DIGITOXOSE AND THE EXISTENCE OF A GLUCORECEPTOR IN THE BETA CELLS OF THE ISLETS OF LANGERHANS OF THE RAT.

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SUMMARY

On the basis that digitoxin is the only drug reported up to now that stimulating insulin release per se, partially inhibits glucose stimulated insulin release, in this work the idea that the competition glucose-digitoxin may be at the level of a hypothetical glucoreceptor in the beta cells (binding of the glycoside sugar molecule) has been investigated. Our findings indicate that the integrity of the chemical structure of digitoxin is needed to exert its pharmacological action on insulin release. We also demonstrate that digitoxose inhibits glucose stimulated insulin release without inhibiting the glucose oxidation rates of the islets. The discovery of a sugar (digitoxose) that inhibits the insulin secretory response to glucose without inhibiting glucose metabolism is a strong evidence that the action of glucose itself is blocked at the level of glucoreceptors located in the cell membrane, which appear to be the regulator sites of insulin release.

INTRODUCTION

In a recent number of papers (1,2,3,4) we have investigated the role of the microtubular-microfilament system in the insulin secretory response. One of the interesting findings of this work (3) has been the fact that a cardiac glycoside, digitoxin (0.25 μM), produces insulin release per se and a partial inhibition of glucose (16 mM) induced insulin release, indicating a competitive action of these two substances at the level of the trigger mechanisms of insulin secretion. At present a widely accepted concept has been that metabolites of glucose or factors associated with glucose metabolism, rather than glucose itself,

trigger insulin release from the beta cells (5,6). Few authors nevertheless are more in favor that the glucose molecule itself exerts its releasing action by stimulating glucoreceptors located in the cell membrane (7). It has also been considered that a serious point of evidence in favor of the regulator site mechanisms would be provided by the discovery of a sugar that inhibits the insulin secretory response to glucose without inhibiting glucose metabolism (8). In this paper we have investigated the inhibition of glucose stimulated insulin release by digitoxin (3), taking into consideration the effects of both parts of the molecule, the aglicone (digitoxigenin) and the sugar (digitoxose), over insulin release and glucose oxidation rates of the islets of Langerhans with the idea of gaining new insights into the regulation site mechanisms of insulin release.

MATERIAL AND METHODS

Islets were isolated by the collagenase technique (9) from male or female albino rats weighing 150 to 200 grs. A minimum of one hundred islets per experiment were perifused following a procedure recently described (10). The perifusion medium, a bicarbonate buffer, had the following ionic composition Na^{+} 139, K^{+} 5, Mg^{2+} 2, Ca^{2+} 2, Cl^{-} 124, and CO_3H^{-} 24 m Equi./l, pH 7.4. Added to the media as required; digitoxigenin (Sigma) (0.25 μM), digitoxose (Sigma) (16, 8, 4, 0.25, 0.1, 0.050 and 0.025 mM). In many of the studies a double chamber technique was used. In these experiments the islets of two rats combined were placed in each chamber. The chambers were perifused simultaneously with the same peristaltic pump. Islets in one of the chambers were perifused with the buffer containing glucose (3.2 or 16 mM), and those in the other chamber with the perifusion media plus digitoxigenin or digitoxose. The perifusion fluid was collected in graduated tubes at one minute intervals, the volumes were recorded and 0.5 ml aliquots removed for insulin assay. The

insulin content of the perifusate was measured by the inmunoassay technique of Wright and col. (11). The rate of insulin secretion was expressed as μU of insulin x islet^{-1} x minute^{-1}. At least six experiments were performed for each experimental situation. A type experiment has been shown graphically.

