Biochemical Pharmacology, Vol. 37, No. 19, pp. 3609–3611, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00
Pergamon Press plc

EFFECT OF PROLONGED ADMINISTRATION OF CYCLOSPORIN A ON (PRO)INSULIN BIOSYNTHESIS AND INSULIN RELEASE BY RAT ISLETS OF LANGERHANS

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(*Received* 27 *January* 1988; *accepted* 12 *April* 1988)

Abstract—One group of rats received, an oral dose of 25 mg/kg body weight of Cyclosporin A daily for 21 consecutive days in olive oil, whereas the control group received an equal amount of the vehicle for the same period. On the 22nd day animals selected at random from both the groups were subjected to glucose tolerance (1.5 g/kg body weight/oral). The blood sugar values indicated glucose intolerance in experimental rats compared to vehicle-treated rats. The remaining animals were killed, and the pancreases were separated for islet isolation. After incubation of islets for 3 hr at 37° with different glucose challenges, a highly significant ($P < 0.001$) lowering of (pro)insulin biosynthesis, significant decrease in IRI release as well as significant inhibition in activities of acid phosphatase and cathepsin B was seen.

The discovery of Cyclosporin A (Cs A) in 1972 as a potent immunosuppressive agent resulted in its wide application in clinical transplantation [1, 4]. It was also seen as a therapeutic alternative in immunosuppressive therapy in insulin-dependent (Type 1) diabetes mellitus [2–5]. Following the clinical use of Cs A, a number of adverse effects (nephrotoxicity, hepatotoxicity [1], glucose intolerance [6], though reversible with the cessation of treatment) were recorded. Cs A was also found to cause a marked decrease in preformed pancreatic insulin content accompanied by reduced insulin release [7, 8]. The adverse effect of Cs A on the islets of Langerhans is partly understood as some data on the effect of Cs A on insulin release have been published [8], but no information is available on its possible effects on (pro)insulin biosynthesis after prolonged exposure. Therefore a detailed study was undertaken with emphasis on (pro)insulin biosynthesis. The target enzymes (acid phosphatase and cathepsin B) concerned in the synthesis and conversion process of (pro)insulin to insulin were also measured. The results thus obtained are presented in this communication.

MATERIALS AND METHODS

Chemicals. Insulin RIA kit and 4,5 [^{3}H]-leucine, sp. act. 6800 mCi/mmol (BARC, Bombay, India), Soluene-250 (Packard Instrument Co., U.S.A.), *N,N*-dimethylated hemoglobin, CN–Br activated Sepharose-4B, Collagenase type V, 3:5 diamino benzoic acid, DNA, Insulin (Sigma Chemical Co., St Louis, MO), β-glycerophosphate sodium (E. Merck, F.R.G.), PPO, POPOP (V.P. Chest Institute, Delhi, India). Other chemicals were of analytical grade.

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Animals. Charles Foster strain male albino rats (200–250 g) were used. The rats were allowed Hind Lever pellet diet and water *ad libitum*. The rats were sized into two groups of sixteen each. One group received Cs A (25 mg/kg body weight/day; part of it is a generous gift from Sandoz Ltd., Basel, Switzerland) in olive orally by intubation for 21 days. The second group received an equivalent amount of olive oil. On the 22nd day glucose tolerance (1.5 g/kg body weight/oral by intubation) was performed in eight 18-hr-fasted rats, selected at random. Blood was collected from tail vein at 0 and 0.5, 1.0 and 3.0 hr after glucose administration. Blood sugar was estimated photometrically [9].

Plasma is separated from an aliquot of blood for the determination of immunoreactive insulin (IRI) by radioimmunoassay [10] utilizing a second antibody and polyethylene glycol. The remaining animals were killed under ether anaesthesia and the pancreases separated. A portion was used for the determination of total pancreatic insulin after repeated acid ethanol extraction and the rest used for islet isolation.

Islet isolation. Pancreatic islets were isolated by collagenase digestion technique [11]. Groups of five islets in 0.5 ml Krebs Ringer biocarbonate (KRB) buffer (pH 7.4, previously gassed with $O_2:CO_2::19:1$) containing 1 mg/ml BSA were incubated for 3 hr at 37° with gentle shaking (90 cycles/min). The media were supplemented with varying concentrations of glucose in results together with 20 μCi [^{3}H]leucine/ml. Incubation media were collected separately. Islets were washed with phosphate buffer saline (PBS; pH 7.4) and subsequently sonicated for 15 sec in 15 μl PBS.

(Pro)insulin biosynthesis. Newly synthesized (pro)insulin in the aliquots of sonicated islet preparations and corresponding incubation media were

Table 1. Effect of prolonged administration of Cyclosporin A (25 mg/kg body weight/day by mouth in olive oil for 21 days) on glucose tolerance (1.5 g/kg body weight orally by intubation) of male albino rats

		Time in hr after glucose 0.0	0.5	1.0	3.0
Control	Blood glucose	70 ± 1.98 (7)	137 ± 3.10 (8)	93 ± 4.00 (8)	68 ± 2.68 (7)
	Plasma IRI	20.01 ± 2.21 (6)	56.62 ± 1.91 (6)	44.00 ± 0.88 (5)	23.17 ± 2.11 (5)
Experimental	Blood glucose	108 ± 3.34*[3] (8)	211 ± 2.30*[3] (8)	176 ± 3.78*[3] (8)	148 ± 5.60*[3] (6)
	Plasma IRI	11.87 ± 2.15*[1] (6)	32.00 ± 1.12*[3] (8)	21.68 ± 1.98*[3] (5)	14.13 ± 1.24*[2] (6)

Figures in parenthesis denote number of observations. Values are mean ± SEM.
*[1] $P < 0.05$; *[2] $P < 0.01$; *[3] $P < 0.001$ (Student's t-test).

determined by the method of Bone and Howell [12]. The total (pro)insulin synthesized in islets were calculated by summing up the radioactive leucine incorporation in homogenates with that of media.

