Direct Effects of Cyclosporin A on Human Pancreatic β-cells

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SUMMARY

Cyclosporin A (CyA) may induce clinical remission in newly diagnosed insulin-dependent diabetes mellitus patients. Recently, however, adverse effects of high doses of CyA on rodent islets have been reported in vivo and in vitro. The possible direct effects of CyA on the human endocrine pancreas were therefore evaluated. Islets isolated from eight necrokidney donors were cultured in the presence of a therapeutically relevant dose of CyA, i.e., 100 ng/ml. During a 5-day culture period the release of insulin was reduced by 36% (range 7–61%), whereas the islets' content of insulin was increased by 59% (range 3–268%). The glucagon content was not affected. Cyclosporin G inhibited the insulin release, whereas dihydrocyclosporin D had no consistent effects. Glucose-stimulated insulin release from perifused islets was markedly depressed in CyA-treated islets. This effect was not fully reversed 48 h after removal of the drug. We concluded that CyA has a direct inhibitory effect on insulin release from human pancreatic islets with a concomitant increase in the residual insulin content. If applicable to the in vivo condition, CyA may therefore, in addition to its immunosuppressive effect, have direct effects on the endocrine pancreas, which may be relevant for clinical application of the drug. DIABETES 1986; 35:1049–52.

Autoimmune processes seem to be involved in the pathogenesis of insulin-dependent diabetes mellitus (IDDM).[1] Investigations in humans suggest that cyclosporin A (CyA) may induce clinical remission in newly diagnosed type I diabetic patients.[2,3] These effects of CyA are supposed to be due to the suppressive action of the drug on the cellular immune system.[4,5] Recent animal studies, however, have revealed that high concentrations of CyA may have toxic effects on the pancreatic islets both in vivo[6,7,8] and in vitro.[9,10] In recipients of pancreatic transplants, CyA has been reported to deteriorate glucose tolerance.[11] The aim of our study was therefore to determine whether CyA and its analogues cyclosporin G (CyG) and dihydrocyclosporin D (CyD) affect isolated human islets maintained in organ culture.

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MATERIALS AND METHODS

Drugs and reagents. Cyclosporin A, cyclosporin G, and dihydrocyclosporin D were kindly supplied by J. F. Borel (Sandoz AG, Basel, Switzerland). The drugs were dissolved to 1 mg/ml in 0.1 ml ethanol and 0.02 ml Tween 80 (polysorbate 80) (Fluka AG, Buchs. Switzerland) followed by slow addition of 0.88 ml RPMI-1640 (Flow Laboratories, Irvine, UK). The corresponding dilution of the solvent was added to the control media.

Isolation and culture of human islets. Islets were isolated from the pancreas of eight necrokidney donors by collagenase digestion, as described previously.[12] In brief, small pieces of tissue were incubated individually with 1–2 mg/ml collagenase (Sigma, St. Louis, MO; or Boehringer, Mannheim, FRG) in Hanks' balanced salt solution (Gibco, Paisley, UK). The treatment was repeated several times, and islets were collected from the supernatants under a dissecting microscope. The islets were precultured for 1–2 days in RPMI-1640 supplemented with 10% newborn calf serum and further cultured in RPMI-1640 supplemented with 0.5% human serum for 7–15 days before the experiments. In each experiment, ~50 islets were placed in bacteriological plastic Petri dishes (Tecnunc, Roskilde, Denmark) containing 5 ml medium with solvent alone or 100 ng/ml CyA, CyG, or CyD. The medium was changed after 2 and 5 days culture and analyzed for insulin. The islets were collected after culture, homogenized, and analyzed for content of insulin and glucagon, as previously described.[13]

Islet perifusion. Islets cultured for 5 days with or without CyA were mixed with Bio-Gel P2 (Bio-Rad, Rockville Center, NY) and placed in a perifusion chamber, as previously described.[14] Krebs-Ringer buffer containing 2 mM L-glutamine

TABLE 1
Effect of 100 ng/ml cyclosporin A (CyA) on insulin release (ng · islet^{-1} · day^{-1}) and insulin and glucagon content (ng/islet) of isolated human islets

