DIRECT EFFECTS OF GOLD THIOGLUCOSE ON INSULIN SECRETION BY ISOLATED RAT ISLETS OF LANGERHANS

Ian D. CATERSON and Keith W. TAYLOR
Department of Biochemistry, University of Sydney, Sydney, NSW 2006, Australia

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1. Introduction

Gold thioglucose (6-aurothioglucose) has been used for some years to produce obesity in rodents, which it is presumed to do by destroying the 'satiety centre' in the hypothalamus and hence inducing excessive food consumption [1,2]. It is presumed to act by binding to the glucose receptor cells in the ventromedical hypothalamus. To date there has been little reported on the direct effects of gold thioglucose on isolated islets of Langerhans, though in [3] it was suggested that, at high concentrations it may have no effect on islet function.

Here, the effects of gold thioglucose on islet function have been investigated in more detail. Two distinct effects on insulin secretion have been found. In the first place, it has been shown that gold thioglucose at a low concentration, by itself, may induce a small stimulation of insulin secretion, though the rate of secretion does not alter as the concentration of gold thioglucose rises.

In addition it has been found that gold thioglucose is able to potentiate the effects of glucose though not D-glyceraldehyde on insulin secretion. Its potentiating effect is moreover accompanied by a rise in intra-islet cyclic AMP levels. The relevance of these findings to the biochemical mechanisms underlying insulin release is briefly discussed.

2. Methods

Islets of Langerhans were isolated from adult male Sprague Dawley rats by the collagenase digestion technique in [4]. Collagenase at 2 mg/ml was used throughout. The rats (250–350 g) were fed with Allied Feed Rat Kubes ad libitum.

Five islets of approximately equal size were then incubated in a buffered bicarbonate medium with varying concentrations of glucose and/or gold thioglucose added for 1 h. At the end of this time the incubation vial was centrifuged at 1500 × *g* for 1 min and the supernatant taken for assay of insulin by radioimmunoassay. Results are expressed as μunits insulin released/islet/min (±SEM).

In the preincubation experiments, 50 islets were incubated in 2 ml buffered bicarbonate medium with either 3 mM gold thioglucose, 5 mM glucose, or both. After 1 h the islets were incubated in groups of 5 as above. Glyceraldehyde was also used as a secretagogue at 5 mM and 30 mM [5].

Cyclic AMP was measured by radioimmunoassay with an antibody to the 2′-*O*-acetyl derivative (substitutions at the 2-*O*-position increase sensitivity) [6]. Fifteen islets were preincubated in 15 μl medium at 37°C. After incubation, 35 μl ice cold 0.1 M HCl was added and the mixture sonicated with a Branson Sonifier Cell Disruptor B15. Samples were taken for measurement of cyclic AMP and insulin. Results are expressed as fmol cAMP/ng insulin/islet.

3. Results and discussion

Two major effects are seen when rat islets of Langerhans are incubated with gold thioglucse.

Using 2 mM gold thioglucose alone, there is a small but statistically significant increase in insulin release. At higher levels, no stimulatory effect is observed as has already been reported [3] (fig.1).

