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Effect of hypothyroidism on the composition and turnover rate of islet phospholipids

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It has already been demonstrated that the pancreatic B cells of hypothyroid rats have a reduced capacity to release insulin in response to glucose (1). This impaired B cell function may be partly due to a diminished rate of glucose oxidation and net calcium uptake associated with ultrastructural alteration of the pancreatic islets (2). To identify further other factors responsible for this diminished B cell secretory function, we studied the composition and the turnover rate of phospholipids in islets obtained from hypothyroid rats.

Materials and methods: Wistar male rats about 100 g b.wt were used, rendered hypothyroid by administration of a single intraperitoneal injection of 700 μCi ^{131}I in saline solution, whilst the remaining animals were used as controls. The experiments were performed at least 5 weeks after the iodine injection. Thyroid function was assessed by the control of individual body weight and the circulating levels of T_3 and T_4 (RIA kit, Diagnostic Prod. Corp., Los Angeles). Serum immunoreactive insulin (IRI) (3) and glucose levels (4) were also measured. Pancreata from both groups of rats were used to obtain isolated islets by collagenase digestion (5).

Insulin secretion by isolated islets was studied by incubating groups of five islets for 60 min at 37°C in 0.6 ml Krebs Ringer bicarbonate (KRB) buffer, pH 7.4 with 1% bovine serum albumin (Sigma Chem. Co., St. Louis, MO), aprotinin 400 IU/ml (Trasylol® kindly provided by Bayer Argentina); and glucose 3.3 or 16.6 mmol/l. The preparation was gassed with a mixture of 5% CO_2:95% O_2. Aliquots from the media were separated for insulin determination by radioimmunoassay (3).

^{3}H-glycerol incorporation into islet phospholipids was studied using groups of fifty islets incubated for 60 min in 1 ml KRB in the presence of 3.3 or 16.6 mM glucose plus the addition of 5 or 25 μCi of ^{3}H-glycerol (2-^{3}H-glycerol, 10 mCi/mmol, New England Nuclear, Boston, Ma), respectively. The tubes were gassed with a mixture of 5% CO_2:95% O_2. At the end of the incubation period, the islets were sonicated and the lipids extracted with 2:1 chloroform:methanol and the washed lower phase was used for the analysis of different lipids. Individual phospholipids were separated by thin layer chromatography (6). Phospholipid areas were visualized with iodine vapour, scraped off from the plates and transferred to counting vials to measure the corresponding radioactivity in a liquid scintillation counter.

Islets phospholipid composition was studied in groups of 600–700 isolated islets disrupted by sonication in 1 ml of 0.05 mM phosphate buffer, pH 7.4. The islet homogenates were extracted and the individual phospholipid fraction isolated as described above. The spots corresponding to each phospholipid fraction were scraped off and eluted with 1.0 M methanol: HCl at 60°C. The eluates were evaporated and the organic phosphorus was mineralized (7) and later measured by a colorimetric reaction (8).

Table 1: Body weights, serum glucose and thyroid hormone levels in control and hypothyroid rats (means ± SEM, n *in parentheses)*

Animals	Body weight (g)		Serum hormone levels			Serum glucose (mg/dl)
	Initial	Final	T_3 (ng/dl)	T_4 (μg/dl)	IRI (μU/ml)	
Controls	125 ± 0.9 (11)	245 ± 4.3 (11)	60.3 ± 4.30 (28)	2.5 ± 0.13 (28)	35.5 ± 4.40 (19)	126.2 ± 6.90 (24)
Hypothyroid	127 ± 5.0 (10)	170 ± 5.4 (10)**	27.5 ± 2.90 (37)**	0.7 ± 0.08 (37)**	24.7 ± 2.60 (19)*	119.0 ± 6.00 (24)

In comparison with controls p <: *0.05; **0.001.

Results and discussion: The final body weight values as well as the serum T_3 and T_4 levels demonstrate the deficient thyroid function in the rats treated with ^{131}I (Table 1). These animals also showed significantly lower serum IRI levels than their corresponding controls. However, there were no significant differences in serum glucose levels between the two groups. This could be due to an enhanced effect of insulin in these animals in the peripheral tissues (9).