Determination of the glucose oxidation rates; groups of 100 islets were incubated for 90 min at 37 ºC in 1.5 ml of the buffer described above, pH 7.4. The incubation medium contained 2.5 μCi/ml of D-(U^{14}C) glucose, specific activity 283 mCi/mM (CEA, France) and glucose (3.2 or 16 mM) plus digitoxin as required (Nativelle, France) (0.25 μM) or digitoxose (16 mM). At the end of the incubation period the flasks were removed from the water bath and chilled. Metabolism was stopped by injection of 1 ml perchloric acid (20%) into the outer vessel containing the islets, followed inmediately by an injection of 0.25 ml of hyamine into the inner vessel. After been shaken for 40 min at 0 ºC the radioactivity trapped in the hyamine was subsequently assayed by liquid scintillation spectrometry using a scintillator composed of toluene

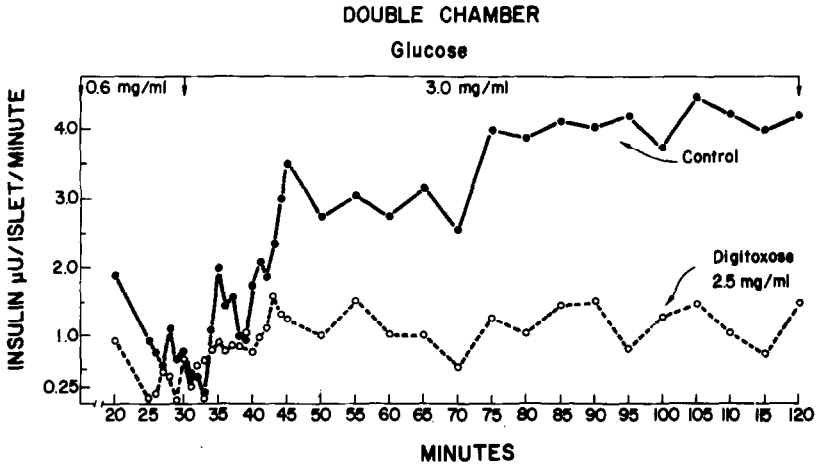

Figure 1. Insulin secretion following stimulation of perifused rat islets with glucose (16 mM) and with glucose (16 mM) plus digitoxose (16 mM). Digitoxose showed an inhibitory effect on glucose induced insulin release.

(1 L), PPO (6 grs) and POPOP (0.2 grs). Blank incubations without islets were carried through each experiment and the blank values were substracted from the experimental values.

RESULTS

Digitoxigenin itself had no effect on insulin secreted values in the presence of glucose (3.2 mM) or glucose (16 mM). Digitoxose (16 mM) had no stimulatory effect on insulin release in the presence of glucose (3.2 mM), and showed a very clear inhibitory effect on glucose (16 mM) stimulated insulin release (figure 1). The insulin values obtained of rat perifused islets following sustained administration of glucose (16 mM) plus digitoxose (16 mM) were 91 $\pm$ 6.51 μU/islet and the values obtained after perifusing the islets with glucose (16 mM) alone were 260 $\pm$

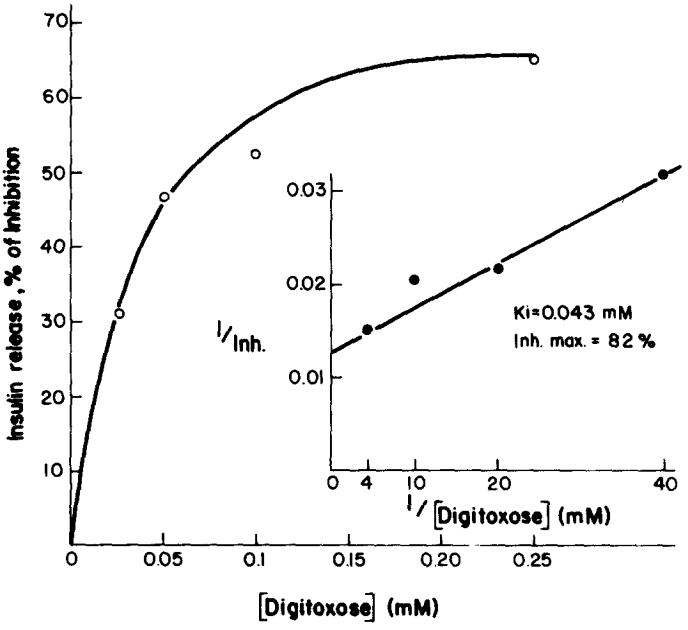

Figure 2. Dose response of the digitoxose mediated insulin release inhibition. In the insert appears a reciprocal plot of the same data plus the numerical values for the inhibition constant (Ki) and maximal inhibition.