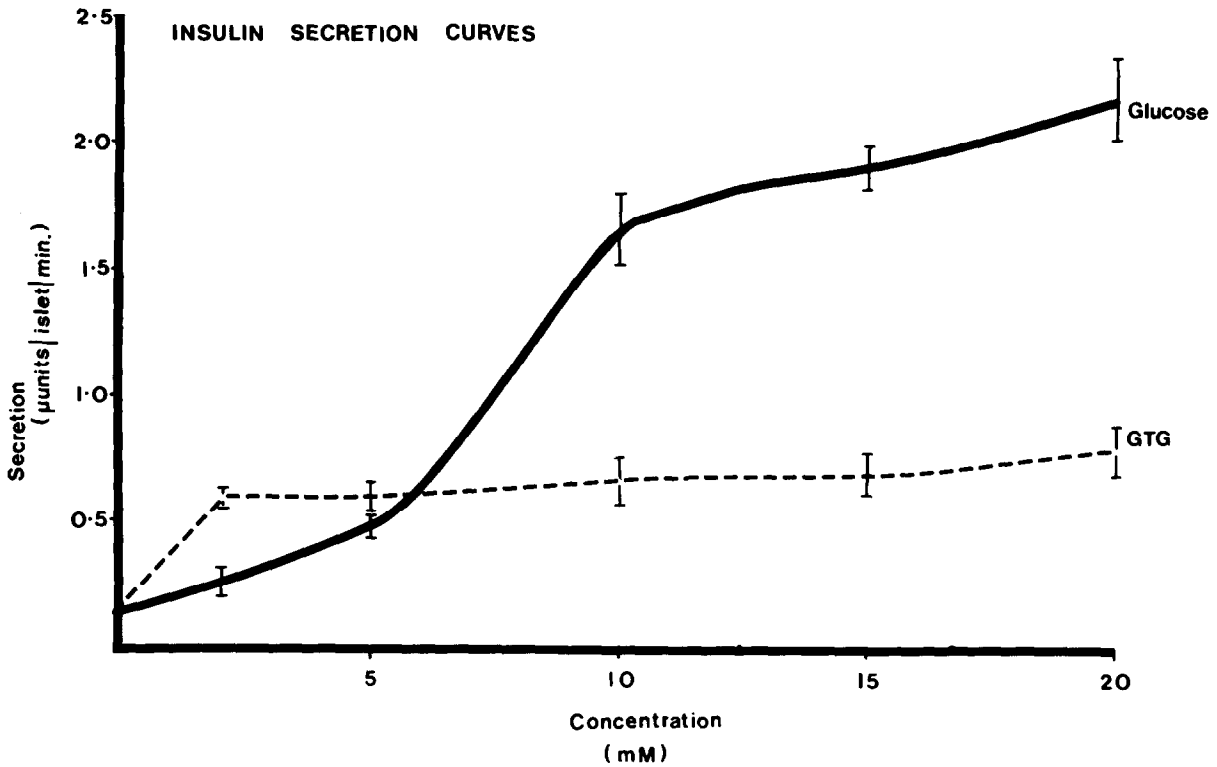

Fig.1. Insulin secretion from isolated rat islets of Langerhans stimulated by glucose or gold thioglucose (GTG). Five islets of Langerhans were incubated in 1 ml buffered bicarbonate medium for 1 h, then centrifuged at 1500 × *g* for 1 min and the insulin concentration in the supernatant measured by radioimmunoassay. All results are expressed as mean ± SEM. Eight observations. When comparing insulin secretion stimulated by glucose to that stimulated by gold thioglucose, $p<.001$ for all points at 5 mM, where the difference is not significant.

When 2 mM gold thioglucose is added to islets incubated with varying concentrations of glucose (5–20 mM) there is potentiation of insulin secretion (fig.2). This potentiation occurs at all concentrations of glucose studied and is an approximately similar increase at each glucose concentration (e.g., at 5 mM glucose, insulin secretion increased from 0.45 ± 0.05 μunits/islet/min to 0.69 ± 0.05 μunits/islet/min with gold thioglucose, and at 15 mM glucose, insulin secretion of 1.54 ± 0.05 was increased to 1.89 ± 0.10

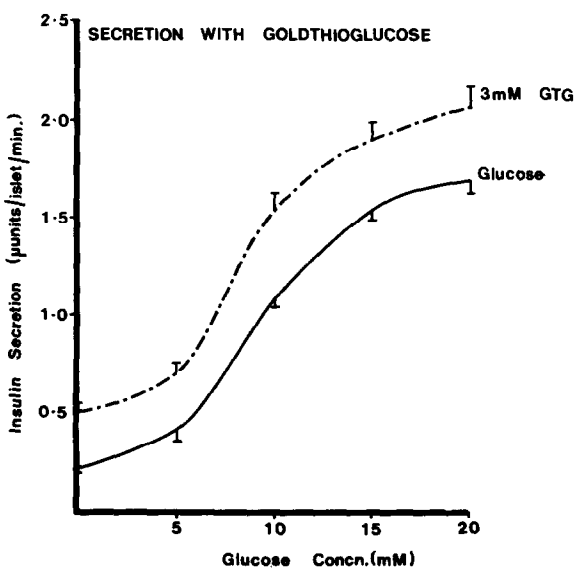

Fig.2. Insulin secretion from isolated rat islets of Langerhans stimulated by glucose alone, or glucose plus gold thioglucose. Five islets were incubated as above for 1 h with either glucose alone in varying concentrations, or with glucose plus 3 mM gold thioglucose. All results expressed as means ± SEM. When comparing the two curves $p<.001$ for all points.