Donor			HLA type			Ischemia time (min)		Preculture	Insulin release		Insulin content		Glucagon content	
No.	Age (yr)	Sex	A	B	DR	Warm	Cold	(days)	Control	CyA	Control	CyA	Control	CyA
1	13	F	1,3	7,8	2,w6	15	95	7	2.5	1.1	0.2	1.0	ND	ND
2	13	M	2,24	5,38	w6,7	23	60	12	11.3	7.4	4.3	5.0	3.7	3.9
3	19	M	2,24	7	2	26	75	13	11.0	8.6	19.5	20.0	2.4	2.1
4	33	F	3,28	7,44	2,5	18	70	8	20.4	9.6	23.1	28.4	11.6	11.4
5	37	F	2,3	7,14	2,7	35	150	13	4.6	4.3	14.1	15.1	5.3	5.9
6	37	F	2,3	15,35	1	15	60	9	2.6	1.1	4.3	6.2	ND	ND
7	37	M	3,28	5,47	w6,7	20	170	15	4.2	2.4	4.2	6.0	ND	ND
8	45	M	2,4	15,35	1	18	540	8	1.5	1.0	2.1	3.3	2.3	2.2
Mean									7.3	4.4	9.0	10.6	5.1	5.1
Wilcoxon's test for paired differences									$P \leq .02$		$P \leq .05$		NS	

Islets were cultured for 5 days in medium RPMI-1640 supplemented with 0.5% normal human serum. Results are means of duplicate cultures. The coefficients of variation were 17.5% for insulin release and 17.2% for insulin content. ND, not determined; NS, not significant.

and 2 mg/ml bovine serum albumin (BSA; Miles, Elkhart, IN) and either 3.3 or 20 mM D-glucose with or without 0.1 mM IBMX (3-isobutyl-1-methylxanthine; Aldrich, Beerse, Belguim) was used as perifusion medium. Residual insulin was extracted by the addition of 3 M acetic acid at the end of the perifusion period. The eluates were analyzed for insulin.

Statistical methods. Wilcoxon's test for single or paired observations was used. Each observation represents the mean of two parallel incubations.

RESULTS

The effect of 100 ng/ml CyA on insulin release and insulin and glucagon content was studied in islets from eight individuals (Table 1). The islets exposed to 100 ng/ml CyA released less insulin ($P \leq .02$) but contained more insulin than the control islets ($P \leq .05$; Table 1). There was no change in the glucagon content.The effects were not related to the age, sex, or tissue type of the eight donors. When CyA, CyG, and CyD were tested on islets from five of the donors, CyA showed a consistent effect on both insulin release and content, whereas CyG only inhibited the release ($P \leq .10$) and CyD showed no consistent effects (Fig. 1).

Islets exposed to CyA for 5 days showed a marked reduction in insulin release in response to perifusion with 20 mM D-glucose (Figs. 2 and 3). The insulin release in the presence of IBMX was also reduced in two of the three islet preparations studied. However, when CyA was removed from the cultures 2 days before the perifusion, a somewhat higher response to IBMX was noted (Fig. 3, *right panel*).

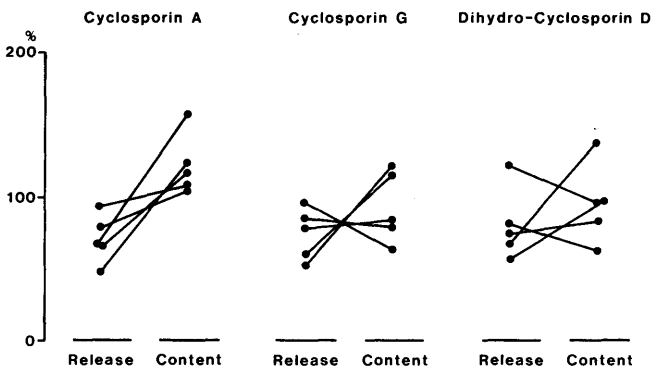

FIG. 1. Effect of 100 ng/ml CyA, CyG, and CyD on insulin release and content of human islets cultured for 5 days in RPMI-1640 supplemented with 0.5% normal human serum. Results are given in % of values for control islets. Donor data are given in Table 1 (no. 2, 3, 4, 5, and 8).