Insulin release. The islets were incubated following the same protocol as above except that tritiated leucine was not added. After incubation, IRI content of the media were measured by RIA [10].

Enzymes. Simultaneous assay of acid phosphatase (EC 3.1.3.2) [13] and cathepsin B (EC 3.4.22.1) [14, 15] enzyme activities were carried out at 37° (pH 5.0).

Islet DNA. Islet DNA content was estimated by an improved fluorometric assay [16].

Statistics. Results are given as mean ± SEM. Data were analyzed by Student's t-test and $P < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Prolonged administration of Cs A to rats resulted in a substantial decrease in (a) (pro)insulin biosynthesis, (b) immunoreactive insulin release, and (c) in the specific activities of acid phosphatase and cathepsin B in islets isolated from treated rats when challenged with different concentrations of glucose. The DNA content of islets isolated from experimental (48.97 ± 2.10 ng/islet, N = 10) as compared to untreated, control (55.42 ± 2.43 ng/islet, N = 10) remained more or less unchanged. It is interesting to note that when glucose is administered to Cs A treated and untreated groups of rats, apart from glucose intolerance there has been a significant decrease in plasma IRI level at 0, 0.5, 1 and 3 hr (Table 1). A lowering in the level of pancreatic IRI ($P < 0.02$) was also observed in treated rats (0.67 ± 0.21 U/g wet weight, N = 6) compared to controls (1.89 ± 0.38 U/g wet weight, N = 6).

Glucose intolerance was observed in man [17] and animals [6] after prolonged administration of Cs A and was attributed to diminished insulin release. A presumption has been made on the possible effects of Cs A on (pro)insulin biosynthesis. Data obtained by us with respect to insulin release is in agreement with the observations made by others [6, 17]. A highly significant ($P < 0.001$) inhibition in pro(insulin) biosynthesis was spotted (Table 2) when islets from treated rats were exposed to different concentrations of glucose, giving definite evidence of interference in synthesis.

Studies on the activities of acid phosphatase and Cathepsin B, the lysosomal enzymes concerned with synthesis and cleavage of (pro)insulin to insulin demonstrated a significant fall in their activities. These data indicate a defect in conversion of (pro)insulin to insulin. The enzyme parameters correlate handsomely to inhibition of (pro)insulin biosynthesis resulted in diminished insulin release (Table 2).

Table 2. Effect of prolonged administration of Cyclosporin A (25 mg/kg body weight/day orally for 21 days in olive oil) on (pro)insulin biosynthesis (cpm × 10^3/islet), immunoreactive insulin release (μU/islet), specific acid phosphatase and cathepsin B enzyme activity (U/min/mg islet protein) by isolated rat islets

Parameter		Glucose concentration (mM) 2.8	11.1	16.7	22.2
(Pro)insulin biosynthesis	Control	3.10 ± 0.17 (6)	4.43 ± 0.13 (6)	5.91 ± 0.24 (7)	5.13 ± 0.27 (5)
	Experimental	1.70 ± 0.09** (7)	2.38 ± 0.15** (6)	3.60 ± 0.30** (6)	3.89 ± 0.41 (5)
IRI release	Control	3.17 ± 0.27 (5)	9.33 ± 0.78 (6)	21.07 ± 1.12 (6)	19.87 ± 1.67 (6)
	Experimental	1.98 ± 0.79 (7)	4.12 ± 0.23** (5)	15.98 ± 0.92* (6)	15.02 ± 1.36 (6)
Acid phosphatase	Control	7.20 ± 0.41 (6)	10.00 ± 2.41 (6)	18.00 ± 1.62 (7)	13.11 ± 0.43 (6)
	Experimental	3.88 ± 0.78* (7)	5.10 ± 0.37 (5)	11.60 ± 0.76* (7)	12.11 ± 0.91 (7)
Cathepsin B	Control	1.80 ± 0.21 (5)	2.70 ± 0.11 (5)	4.20 ± 0.31 (6)	3.12 ± 0.72 (5)
	Experimental	0.72 ± 0.61 (5)	1.06 ± 0.29* (8)	2.64 ± 0.16* (6)	2.11 ± 0.23 (7)

Figures in parenthesis denote number of observations. Values are mean ± SEM.
*$P < 0.01$; **$P < 0.001$ (Student's t-test).

Islets obtained from untreated healthy animals when exposed to Cs A in doses above therapeutic to toxic levels for short as well as prolonged periods showed no effect on the morphological and functional activity of the islets [18]. But in contrast, we observed that *in vivo*, Cs A adversely affects islet insulin synthesizing capacity.

Besides its extensive use to prevent graft rejection clinically, Cs A has been used in non-specific immunosuppressive therapy of type 1 diabetes with very satisfactory results [3, 5]. The dose and duration of Cs A had varied from 5 to 10 mg/kg body weight for a period of 1 to 5 years. Although it will not be fair to extrapolate the rat data (i.e. glucose intolerance, suppression of (pro)insulin biosynthesis and IRI release) to man, the present observations at least warrant monitoring of insulin level in blood during prolonged clinical use of Cs A in immunosuppression.

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