± 0.17 (10)	1.60 ± 0.12 (10)	
$^{14}CO_2$ output from prelabelled islets (cpm/islet per 120 min)	0	56.5 ± 6.5 (10)	52.5 ± 4.0 (10)	
[U-^{14}C]Glucose oxidation (pmol/islet per 120 min)	5.6	14.3 ± 1.9 (8)	12.1 ± 2.5 (8)	
	11.1	49.4 ± 4.7 (8)	46.1 ± 2.8 (8)	
	16.7	73.1 ± 3.9 (8)	70.7 ± 5.4 (8)	
^{45}Ca net uptake (pmol/islet at 90th minute)	0	1.53 ± 0.12 (27)	2.67 ± 0.18 (22)	***
	5.6	2.08 ± 0.11 (12)	3.40 ± 0.18 (12)	***
	8.3	2.39 ± 0.14 (12)	4.66 ± 0.27 (11)	***
	11.1	4.40 ± 0.25 (11)	5.38 ± 0.19 (12)	**
	16.7	5.63 ± 0.39 (12)	5.83 ± 0.38 (12)	
^{86}Rb net uptake (pmol/islet at 90th minute)	0	147 ± 10 (15)	160 ± 11 (15)	
	2.8	194 ± 17 (8)	193 ± 15 (8)	
	16.7	230 ± 17 (16)	225 ± 16 (15)	
^{22}Na net uptake (pmol/islet at 90th minute)	0	82 ± 23 (9)	140 ± 26 (8)	
[^{3}H]Leucine incorporation in TCA-precipitable material (fmol/islet per 120 min)	2.8	140 ± 13 (10)	162 ± 12 (10)	
	8.3	237 ± 18 (10)	244 ± 13 (10)	
	16.7	542 ± 32 (10)	551 ± 21 (10)	
[^{3}H]Leucine incorporation in immunoreactive peptides (fmol/islet per 120 min)	2.8	20 ± 2 (10)	27 ± 2 (10)	*
	8.3	46 ± 3 (10)	49 ± 2 (10)	
	16.7	104 ± 8 (10)	118 ± 4 (10)	
[^{3}H]IRI/[^{3}H]TCA-precipitable material (%)	2.8	14.8 ± 1.8 (10)	17.1 ± 0.8 (10)	
	8.3	19.6 ± 1.0 (10)	20.4 ± 1.0 (10)	
	16.7	19.3 ± 1.0 (10)	21.6 ± 0.7 (10)	

* $P < 0.025$.
** $P < 0.005$.
*** $P < 0.001$.

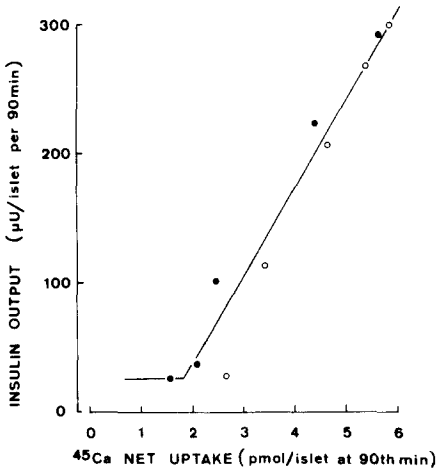

Fig. 9. Relationship between insulin output and ^{45}Ca net uptake in islets incubated in the presence of glucose (0, 5.6, 8.3, 11.1 and 16.7 mM) with (open circles) or with (closed circles) quinine (100 μM). The horizontal line in the lower left corner corresponds to the basal insulin output, whereas the ascending line was calculated by regression analysis.

0.01 and 0.12 ± 0.01 nmol/islet per h ($n = 8$ in all cases) in the absence and presence of 5, 100 and 500 μM quinine, respectively. Under the same experimental conditions, the enzyme activity of the islets themselves as measured in tissue homogenates averaged 21.8 ± 2.1, 30.1 ± 4.1, 26.2 ± 3.0 and 23.3 ± 2.1 nmol/islet per h. Thus, over 90 min incubation, the leakage of enzyme represented no more than 0.4—0.5% of the tissue content, and was unaffected by the presence of quinine.

Over prolonged incubations of 90 min, quinine (0.1 mM) failed to significantly affect the basal release of insulin, but augmented glucose-stimulated insulin output. The enhancing effect of quinine upon insulin release faded out at high glucose concentrations, Tetraethylammonium (10.0 mM) did not further enhance insulin release evoked by glucose in the presence of quinine (Table I).

Quinine (0.1 mM) failed to affect both $^{14}CO_2$ output from islets prelabelled with [U-^{14}C]palmitate and [U-^{14}C]glucose oxidation (Table II).