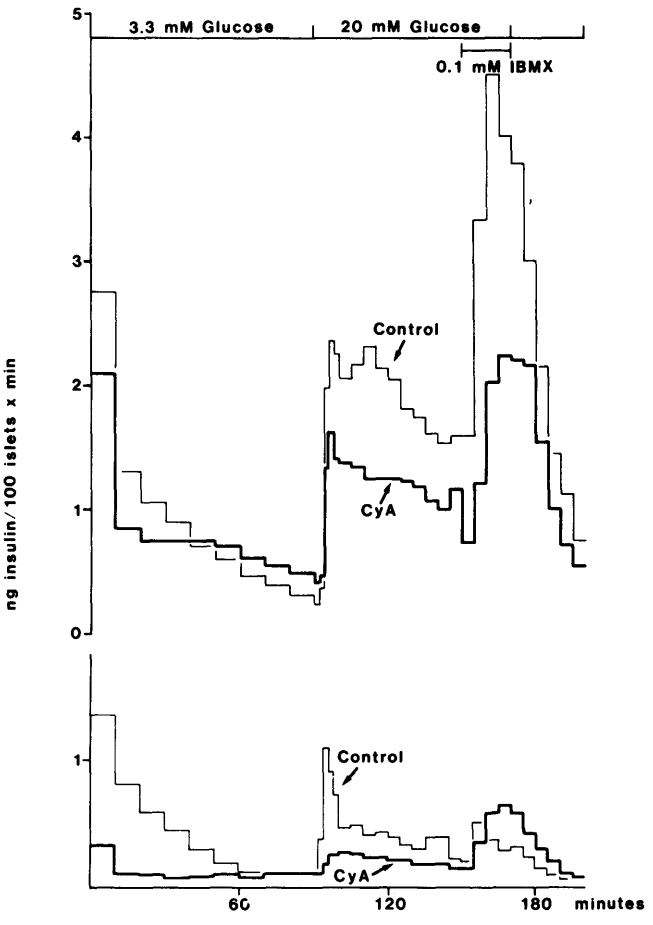

FIG. 2. Insulin release from human islets cultured for 5 days in presence or absence of 100 ng/ml CyA. Islets were perifused with 3.3 mM glucose for 90 min, 20 mM glucose for 60 min, and 20 mM glucose plus 0.1 mM IBMX for 20 min. *Upper panel*: islets from donor no. 7 (see Table 1). *Lower panel*: islets from donor no. 1 (see Table 1).