11.08 μU/islet. Figure 2 shows the results obtained when digitoxose was constantly perifused at different concentrations. Glucose stimulated insulin release was inhibited at digitoxose concentrations between 16 and 0.25 mM, showing inhibition of approximately 65% (figure 2). From there on, decreasing concentrations of digitoxose decreased the inhibition over glucose (16 mM) stimulated insulin release (figure 2). A plot of reciprocals showed a constant inhibition at 0.043 mM and a maximum inhibition of 82% (figure 2). The rates of glucose oxidation were measured by the formation of $^{14}CO_2$ from D-(U^{14}C) glucose, and are expressed in table 1. When the islets were incubated in the presence of digitoxin (0.25 μM) and glucose (3.2 mM or 16 mM) there was an increase in the glucose oxidation rates (table 1). The same result was obtained when the islets were incubated in the presence of digitoxose (16 mM) plus glucose (16 mM) (table 1).

DISCUSSION

Like all other cardiac glycosides, digitoxin represents the combination of an aglycone or genin (digitoxigenin) and a sugar (2-6 dideoxy-D-ribohexose). In a recent work (3) it has been described that digitoxin produces insulin release per se and inhibition of glucose stimulated insulin release. Our results expressed above indicate that digitoxigenin and digitoxose are not capable of eliciting insulin release in the presence of glucose (3.2 mM), finding that indicates also that integrity in chemical structure is needed for the glycoside (digitoxin) to stimulate insulin release and that this pharmacological action of the glycoside molecule may reside in the aglycone (digitoxigenin) but that the sugar (digitoxose) may be needed for cell permeability (12). An interesting finding was that digitoxose partially inhibited glucose stimulated insulin release without inhibiting the glucose oxidation rates of the incubated islets of Langerhans. This result demonstrates that the inhibitory effect of glucose

TABLE 1

EFFECT OF DIGITOXIN AND DIGITOXOSE OVER GLUCOSE OXIDATION RATES BY ISOLATED RAT ISLETS

	CONTROL	DIGITOXIN (0.25 μM)	DIGITOXOSE (16 mM)
	Glucose oxidation rates (nMoles/100 islets/hr.)		
GLUCOSE 3.2 mM	0.092	0.24	---
16 mM	1.36	2.84	2.60

Table 1: Glucose oxidation by rat islets in incubation media containing a low (3.2 mM) or a high (16 mM) concentration of glucose. The glucose oxidation rates were calculated from the CO_2 output during a ninety-minute incubation period as described in Material and Methods.

stimulated insulin release is at the level of the glycoside sugar molecule without inhibiting glucose metabolism. At present several current hypotheses, concerning the underlying molecular processes of insulin release are based on the concept that the metabolism of glucose or factors associated with glucose metabolism rather than glucose itself, trigger insulin release from the beta cells (5,6). Nevertheless the regulator site hypothesis, direct interaction of glucose with a specific receptor has also been implied as being the mechanism of insulin release (7). The idea has been raised that a serious point of evidence for the existence of this latter mechanism, would be provided by the discovery of a sugar that inhibits the insulin secretory response to glucose without inhibiting glucose metabolism (8). The only sugar known to offer this characteristic would be digitoxose which most probably exerts its inhibitory action over glucose stimulated insulin release by blocking the action of glucose itself at the level of glucoreceptors located in the cell membrane.

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