QUININE-INDUCED MODIFICATIONS OF INSULIN RELEASE AND GLUCOSE METABOLISM BY ISOLATED PANCREATIC ISLETS

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1. Introduction

The striking analogies between the processes regulating secretion and contraction led Douglas and Rubin [1] to introduce the term 'stimulus-secretion coupling' to parallel the concept of 'excitation-contraction coupling' proposed earlier by Sandow [2]. The effects of several alkaloids on muscle contraction have long been recognized and their site and mechanism of action extensively studied [3]. Among these alkaloids, methylxanthines are well-known potentiators of contraction [4] and, more recently, have been shown to increase secretion in various glands, in particular insulin release [5–7]. The effects of quinine and of its optical isomer quinidine on contraction are, in some respects, similar to those of methylxanthines, but are more complex, depending on the concentration used and on the type of muscle. As no attention has been paid on possible modifications of secretion by these latter alkaloids, we investigated the action of quinine on isolated islets of Langerhans. In this report, we show that the drug stimulates, potentiates or inhibits insulin release according to the experimental conditions and also alters glucose metabolism by islet cells.

2. Materials and methods

All experiments were performed with islets of Langerhans isolated by collagenase digestion [8] of the pancreas of fed male Wistar R rats (Mol, Belgium) weighing 275–300 g. The medium utilized was a Krebs–Ringer bicarbonate buffer (KRB), pH 7.4, gassed with O_2/CO_2 (94/6) and supplemented with 0.5% (w/v) bovine serum albumin.

2.1. *Insulin release*

Insulin secretion was studied either in static incubations carried out for 60 min in a shaking incubator or in a perifusion system that particularizes the dynamics of release [9,10]. Specific experimental conditions are given in the legends. Immunoreactive insulin (IRI) was measured by a double antibody assay [11] with rat insulin (21 IU/mg) as standard. No interference of the substances studied could be detected.

2.2. *Glucose metabolism*

Glucose oxidation by islet cells was measured as the formation of $^{14}CO_2$ from D-[U-^{14}C]glucose. Batches of 10 islets were incubated, for 2 h at 37°C, in 50 μl KRB contained in small polyethylene tubes suspended in glass counting vials that were tightly stoppered after being gassed. Metabolism was arrested by injection, through the stopper, of 10 μl 1 N HCl into the polyethylene tube and CO_2 was collected during 4 h in 0.5 ml Hyamine injected in the vial.

Glucose utilization was measured as the formation of [^{3}H] water from D-[5-^{3}H]glucose [12]. Batches of 10 islets were incubated in 20 μl KRB in the same conditions as described above. Metabolism was arrested by injection of 10 μl 0.2 N HCl, and [^{3}H]water formed was allowed to equilibrate overnight with 0.5 ml water contained in the counting vial.

Incubations without islets provided blank values for all experimental conditions. Samples of incubation media were used as external standards to translate the observed counts (after blank substraction) into

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picomoles of glucose oxidized or utilized/hour/islet [12].

2.3. *Chemicals*

Quinine hydrochloride was from Sigma Co. (St Louis, USA); D-glucose and atropine sulfate from Merck, A. G. (Darmstadt, Germany); D-[U-^{14}C]glucose (283 mCi/mmol) and D-[5-^{3}H]glucose (1 Ci/mmol) from the Radiochemical Centre (Amersham, England), Hyamine hydroxide from Packard Instrument Co. (Downers Grove, USA).

3. Results

3.1. *Insulin release*

As shown in fig.1, quinine exerted a dual effect on glucose-induced IRI release. Low concentrations of the alkaloid (5 to 100 μM) potentiated the insulinotropic action of the sugar. 50 μM quinine increased the amount of IRI released by more than 12 times in the presence of 100 mg glucose/100 ml and by 1.8 times at glucose 300 mg/100 ml. By contrast, 0.5 and 1 mM quinine markedly depressed the effect of the highest concentration of the sugar. In the absence of glucose, IRI release was significantly augmented ($p < 0.001$) from 0.44 ± 0.05 to 0.91 ± 0.06 and 1.27 ± 0.09 ng/islet/60 min (n= 12) by 25 and 100 μM quinine, respectively.

The possibility that quinine unspecifically augmented β-cell membrane permeability and caused passive leakage of insulin could be ruled out by experiments carried out at low temperature. Table 1 demonstrates that, at 22°C, glucose failed to stimulate IRI release both in the absence and the presence of the alkaloid.

