Synthesis-Secretion Coupling of Insulin
Effect of Cyclosporin

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This study investigated the effects of cyclosporin (Cs) on insulin secretion and synthesis from the endocrine pancreas. With in vitro perfused pancreases from control and Cs-treated rats (1, 5, 10, or 25 mg · kg^{-1} · day^{-1} for 2 wk), a dose-response relationship between Cs dose and inhibition of insulin secretion was demonstrated. Examination of the dynamic secretory response to a glucose stimulus (200 mg/dl) over a 3-h perfusion revealed an inhibition of all three secretory phases. Similarly, the ability of the pancreases to synthesize insulin decreased as a function of Cs dose. Reversibility of Cs toxicity on the pancreas was established by 2 wk after cessation of treatment. To evaluate the effect of Cs treatment in vivo, intravenous glucose tolerance tests were performed. Rats treated with 25 mg · kg^{-1} · day^{-1} Cs for 2 wk had significantly lower *k* values (slope of log glucose concentration over time) than controls. At 10 mg · kg^{-1} · day^{-1}, although curves that appeared abnormal were observed, *k* values were not significantly different from those of controls. In summary, this study demonstrates the profound inhibitory effect of Cs on the endocrine pancreas.
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The effects and side effects of the powerful immunosuppressive agent cyclosporin (Cs) have received widespread investigation. This cyclic undecapeptide has been clinically used for <10 yr; thus, full knowledge of its effects has yet to be determined. Although there are several well-documented toxic effects of Cs at high doses (1), its efficiency as an immunosuppressive agent and its apparent lack of unmanageable side effects have made Cs the drug of choice in many transplantation procedures.

Recently, however, there has been increasing evidence that Cs has a toxic effect on the endocrine pancreas. In 1983, Gunnarrson et al. (2) described impaired glucose metabolism when four transplantation patients were switched from azathioprine immunosuppression to Cs. When the patients were put on Cs therapy, the *k* values (slope of log glucose concentration over time) of intravenous glucose tolerance tests (IVGTTs) declined, but circulating C-peptide levels increased. Therefore, the assumption was made that Cs was not interfering with β-cell secretory function but in some unknown way was promoting peripheral insulin resistance. Similar results were reported by Engfeldt et al. (3). Others observed a decrease in plasma insulin and an accompanying glucose intolerance (4,5). In an intriguing study performed by Merrell et al. (6), canine pancreatic islet–autotransplanted animals were treated with Cs and compared with autotransplanted controls that received no Cs. Cs-treated animals showed a much higher graft failure rate and significantly reduced fasting insulin levels. However, there was no histological evidence of rejection, thus implying a direct toxic effect of Cs on the islets. Finally, Helmchen et al. (7) demonstrated, at a Cs dose of 50 mg · kg^{-1} · day^{-1}, severe degranulation and degeneration of rat islets accompanied by in vivo hyperglycemia and hypoinsulinemia.

Although there are several reports that have indicated no effect of Cs on β-cell function (8,9), there are far more that have led investigators to pursue this potential side effect. In vitro studies of islet function in the presence of Cs have been conflicting. Some investigators have shown that Cs inhibits glucose-induced insulin release in both isolated islets (10–12) and a glucose-sensitive clonal β-cell line (12). Laube and Hahn (13), however, showed no reduction in insulin secretion over a wide range of Cs concentrations.

The purpose of this study was to focus on the characteristics of Cs action directly on the endocrine pancreas. Specifically, we asked four questions: *1*) Does Cs treatment alter glucose-induced insulin output when given acutely and/or

Glucose 1 mM = 18 mg/dl Insulin 1 pM = 0.139 μU/ml

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chronically? 2) If present, is this Cs-mediated effect due to changes in secretion or synthesis of insulin or both? 3) Does Cs alter pancreatic endocrine function in a dose-dependent manner? and 4) Is the effect reversible? The in vitro pancreas-perfusion technique allowed us to investigate the dynamics of insulin synthesis-secretion coupling in response to glucose introduction into the normal arterial supply to the endocrine pancreas. This more closely mimics the in vivo response of the whole pancreas than an in vitro islet or cell preparation. At the same time, this technique allowed us to look at the organ in isolation, thus enabling distinction between direct pancreatic effects and peripheral effects. We also examined the ability of the intact animal to metabolize glucose in the presence of Cs. These two approaches, when combined, allowed us to determine the extent of toxicity of the drug on the pancreas and to infer how it is manifested in vivo.

