MOLECULAR PHARMACOLOGY, 18, 243-246

Inhibition by Quinine of Insulin Release and Calcium Ionophoresis

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Received January 17, 1980; Accepted April 2, 1980

SUMMARY

COUTURIER, E., A. SENER, K. ANJANEYULU AND W. J. MALAISSE. Inhibition by quinine of insulin release and calcium ionophoresis. *Mol. Pharmacol.* 18: 243-246 (1980).

Quinine at low concentrations (0.05–0.1 mM) facilitated glucose-stimulated ^{45}Ca net uptake and insulin release in isolated rat pancreatic islets. At higher concentrations (0.25–0.5 mM), quinine inhibited both ^{45}Ca net uptake and insulin release. Quinine caused a dose-related inhibition of the translocation of Ca from an aqueous medium into an organic immiscible phase, as evoked by either the antibiotic ionophore X537A or an islet extract. Quinine also inhibited the process of X537A-mediated Na–Ca countertransport. It is proposed that the inhibitory effect of quinine, at high concentrations, upon insulin release may be due to a direct interference with the transport of Ca across the membrane system(s) in the islet cells.

INTRODUCTION

Quinine is currently used to explore the influence of changes in plasma membrane K conductance upon cationic (1), bioelectrical (2), and secretory (3) events in the pancreatic B cell. Such studies are based on the following premises. The process of glucose-induced insulin release involves a decrease in K outflow from islet cells (4–7), leading to membrane depolarization (8) and subsequent gating of voltage-dependent Ca channels (9, 10). This sequence results in the cellular accumulation of Ca and activation of the effector system for exocytosis of insulin secretory granules (11). Quinine (0.05–0.1 mM) mimics in several respects the effects of glucose upon islet function: It also decreases K conductance (1), causes B-cell depolarization (2), and stimulates insulin release (3). However, at higher concentrations (0.5 mM), quinine inhibits glucose-stimulated insulin release (3). In the present work, it is proposed that the latter inhibitory effect may be due to a direct interference of quinine with the transport of Ca across the membrane system(s) in the islet cells.

MATERIALS AND METHODS

The release of insulin (12), net uptake of ^{45}Ca (13), and oxidation of glucose (14) by isolated pancreatic islets removed from fed female albino rats were measured as reported elsewhere. The methods used to measure the translocation of ^{45}Ca into (15) or across (16) an organic phase containing either the ionophore X537A or an islet extract (17) were previously described. Quinine hemisulfate was used in all experiments.

All results are expressed as the mean (±SEM) together with the number of individual determinations (N).

RESULTS

Effect of quinine upon insulin release. At low concentrations (0.05–0.1 mM), quinine failed to significantly affect insulin release in the absence of glucose and at either a low (2.8 mM) or a very high (16.7 mM) concentration of the sugar (Table 1, Fig. 1). However, quinine dramatically enhanced insulin release at intermediate glucose concentrations (5.6–11.1 mM). When used at higher concentrations (0.25–1.00 mM), quinine caused a dose-related inhibition of glucose-stimulated insulin release (Table 1).

Effect of quinine upon ^{45}Ca net uptake and glucose oxidation by the islets. Whether in the absence or the presence of glucose, quinine stimulated ^{45}Ca net uptake when used at a 0.1 mM concentration (Table 2). However, at concentrations of 0.25 and 0.5 mM, quinine inhibited glucose-stimulated ^{45}Ca net uptake (Table 2). There was a highly significant correlation ($r = 0.9778$, $N = 6$, $P < 0.001$) between the mean values for ^{45}Ca net uptake and insulin release, respectively. At high concentrations (0.5 mM), quinine also caused a modest decrease in [U-^{14}C]glucose oxidation by the islets (Table 3).

Effect of quinine upon ionophore-mediated Ca translocation. X537A provoked the translocation of Ca from a Hepes-buffered aqueous solution (25 mM; pH 7) initially containing Na^+ 123, K^+ 5, and Cl^- 120 mEq/liter and ^{45}Ca (1.2 μM) into an organic phase (toluene–butanol; 7/

Supported in part by grants from the Belgian Foundation for Scientific Medical Research and the Belgian Institute for Scientific Research in Industry and Agriculture.