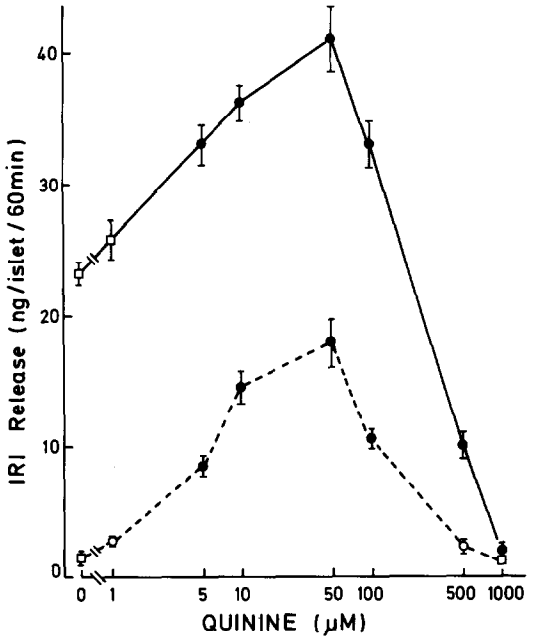

Fig.1. Effect of quinine on glucose-induced IRI release. After preincubation for 15 min in KRB containing 50 mg glucose/100 ml, batches of 3 islets were transferred into 1 ml medium supplemented with various concentrations of quinine and glucose 100 mg/100 ml (broken line) or 300 mg/100 ml (solid line). IRI was measured in an aliquot of medium taken at the end of the 60 min of incubation at 37°C. Values are means ± SEM of 12 observations. The significance of difference between experimental groups and controls without quinine is denoted by □ = $p > 0.05$, ○ = $p < 0.05$ and ● = $p < 0.001$.

Table 1
Effect of quinine on glucose-induced insulin release at 37°C and 22°C

Glucose concentration (mg/100 ml)	IRI released after 60 min of incubation (ng/islet)			
	No Quinine		Quinine (25 μM)	
	37°C	22°C	37°C	22°C
100	1.28 ± 0.12	0.78 ± 0.10[a]	10.52 ± 0.94[b]	0.68 ± 0.09
300	26.11 ± 1.16	0.89 ± 0.09[a]	38.74 ± 2.11[b]	0.93 ± 0.08

Values are means ± SEM of 12 observations. Significance levels: a = $p < 0.001$ for difference from appropriate control at 37°C; b = $p < 0.001$ for difference from appropriate control without quinine. Experimental conditions were similar to those described in fig.1, except for temperature of incubation.

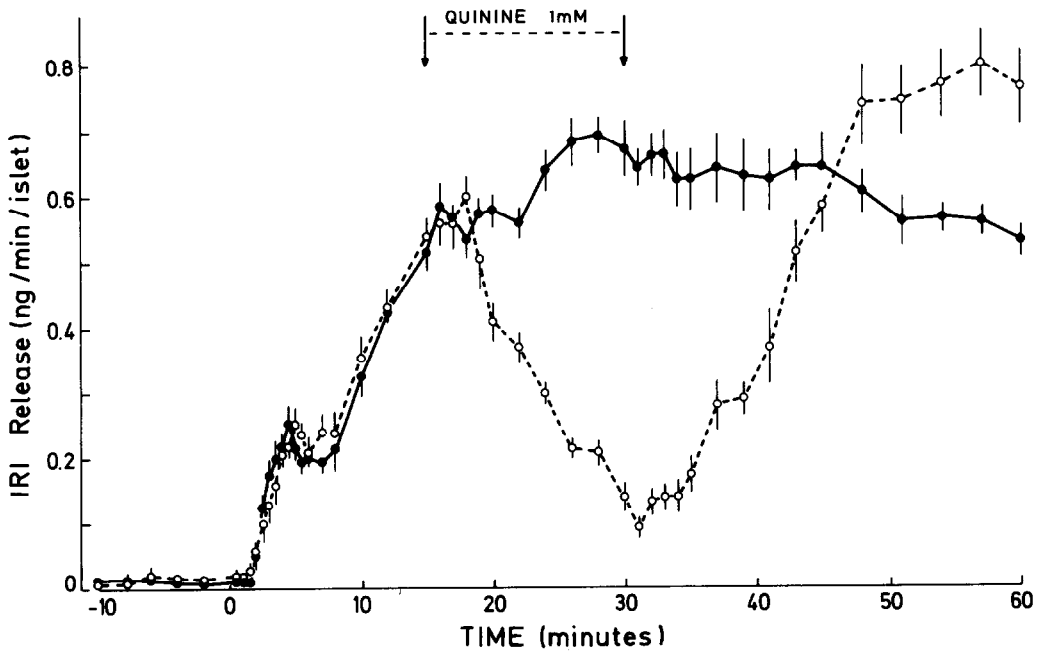

Fig.2. Reversibility of the inhibitory effect of 1 mM quinine on glucose-induced IRI release. Pools of 20 islets were perifused in parallel chambers with KRB containing 50 mg glucose/100 ml from min −25 to 0. At min 0, glucose concentration was suddenly increased to 300 mg/100 ml and maintained at this level until the end of the perifusion. The experimental group of islets (o—o) was further exposed to 1 mM quinine between min 15 and 30. Values are means ± SEM of 5 experiments.