Table 1
3′5′-cyclic AMP levels in islets of Langerhans incubated with glucose at varying concentrations with or without 3 mM gold thioglucose

Glucose mM	cAMP (fmol/ng insulin/islet)	
	Glucose alone	+3 mM gold thioglucose
0	0.09 ± 0.01 (7)	0.26 ± 0.03 (7)*
5	0.17 ± 0.02 (11)	0.28 ± 0.02 (9)*
10	0.28 ± 0.06 (5)	0.35 ± 0.04 (7)
20	0.23 ± 0.03 (8)	0.39 ± 0.06 (9)**

All results expressed as mean ± SEM. Number of observations shown in brackets. For difference between secretion with glucose alone and glucose plus gold thioglucose, statistical significance is shown as follows: [a] $p<.01$, [b] $p<.05$

Table 2
Secretion of insulin from rat islets of Langerhans in the presence of D-glyceraldehyde with or without 3 mM gold thioglucose

Glyceraldehyde mM	Secretion of insulin (μunits/islet/min)	
	Glyceraldehyde alone	+3 mM gold thioglucose
5	0.49 ± 0.02 (8)	0.48 ± 0.01 (8)
30	1.46 ± 0.07 (8)	1.46 ± 0.05 (8)

All results expressed as mean ± SEM. Number of observations shown in brackets

μunits/islet/min with gold thioglucose). The gold thioglucose does not appear to compete with glucose for surface receptors as even at 20 mM glucose, insulin secretion is potentiated by gold thioglucose.

The cyclic AMP content of islets which had been incubated for 15 min with glucose alone as secretagogue, increases concomitantly with increasing concentrations of glucose (see table 1), an effect which has been reported with islets of fed rats by many other workers [7–9]. There is also an increase in cyclic AMP when islets are incubated with varying concentrations of glucose plus 3 mM gold thioglucose over those levels obtained with glucose alone. These observations are consistent with the hypothesis that increased insulin release due to many secretagogues and potentiators is associated with increased cyclic AMP. It is reasonable to suppose that gold thioglucose may directly affect adenyl cyclase or diesterase activity to increase islet cyclic AMP, and thus potentiate insulin secretion. As it has been shown in brain cells that gold thioglucose is bound to the cell membrane [10], it is possible in islets of Langerhans that gold thioglucose binds to the cell surface at a site different from the glucose receptor and effects adenyl cyclase. The subsequent rise in cyclic AMP will then potentiate glucose-stimulated insulin secretion. This effect of gold thioglucose is seen at, and is maximal at, low concentrations of the substance ($\leqslant$2 mM). The effect of gold thioglucose is not due to islet damage because in separate experiments, islets pre-incubated for 1 h in 3 mM gold thioglucose, 5 mM glucose, or 3 mM gold thioglucose plus 5 mM glucose all secrete the same amount of insulin when subsequently tested for secretion.

When secretion is measured with glyceraldehyde in the presence of gold thioglucose there is no difference from the values obtained with glyceraldehyde alone, even though glyceraldehyde is known to raise islet cyclic AMP [11,12]. This points to a different mechanism of secretion by glyceraldehyde from that of glucose. Similar differences in the islet response to glucose and glyceraldehyde have been reported [13]. Perhaps gold thioglucose is effective either at the cell surface or at an early stage in glycolysis above the triose-phosphate level.

The concentration of gold thioglucose chosen (3 mM) was the maximum theoretical concentration reached after injection of 0.6 mg/g intraperitoneally, the dose frequently used to produce hypothalamic damage. The effects of this dose on insulin release in rodents is therefore transient and reversible. This short-lived effect is of considerable biochemical interest. It does however not preclude the use of this drug in the induction of experimental obesity.

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