0026-895X/80/050243-04$02.00/0

TABLE 1

Effect of quinine upon insulin release (μU/islet per 90 min)

Glucose	Quinine (mM)					
	Nil	0.05	0.10	0.25	0.50	1.00
mM						
Nil	27.7 ± 3.7 (20)		25.0 ± 2.5 (10)			
2.8	22.4 ± 5.1 (8)		31.3 ± 2.7 (8)			
5.6	37.7 ± 5.5 (29)	98.3 ± 1.0 (10)	113.7 ± 5.5 (20)	18.6 ± 2.7 (8)	19.8 ± 3.0 (9)	
8.3	102.0 ± 8.9 (29)	209.0 ± 6.4 (9)	206.9 ± 7.1 (31)	38.4 ± 2.6 (8)	17.0 ± 3.7 (8)	
11.1	224.3 ± 6.0 (29)	281.4 ± 8.1 (10)	268.9 ± 10.8 (29)	70.4 ± 8.1 (17)	18.7 ± 3.2 (20)	10.4 ± 4.3 (10)
16.7	292.4 ± 11.8 (18)		300.1 ± 10.6 (18)	98.0 ± 12.1 (8)		

3, v/v) containing the antibiotic ionophore (1.4 mM). Quinine, when added to the initial aqueous phase, caused a dose-related inhibition of Ca translocation (Fig. 2). Likewise, quinine inhibited the translocation of Ca evoked by an islet extract (Table 4). The relative magnitude of such an inhibitory effect was inversely related to the Ca^{2+} concentration of the initial aqueous medium.

Effect of quinine upon X537A-mediated Na–Ca countertransport. When added to the toluene–butanol mixture, X537A (0.07–0.28 mM) stimulated, in a dose-related fashion, the uphill transport of Ca from one to the other of two triethanolamine-buffered aqueous solutions (20 mM; pH 7.0) initially containing 0.2 mM $CaCl_2$ and either 2.5 or 100.0 mM NaCl, Ca being translocated across the organic mixture from the aqueous medium of low to that of high NaCl concentration (Table 5, Fig. 3). Quinine (0.1 mM), when added to both aqueous solutions, inhibited X537A-mediated Na–Ca countertransport.

DISCUSSION

The present data indicate that quinine, at low concentrations (0.05–0.1 mM), mimics the effect of glucose to stimulate both ^{45}Ca net uptake and insulin release in isolated pancreatic islets. The effect of guinine upon insulin secretion at different glucose concentrations corresponded to a shift to the left of the sigmoidal curve relating the secretory rate to the glucose concentration, without any significant change in either basal or maximal insulin output. The magnitude of such a shift indicates that 0.1 mM quinine mimics the effect of 2.6 mM glucose

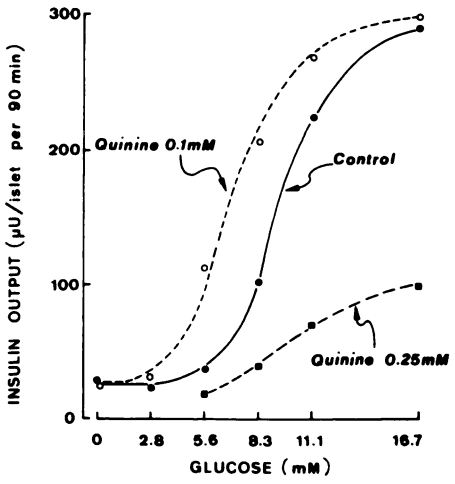

FIG. 1. *Effect of quinine (0.1 and 0.25 mM) upon glucose-induced insulin release in isolated pancreatic islets*

TABLE 2

Effect of quinine upon ^{45}Ca net uptake (pmol/islet per 90 min)

Quinine	Glucose (mM)	
	Nil	11.1
mM		
Nil	1.25 ± 0.09 (24)	4.40 ± 0.25 (11)
0.10	2.12 ± 0.25 (7)	5.38 ± 0.19 (12)
0.25	1.13 ± 0.08 (12)	1.91 ± 0.16 (12)
0.50	1.14 ± 0.07 (12)	1.65 ± 0.10 (12)

(Fig. 1). For reasons already mentioned, the stimulatory effect of quinine upon insulin release is likely to be attributable to a drug-induced decrease in K conductance and subsequent facilitation of Ca entry into the islet cells through voltage-dependent Ca channels (1, 2, 9, 10).