Table 2
Effect of quinine on glucose metabolism by islet cells

Quinine concentration (μM)	Glucose oxidized (pmol/hr/islet)		Glucose utilized (pmol/hr/islet)	
	Glucose 100 mg/100 ml	Glucose 300 mg/100 ml	Glucose 100 mg/100 ml	Glucose 300 mg/100 ml
0	20.21 ± 1.06 (10)	41.47 ± 3.63 (8)	47.65 ± 1.45 (10)	100.08 ± 3.50 (16)
50	19.88 ± 1.02 (10)	39.15 ± 3.31 (9)	47.36 ± 1.67 (10)	94.03 ± 2.00 (8)
500	17.30 ± 0.82[a] (10)	39.36 ± 2.81 (9)	41.96 ± 1.05[a] (10)	96.43 ± 4.63 (8)
1000	5.75 ± 0.36[b] (10)	7.31 ± 0.48[b] (9)	25.06 ± 1.27[b] (10)	48.50 ± 4.25[b] (8)

After 15 min of preincubation in the absence of glucose, islets were incubated as described in the section 'Materials and methods'. To the incubation media were added 1 μCi D-[-U-^{14}C]glucose/50 μl KRB and 0.1 μCi D-[-5-^{3}H]glucose/20 μl KRB, respectively for oxidation and utilization measurements.
Values are means ± SEM of the number of observations indicated in parentheses. Significance levels : a = $p < 0.05$ and b = $p < 0.001$ for difference from appropriate control without quinine.

1 μM atropine, a concentration higher than that required to block the effect of acetylcholine in the islets [13,14], did not alter the potentiation by 25 μM quinine of IRI release induced by 100 g glucose/100 ml (13.37 ± 0.86 and 13.60 ± 0.59 ng/islet/60 min, $n = 16$, without and with atropine).

The dynamics and the reversibility of quinine-induced inhibition of IRI release are illustrated by fig.2. The solid line depicts the biphasic secretory response of control islets stimulated with 300 mg glucose/100 ml. As evidenced by the broken line, addition of 1 mM quinine to the perifusate was followed by a rapid decline of IRI secretion rate. Upon removal of the drug, the rate of secretion not only returned to control values but reached levels significantly higher.

3.2. *Glucose metabolism*

The effect of quinine on glucose metabolism by islet cells, is detailed in table 2. 50 μM quinine did not change glucose oxidation or utilization, whereas both metabolic parameters were inhibited by 1 mM of the drug. It is of note that oxidation was more depressed than utilization and that 500 μM quinine, which clearly inhibited IRI release (fig.1), caused only a minor decrease of glucose metabolism in the presence of 100 mg of the sugar/100 ml (table 2).

4. Discussion

In cardiac muscle, the main and best established effect of quinine (and of its isomer) is to depress contractility [15,16], but in some conditions, low concentrations of the drug also produce a positive inotropic effect [17]. This latter property is comparable to the potentiation of twitch tension provoked by the alkaloid (10^{-5} to 10^{-4} M) in skeletal muscle [18].

The results reported here show that low concentrations of quinine slightly stimulate insulin release in the absence of glucose and powerfully potentiate the insulinotropic effect of the sugar, mostly at glucose levels close to the threshold for stimulation of secretion. The first observation contrasts but the second concurs with the effects of methylxanthines [7], which, however, must be used at higher concentrations. Preliminary experiments indicating the Ca-dependency of this potentiating effect of quinine point out another difference between the mode of action of both types of alkaloids. At the present time, the exact mechanism of action of quinine is not elucidated, but does not seen to involve changes of glucose metabolism although requiring its normal fonctioning as indicated by the experiments carried out at low temperature. The possibility that the recorded effects of quinine are indirect of quinine are indirect and mediated by stimulation of neurotransmitter release from cholinergic terminals in the islets is also unlikely.

The inhibition of glucose-stimulated insulin secretion by high concentrations of quinine seems to be accounted for by the alteration of glucose metabolism. However, another mechanism cannot be ruled out since 0.5 mM quinine already reduced secretion with very little effect on metabolism. This would be in keeping with conclusions reached for cardiac muscle, where the inhibition of carbohydrate and lipid metabolism by quinine and quinidine [19,20] has not been considered as a valid or the sole explanation for the depressant effect of these drugs on contractility.

In conclusion, the similarities between the effects of quinine on muscle cells and pancreatic β-cells reinforce the view that contraction and secretion share similar basic processes of control. Quinine might thus prove to be a useful tool to gain further insight in some steps of the stimulus-secretion coupling leading to exocytosis of various secretory products including insulin. Since many effects of the alkaloid on muscle have been related to its ability to release Ca from intracellular stores or to inhibit uptake of the cation at this level [21,23], quinine might help to evaluate a possible involvement of these pools in the secretory process.

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