Quinine significantly augmented ^{45}Ca net uptake in the absence or presence of glucose, except at a very high concentration of the sugar (16.7 mM). There was a highly significant correlation between insulin output and ^{45}Ca net uptake ($r = 0.972$; $P < 0.001$; Fig. 9). Quinine failed to affect significantly ^{86}Rb net uptake, whether in the absence or presence of glucose. There was a trend for quinine to augment ^{22}Na net uptake in glucose-deficient islets; within the same experiments, ouabain augmented ^{22}Na net uptake from a basal value of 82 ± 23 to 224 ± 48 pmol/islet per 90 min ($n = 9$ in both cases; $P < 0.02$).

Quinine slightly increased proinsulin biosynthesis at a low glucose concentration (2.8 mM), whilst failing to affect significantly the islet biosynthetic activity at higher concentrations of glucose.

Discussion

Quinine, ^{45}Ca net uptake and insulin release. At the concentration of 100 μM, quinine has been shown to reduce K^+ permeability from glucose-deficient islets to the same extent as glucose at a concentration of 16.7 mM [11]. At this concentration, the drug was also reported to depolarize the membrane of B-cells perifused in the absence of glucose and to induce continuous electrical activity [23]. The present results show that 100 μM quinine produced a moderate but significant increase in insulin release from islets perifused in the absence of glucose. Like the electrical activity, the secretory response evoked by quinine was monophasic, delayed and not rapidly reversible. However, it represented a transient phenomenon, since insulin release occurred at basal value after prolonged exposure (45 min) of the islets to the drug (Fig. 3). The latter observation, taken together with the modest magnitude of the initial secretory response to the drug, may explain why no significant effect of quinine upon insulin release was detected over prolonged static incubation (90 min, see Table I). During such static incubation, quinine shifted to the left the sigmoidal curve normally relating insulin output to the ambient glucose concentration. Thus, quinine dramatically enhanced the insulinotropic potency of intermediate glucose concentrations (5.6 and 8.3 mM). This enhancing effect was less pronounced at a higher glucose concentration (11.1 mM) and virtually absent at 16.7 mM glucose.

In good agreement with these secretory data, quinine produced a modest increase in ^{45}Ca net uptake in the absence of glucose and markedly enhanced ^{45}Ca net uptake at intermediate glucose concentrations (5.6—8.3 mM). The drug exerted a lesser enhancing action upon ^{45}Ca net uptake in islets exposed to 11.1 mM glucose and failed to affect ^{45}Ca net uptake when the islets were incubated in the presence of 16.7 mM glucose. Taken as a whole, these data suggest that quinine, by reducing the B-cell membrane conductance to potassium, reproduces to a limited extent the effect of glucose to stimulate calcium entry into the islet cells.

Quinine and the glucose induced increase in ^{45}Ca efflux. In previous reports, it was shown that the secondary rise in ^{45}Ca efflux induced by glucose depends on the ability of the sugar to decrease potassium conductance and to depolarize the B-cell membrane [7,9]. This secondary rise in ^{45}Ca efflux was proposed to reflect an increased entry of calcium through gated calcium channels, leading to a process of calcium-calcium exchange [14,24]. The idea that quinine acts in a comparable manner is supported by the effect of the drug on ^{45}Ca efflux as characterized in the present study. When the islets were perifused in the absence of glucose, quinine induced a dramatic increase in ^{45}Ca efflux. This increase, like that evoked by glucose, apparently reflected a stimulation of calcium entry into the islet cells. Indeed, when the same experiments were carried out in the absence of extracellular calcium, quinine failed to stimulate ^{45}Ca efflux. Furthermore, quinine only produced a modest and transient enhancement of ^{45}Ca efflux when the islets were already exposed to glucose. The latter finding argues for a common mechanism of action of quinine and glucose to stimulate calcium entry via a change in K^+ conductance.

The increase in ^{45}Ca efflux induced by quinine was a transient phenomenon.

This is indicated both by the rapid decline in ^{45}Ca efflux observed after about 15 min of exposure of the islets to the drug (Fig. 1) and by the absolute value for the ^{45}Ca fractional outflow rate which, after the 40th to 44th minute of perifusion in the presence of quinine, was similar to that observed in control experiments carried out in the absence of the drug. Such a transient effect of quinine on ^{45}Ca efflux, which is consistent with the short-lasting action of the drug on insulin release, is apparently not due to any untoward effect of quinine on the viability or metabolism of islet cells. Quinine failed to increase the release of lactate dehydrogenase from the islet cells. The drug also failed to impair endogenous nutrient metabolism, glucose-induced proinsulin biosynthesis and glucose oxidation in the islets. The transient nature of the quinine-induced stimulation of insulin release and ^{45}Ca efflux contrasts with the sustained inhibitory effect of the drug upon ^{86}Rb outflow (data not shown) and could suggest a late refractoriness of the calcium channels. In frog auricular fibres, quinine impairs the reactivation kinetics of the slow inward current and reduces calcium conductance [25].