The inhibitory effect of quinine at higher concentrations (0.25–1.00 mM) upon glucose-stimulated insulin release cannot be ascribed to a primary change in glucose metabolism. The modest inhibition in [U-^{14}C]glucose oxidation seen in the presence of 0.5 mM quinine (Table 3) might be secondary to alteration in Ca handling by the islets, a comparable inhibitory effect being observed in islets exposed to media either deprived of Ca^{2+} (18) or containing Mg^{2+} at a high concentration (19).

The quinine-induced inhibition of insulin release was obviously linked to a decrease in ^{45}Ca net uptake (Table 2). The normal relationship between ^{45}Ca net uptake and insulin release, as previously documented in the present system (13), was not altered by quinine. This suggests that quinine did not affect the functional capacity of the effector system which causes exocytosis of secretory granules in response to intracellularly accumulated Ca^{2+}.

A possible explanation for the inhibitory effect of quinine upon glucose-stimulated insulin release would be that the drug interferes with the native system(s) mediating the transport of Ca across the plasma membrane and/or membranes of intracellular organelles in the islet cells. This view is compatible with the observation that quinine inhibited the translocation of Ca mediated by

TABLE 3

Effect of quinine upon glucose oxidation

Glucose	Quinine	[U-^{14}C]Glucose oxidation
mM	*mM*	*pmol/islet per 120 min*
5.6	Nil	14.3 ± 1.9 (8)
11.1	Nil	49.4 ± 4.7 (8)
11.1	0.1	46.1 ± 2.8 (8)
11.1	0.5	35.6 ± 2.2 (8)

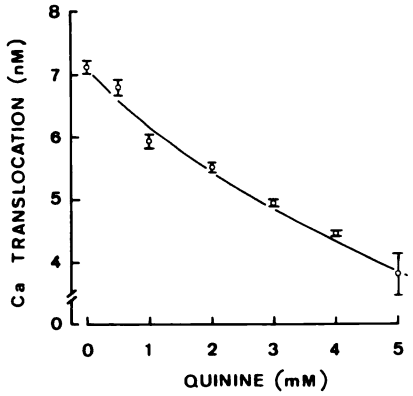

FIG. 2. *Dose-action relationship for the effect of quinine upon X537A-mediated Ca translocation in a two-phase system*

Each value for the concentration of Ca in the organic phase is derived from three individual determinations.

either the antibiotic ionophore X537A (Figs. 2 and 3) or native ionophores extracted from the islet cells (Table 4). As is the case with other inhibitors of ionophore-mediated Ca translocation (17), Ca^{2+} itself protected against the inhibitory effect of quinine (Table 4). Incidentally, the apparently higher sensitivity to quinine of the islet extract as distinct from X537A may be related to a different affinity of these two preparations toward Ca^{2+} and quinine, respectively.

In comparing the dose-action relationship for the effect of quinine upon insulin release and other parameters, it is obvious that the situation found at increasing concentrations of the drug will depend on the balance between opposite phenomenon, such as (i) a decrease in K conductance (1) with gating of the voltage-dependent Ca channels and (ii) an inhibition of carrier-mediated Ca transport, which may affect not only Ca inflow but also the process of Na-Ca countertransport responsible for Ca outflow from the islet cells (10).

We have previously shown that the native ionophoretic material extracted from the islets is derived mostly from a membrane-rich subcellular fraction (17). The influence of such factors as Na^+, H^+, Li^+, organic Ca antagonists, hypoglycemic sulfonylureas, and the hyperglycemic sulfonamide diazoxide upon Ca transport, as mediated by this native ionophoretic material, coincides qualitatively with the effect of the same factors upon Ca handling in intact islet cells (17, 20, 21). The present work extends this series of analogies to the situation found at high concentrations of quinine.