Table 2: Insulin secretion by islets, isolated from control and hypothyroid rats, incubated with two different glucose concentrations (means ± SEM, n *in parentheses)*

Glucose concentration	Insulin secreted (μU/islet/h)	
	Control	Hypothyroid
3.3 mM	10.8 ± 1.10 (18)	16.8 ± 2.86 (9)
16.6 mM	317.8 ± 32.5 (20)**	153.8 ± 17.2 (9)*

**In comparison with the value for the 3.3 mM glucose* p <: *0.05; **0.001.

The insulin release by isolated islets was not significantly different in the hypothyroid rats as compared to the controls in the presence of 3.3 mM glucose. However, the amount of insulin released by islets from hypothyroid rats in the presence of 16.6 mM glucose was significantly lower than that secreted by control islets (Table 2). These results confirm the diminished B cell capacity to release insulin observed in hypothyroid animals (1).

The phosphorus content of islet phospholipids was significantly lower in hypothyroid rats. This difference was mainly due to changes in the phosphatidylinositol (PI) and phosphatidylcholine (PC) fractions (Table 3).

The [^{3}H]-glycerol incorporation into islet phospholipids was similar in both groups when the islets were incubated in the presence of 3.3 mM glucose (267.4 ± 13.9 for controls, vs 254.5 ± 18.6 for hypothyroid rats (dpm/islet/h)). Conversely, in the presence of 16.6 mM glucose, control islets incorporated significantly larger amounts of ^{3}H-glycerol than islets from hypothyroid rats (486.2 ± 30.3 vs 340.9 ± 12.4 dpm/islet/h respectively; $p < 0.001$).

Table 3: Phosphorus content of phospholipids from islets isolated from control and hypothyroid rats (means ± SEM, n = 2†*)*

	Phosphorus (ng/islet)	
Phospholipid	Control	Hypothyroid
PS	0.8 ± 0.16	0.5 ± 0.02
PI	0.7 ± 0.02	0.5 ± 0.02**
PC	2.0 ± 0.04	1.4 ± 0.09*
PE	1.4 ± 0.06	0.9 ± 0.12
TPL	4.9 ± 0.14^c	3.3 ± 0.03^f

PE, phosphatidylethanolamine; TPL, total phospholipids. †n *represents the number of experiments performed in triplicate with islets obtained from at least six animals. In comparison with value for controls* p < *: *0.05; **0.01.*

The percentages of [^{3}H]-glycerol incorporation into the different islet phospholipid fractions also showed a similar pattern (Table 4). No differences were seen in the presence of low glucose, but with 16.6 mM glucose, control islets showed a significant increase in the PI fraction, a modest one in the phosphatidylserine (PS), and a marked decrease at the PC level. High glucose levels did not induce changes in islets from hypothyroid rats.

Table 4: Percentage distribution of [^{3}H]-glycerol incorporation into phospholipids in islets isolated from control and hypothyroid rats incubated with two different concentrations of glucose (means ± SEM, n = 3†*)*

	% [^{3}H]-glycerol incorporated			
Group	PS	PI	PC	PE
3.3 mM glucose				
Control	2.5 ± 0.33	5.9 ± 0.51**	66.2 ± 2.34*	25.4 ± 2.65
Hypothyroid	2.1 ± 0.22	6.1 ± 0.85	66.3 ± 3.11	25.5 ± 0.82
16.6 mM glucose				
Control	3.7 ± 0.59	13.3 ± 0.21	58.8 ± 2.04	24.3 ± 2.81
Hypothyroid	1.7 ± 0.04^a	6.4 ± 0.43^c	65.8 ± 0.56^b	26.0 ± 0.42

†n *represents number of experiments performed by triplicate with islets obtained from at least six animals. In comparison with control group with 16.6 mM glucose* p < *: *0.05; **0.001. In comparison with corresponding control group* p < *: ^{a}0.05; ^{b}0.02; ^{c}0.001.*

The importance of the islet phospholipid metabolism, particularly the PI fraction, as a regulator of the glucose-induced insulin secretion has been described in detail in a recent review (10). Moreoever, we have demonstrated that PS turnover rate is also involved in this mechanism (11). According to our results, the reduced effect of glucose on insulin secretion in the hypothyroid state may be due partly to the altered phospholipid composition and the impaired phospholipid turnover rate observed in the pancreatic islets. Otherwise, these results are the first evidence of a hormonal control of the islet phospholipid composition and metabolism. Further studies are needed in order to explain the underlying mechanism of such effects.

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