Like quinine, 9-aminoacridine stimulated insulin release and ^{45}Ca outflow rate from islets perifused in the presence of extracellular Ca^{2+}. However, the relevance of these findings to changes in the rate of calcium entry into islet cells may be questioned, since the drug-induced increase in ^{45}Ca efflux was not abolished in the absence of extracellular Ca^{2+}. This suggests that 9-aminoacridine, as distinct from quinine, may also affect the intracellular distribution of Ca^{2+}.

Quinine and the glucose-induced decrease in ^{45}Ca efflux. The increase in ^{45}Ca efflux was not the sole effect of quinine upon ^{45}Ca outflow from the perifused islets. When the islets were perifused in the presence of extracellular Ca^{2+}, the increase in ^{45}Ca efflux was preceded by a modest inhibition of ^{45}Ca efflux (Fig. 1). Such a biphasic response is strikingly similar to that evoked by glucose [4,5,26]. In the presence of extracellular Ca^{2+}, the initial fall in ^{45}Ca efflux observed in response to quinine appeared transient. However, the inhibitory effect of quinine, like that of glucose, was truly a sustained phenomenon masked by the later increase in ^{45}Ca efflux. Indeed, when the islets were perifused in the absence of extracellular calcium, quinine reduced ^{45}Ca efflux in a sustained and reversible manner. The inhibitory effect of quinine upon ^{45}Ca efflux from islets deficient in extracellular Ca^{2+} did not reach rapidly its full magnitude; the progressive time course for such an inhibitory effect resembled that characterizing the effect of the drug upon K^+ conductance [11].

These comparisons could be taken as evidence that glucose reduces ^{45}Ca efflux as a consequence of its action on K^+ conductance. Such an interpretation would appear compatible with the observation that the inhibitory effects of quinine and glucose on ^{45}Ca efflux were not additive to one another. Quinine failed to reduce ^{45}Ca efflux from islets perifused in the presence of 16.7 mM glucose (Fig. 4) and, conversely, the inhibitory effect of glucose on ^{45}Ca efflux, although not totally abolished, was minimized when the islets were already exposed to quinine (Fig. 4).

However, several findings indicate that, despite their analogy, the inhibitory effects of glucose and quinine upon ^{45}Ca efflux cannot be ascribed to a change in K^+ conductance. Firstly, when used at a low concentration (5 μM), quinine

failed to reduce ^{45}Ca outflow despite a sizeable decrease in K^+ conductance. Secondly, 9-aminoacridine, which inhibits ^{86}Rb outflow to the same extent as quinine (data not shown), also failed to provoke an initial fall in ^{45}Ca fractional outflow rate. Finally, no inhibition of ^{45}Ca outflow occurs when K^+ conductance is decreased by used of tetraethylammonium [9].

In a previous report in this series [6], we suggested that glucose may exert its inhibitory effect on ^{45}Ca efflux from perifused islets by reducing calcium extrusion by Na-Ca countertransport. The present finding that the inhibitory effect of quinine upon ^{45}Ca efflux was impaired when the islets were perifused at a low extracellular Na^+ concentration (24 mM) strongly suggests that quinine also reduces ^{45}Ca efflux by inhibiting Na-Ca countertransport. This may explain why the inhibitory effects of glucose and quinine on ^{45}Ca efflux were not additive. Our data are compatible, therefore, with the view that quinine exerts its inhibitory effect on ^{45}Ca efflux by interfering directly (i.e., independently of its effect on potassium conductance) with the process of Na-Ca countertransport. Such a concept is supported by the recent observation that quinine at the concentration of 100 μM inhibits Na-Ca countertransport in an artificial system for the study of ionophoresis [27]. The ability of quinine to reduce Na-Ca countertransport in islet cells seems less pronounced, however, than that of 16.7 mM glucose, since a reduction of the extracellular Na^+ concentration from 139 to 24 mM further reduced ^{45}Ca efflux from islets perifused in the presence of quinine.

In conclusion, quinine, by reducing K^+ conductance in islet cells, reproduces the effect of and acts synergistically with glucose to activate the voltage-sensitive calcium channels and to stimulate the entry of calcium in the islets. Quinine also mimics the effect of glucose to reduce ^{45}Ca from perifused islets, but apparently exerts such an inhibitory effect independently of its action on K^+ conductance. These observations confirm the idea that, in part, glucose stimulates calcium entry in the islets at least through its effect on K^+ conductance and membrane depolarization while the latter changes cannot account for the glucose-induced decrease in calcium extrusion from the islet cells.

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