In conclusion, we would like to suggest that the inhibitory effect of quinine, at high concentrations, upon insulin release may be due, at least in part, to a direct interference of the drug with the process of Ca transport across the membrane system(s) in the islet cells.

TABLE 4

Effect of quinine (1.0 mM) upon the translocation of Ca from a triethanolamine-HCl-buffered solution (20 mM; pH 7) initially containing 2 mM NaCl and increasing concentrations of $CaCl_2$ into an organic mixture (toluene-butanol; 7/3, v/v) containing an islet extract (each ml of the organic phase contained ionophoretic material derived from 125 islets)

The last column refers to the mean inhibitory effect of quinine expressed as a percentage of the control value.

Expt	Ca^{2+}	Ca translocation (fmol/islet)		Inhibition
		Control	Quinine (1.0 mM)	
	μM			%
1	0.4	61.0 ± 2.9 (9)	0.5 ± 0.2 (9)	99.2
2	0.4	45.6 ± 1.6 (9)	1.1 ± 0.1 (9)	97.6
2	100.0	3442 ± 67 (9)	630 ± 142 (9)	81.6
2	1000.0	8504 ± 368 (9)	2557 ± 307 (9)	69.9

TABLE 5

Effect of quinine upon X537A-mediated Na-Ca countertransport

The rate of Ca translocation (expressed as the percentage radioactivity translocated per sample) was calculated by linear regression analysis of data such as those illustrated in Fig. 4 (samples 1 to 10 inclusive).

X537A	Quinine	Ca translocation
mM	mM	% per sample
0.07	—	1.45 ± 0.09 (2)
0.28	—	9.17 ± 0.27 (3)
0.28	0.1	4.10 ± 0.09 (3)

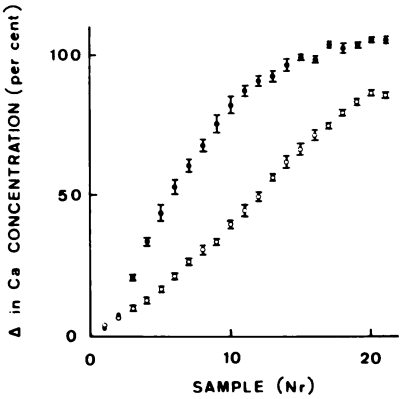

FIG. 3. *The phenomenon of Na-Ca countertransport, as mediated by the ionophore X537A (0.28 mM), is expressed as the percentage of total initial radioactivity translocated from a medium of low NaCl concentration (2.5 mM) to a medium of high NaCl concentration (100.0 mM), both media initially containing $^{45}CaCl_2$ at the same concentration (0.2 mM)*

Samples were removed from these media after each two back-and-forth transfers of the organic phase containing the ionophore, the experiments ($N = 3$ in each case) being carried out in the absence (filled circles) or presence (open circles) of quinine (0.1 mM).

ACKNOWLEDGMENTS

The authors are grateful to M. Mahy, S. Procureur, J. Schoonheydt, and M. Urbain for technical assistance and C. Demesmaeker for secretarial help.

REFERENCES

1. Carpinelli, A. R., and W. J. Malaisse. Regulation of $^{86}Rb^+$ outflow from pancreatic islets. I. Reciprocal changes in the response to glucose, tetraethylammonium and quinine. *Mol. Cell. Endocrinol.* **17:** 103-110 (1980).
2. Atwater, I., C. M. Dawson, B. Ribalet and E. Rojas. Potassium permeability activated by intracellular calcium ion concentration in the pancreatic B-cells. *J. Physiol. (London)* **288:** 575-588 (1979).
3. Henquin, J. C., B. Horemans, M. Nenquin, J. Verniers and A. E. Lambert. Quinine-induced modifications of insulin release and glucose metabolism by isolated pancreatic islets. *FEBS Lett.* **57:** 280-284 (1975).
4. Sehlin, J., and I.-B. Täljedal. Transport of rubidium and sodium in pancreatic islets. *J. Physiol. (London)* **242:** 505-515 (1974).
5. Boschero, A. C., S. Kawazu, G. Duncan and W. J. Malaisse. Effect of glucose on K^+ handling by pancreatic islets. *FEBS Lett.* **83:** 151-154 (1977).