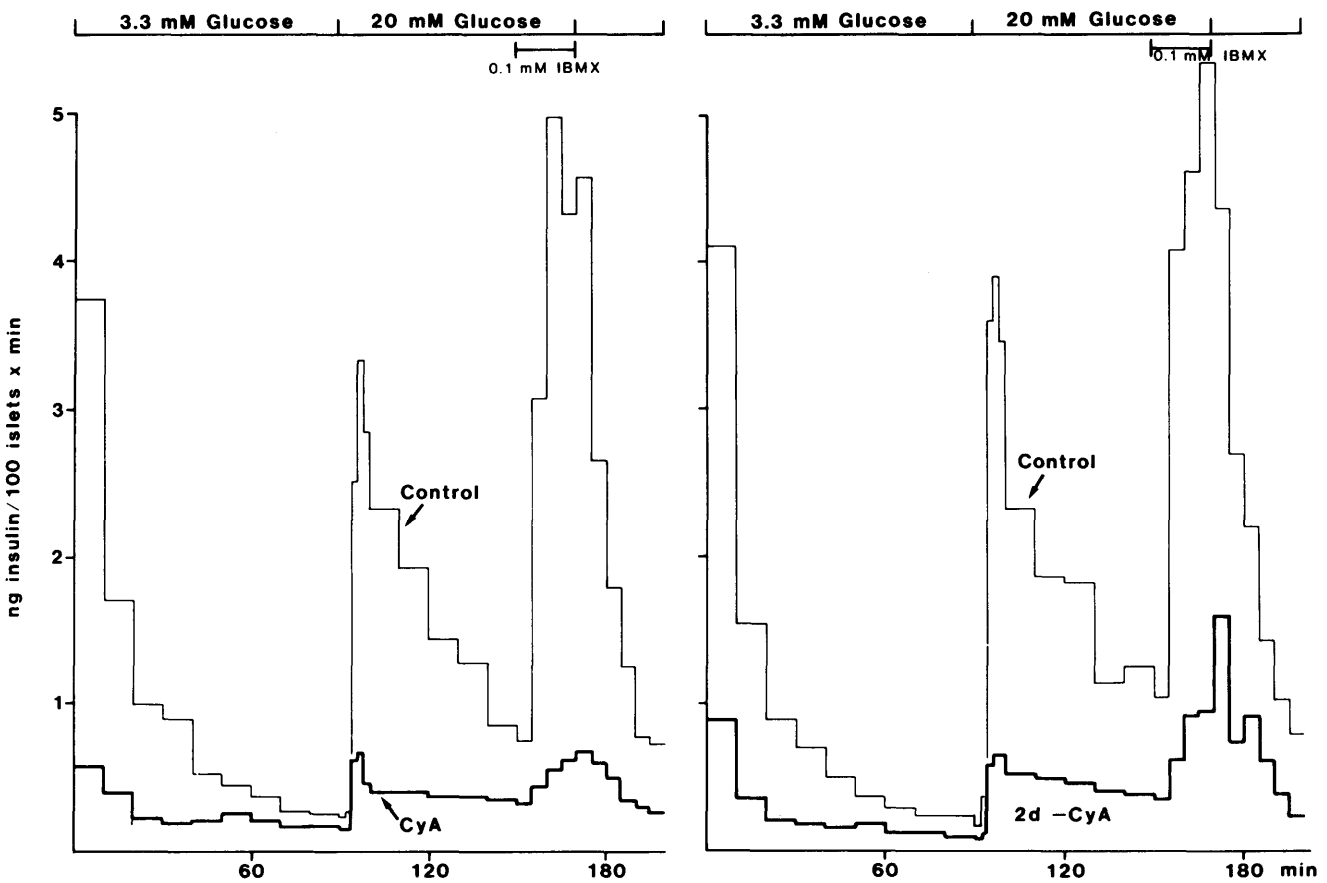

FIG. 3. Insulin release from human islets cultured for 5 days in presence or absence of 100 ng/ml CyA (*left panel*) and parallel islets cultured for additional 2 days in absence of CyA (*right panel*). Perifusion was performed as described in legend to Fig. 2. Donor was no. 6 (see Table 1).

DISCUSSION

Our study demonstrates that the immunosuppressive drug cyclosporin A has a direct inhibitory effect on insulin release from isolated human islets. The CyA concentration of 100 ng/ml is similar to the blood concentrations found in diabetic patients treated with CyA, e.g., 173 ± 32 ng/ml[3] and 100–200 ng/ml.[2] In 4 pancreatic graft recipients treated with CyA and prednisolone, decreased glucose tolerance was observed,[15] and plasma concentrations of 660 and 770 ng/ml were found in 2 of the patients.[11] Glucose tolerance was not affected, however, in 12 patients receiving pancreas and renal transplants.[16] Alterations in vivo are difficult to interpret because simultaneously administered prednisolone may contribute to changes in glucose metabolism and islet function.[17]

In the diabetes-prone BB rat, treatment with CyA has been shown to prevent the development of the diabetic syndrome when given before onset of the disease.[18,19] Reversible glucose intolerance, however, was found in normal rats with blood levels of CyA ~250 ng/ml,[8] whereas manifest diabetes was induced in diabetes-resistant lines of BB rats.[7] Severe morphologic and diabetogenic changes were seen in pancreata from rats with blood concentrations of 4990 ± 454 ng/ml.[6] In vitro, no acute reduction in insulin secretion from rat islets was obtained with 2000 ng/ml CyA,[10] whereas insulin biosynthesis and release as well as DNA synthesis were impaired in mouse islets cultured for 7 days with 1000 ng/ml CyA (but not with 100 ng/ml, which only reduced the insulin content of the islets).[9,20]

These results indicate that species differences and dosages must be considered when evaluating the CyA effects both in vivo and in vitro. In our experiments the variation in the effects of both CyA and the analogues CyG and CyD on human islets may also indicate variation among individuals. No relation to age, sex, or HLA type was demonstrable, but the number of individuals is too small to draw firm conclusions.

The relative purity of the islet preparations from each individual was ascertained by a preculture period of 7–15 days, after which the number of islets and the rate of insulin secretion per islet remained constant.[12] The homogeneity of each preparation is further supported by the small coefficients of variation between the duplicates, which were obtained from separate parallel cultures (Table 1). Although electron microscopy of similar preparations only revealed the presence of endocrine cells,[12] it cannot be ruled out that some of the preparations contained other cell types; the contents and release of hormones, however, strongly suggest that the proportion of endocrine tissue in each preparation remained constant throughout the study.

The variation in insulin release and content between the individual islet isolates, however, may also be due to the differences in ischemia time and collagenase digestion.[12] The inhibitory effect of CyG and the lack of consistent effects of

CyD may reflect the difference in immunosuppressive potency between these analogues.[21]

The increased or unchanged insulin content in the islets exposed to CyA and the possible tendency to recovery of the release after removal of the drug do not indicate, however, total destruction of the β-cells by 100 ng/ml CyA. Recently, CyA was shown to bind to calmodulin,[22] which may explain the CyA effect on insulin secretion. In accordance with the effect of the calmodulin inhibitor trifluoperazine on rat islets,[23] the synergism between glucose and IBMX was retained in the CyA-treated human islets.

The conclusion from the present study, however, suggests that CyA at a therapeutic concentration has a direct inhibitory effect on insulin secretion, which may be relevant for clinical application of the drug.

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