6. Malaisse, W. J., A. C. Boschero, S. Kawazu and J. C. Hutton. The stimulus-secretion coupling of glucose-induced insulin release. XXVII. Effect of glucose on K^+ fluxes in isolated islets. *Pflügers Arch.* **373:** 237–242 (1978).
7. Henquin, J. C. D-Glucose inhibits potassium efflux from pancreatic islet cells. *Nature (London)* **271:** 271–273 (1978).
8. Matthews, E. K. Calcium and stimulus-secretion coupling in pancreatic islet cells, in *Calcium Transport in Contraction and Secretion* (E. Carafoli, F. Clementi, W. Drabikowski and A. Margreth, eds.). North-Holland, Amsterdam, 203–210 (1975).
9. Meissner, H. P., and M. Preissler. Glucose-induced changes of the membrane potential of pancreatic B-cells: Their significance for the regulation of insulin release, in *Treatment of Early Diabetes* (R. A. Camerini-Davalos and B. Hanover, eds.). Plenum, New York, 97–107 (1979).
10. Herchuelz, A., E. Couturier and W. J. Malaisse. Regulation of calcium fluxes in pancreatic islets: Glucose-induced calcium-calcium exchange. *Am. J. Physiol.* **238:** E96–E103 (1980).
11. Malaisse, W. J., A. Herchuelz, G. Devis, G. Somers, A. C. Boschero, J. C. Hutton, S. Kawazu, A. Sener, I. Atwater, G. Duncan, B. Ribalet and E. Rojas. Regulation of calcium fluxes and their regulatory roles in pancreatic islets. *Ann. N.Y. Acad. Sci.* **307:** 562–582 (1978).
12. Malaisse, W. J., G. Brisson and F. Malaisse-Lagae. The stimulus-secretion coupling of glucose-induced insulin release. I. Interaction of epinephrine and alkaline earth cations. *J. Lab. Clin. Med.* **76:** 895–902 (1970).
13. Malaisse-Lagae, F., and W. J. Malaisse. The stimulus-secretion coupling of glucose-induced insulin release. III. Uptake of ^{45}calcium by isolated islets of Langerhans. *Endocrinology* **88:** 72–80 (1971).
14. Malaisse, W. J., A. Sener and M. Mahy. The stimulus-secretion coupling of glucose-induced insulin release. XVIII. Sorbitol metabolism in isolated islets. *Eur. J. Biochem.* **47:** 365–370 (1974).
15. Couturier, E., and W. J. Malaisse. Ionophore-mediated cation translocation in artificial systems. II. X537A-mediated calcium and sodium translocation. *Biochimie* **62:** 177–180 (1980).
16. Malaisse, W. J., K. Anjaneyulu, R. Anjaneyulu and E. Couturier. Ionophore-mediated Ca^{2+} countertransport: Role of Na^+, Li^+ or H^+ gradient. *Mol. Cell. Biochem.* **30:** 67–70 (1980).
17. Valverde, I., and W. J. Malaisse. Ionophoretic activity in pancreatic islets. *Biochem. Biophys. Res. Commun.* **89:** 386–395 (1979).
18. Malaisse, W. J., J. C. Hutton, A. Sener, J. Levy, A. Herchuelz, G. Devis and G. Somers. Calcium antagonists and islet function. VII. Effect of calcium deprivation. *J. Membrane Biol.* **38:** 193–208 (1978).
19. Malaisse, W. J., G. Devis, A. Herchuelz, A. Sener and G. Somers. Calcium-antagonists and islet function. VIII. The effect of magnesium. *Diab. Metab.* **2:** 1–4 (1976).
20. Anjaneyulu, K., R. Anjaneyulu and W. J. Malaisse. The stimulus-secretion coupling of glucose-induced insulin release. XLIII. Na-Ca countertransport by pancreatic islet native ionophores. *J. Inorg. Biochem.*, in press (1980).
21. Anjaneyulu, K., R. Anjaneyulu, E. Couturier and W. J. Malaisse. Opposite effects of hypoglycemic and hyperglycemic sulfonamides upon ionophore-mediated calcium transport. *Biochem. Pharmacol.* **29:** 1879–1882 (1980).

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