MATERIALS AND METHODS

Experimental animals. Male Sprague-Dawley rats weighing ~300 g were used in this study. Animals were housed under 12-h light-dark conditions with access to food and water ad libitum. GTTs were performed on over-night-fasted rats. All other experiments were performed on nonfasted animals. Each group of rats was injected each morning with vehicle (Cremaphor EL, polyoxyethylated castor oil) containing the same dose of Cs (either 1, 5, 10, or 25 mg · kg^{-1} · day^{-1} i.m.) for 14 days. After treatment, the animals were used for one or more of the following experiments: in vitro pancreas perfusion followed by pancreatic insulin content extraction, insulin content extraction without perfusion, and/or GTT. Control animals received neither Cs nor vehicle, because we ascertained that vehicle injection for 14 days has no effect on the pancreas (data not shown).

In vitro pancreas perfusion. Rats were anesthetized with 65 mg/kg i.p. pentobarbital sodium. The pancreas-perfusion technique was performed as previously described (14). Briefly, the pancreas, proximal duodenum, spleen, and stomach were removed, and the celiac artery was cannulated for arterial inflow. The venous effluent was collected through a portal vein cannula. The perfusate consisted of a dextran–Kreb's bicarbonate buffer solution (15) containing 200 mg/dl glucose and Ca^{2+} and Mg^{2+} at 3.25 and 1.3 meq/L, respectively (free Ca^{2+}-to-Mg^{2+} ratio of 2.5; 15). The constant flow rate through the preparation was ~5 ml/min with a Cole-Parmer Masterflex pump. The effluent was collected continuously for 3 h with samples collected at the following times: min 1–10, 12, 15, 20, and 25 and every 10 min from min 30 to 180. Effluent samples were stored at −20°C until subsequent insulin determination. There were seven experimental groups of rats: control rats, rats treated with one of the four indicated doses of Cs, nontreated rats in which pancreases were perfused with 1 μg/ml Cs in the perfusate, and animals treated with 10 mg · kg^{-1} · day^{-1} Cs followed by 2 wk of recovery. Cs was obtained from Sandoz (Basel).

Insulin radioimmunoassay (RIA). Insulin concentrations of the venous effluents given above were determined by RIA with the method of Grodsky and Forsham (16). Radiolabeled human insulin was purchased from Amersham (Arlington Heights, IL), and guinea pig anti-insulin serum was produced in this laboratory. Purified rat insulin for standard was purchased from Novo (Copenhagen). Insulin secretory rates were calculated by multiplying the measured perfusate insulin concentration (ng/ml) by the flow rate (ml/min).

Pancreas insulin content. On completion of the 3-h perfusion, total pancreas insulin content (IC) was extracted as previously described (17). Briefly, pancreases were dissected from the surrounding tissues and frozen in saline. After thawing, the pancreases were minced in an ice-cold acid/ethanol solution containing Trasylol (400 KIU/ml), sonicated with a W-375 sonicator (Heat Systems-Ultrasonics), and centrifuged. The supernatant was removed, and sonication and extraction were repeated twice more. The supernatant volumes were combined and assayed for insulin by RIA. This procedure was also performed on pancreatic preparations that were not perfused, thus providing pancreatic IC data for time zero ($IC_{t=0}$). $IC_{t=0}$ was performed on five groups of rats receiving one of the following 14-day Cs doses: 0, 1, 5, 10, or 25 mg · kg^{-1} · day^{-1}.

Insulinogenesis (IG). To calculate the amount of net insulin synthesized (IG) during the 3-h perfusion, the average $IC_{t=0}$ from nonperfused control pancreases was subtracted from the sum of the insulin released (IR) throughout the perfusion and the IC of the pancreas after termination of the perfusion. Therefore, $IG = IR + IC - IC_{t=0}$ (17). The logical assumption in this calculation is that the IC of the pancreases at time zero is equivalent to the $IC_{t=0}$ of the 3-h–perfused pancreases. In addition, this technique does not measure intracellular insulin degradation, hence the term *net IG* is used. Degradation, however, has proven to be a negligible factor in this organ preparation over the 3-h perfusion (17). Also, from a physiological viewpoint, pancreatic IC at any point in time is determined by net IG (total amount of insulin synthesized minus amounts of insulin degraded and secreted).

IVGTTs. IVGTTs were performed on control animals and animals from each Cs-dose group. Because of the added variability introduced in an oral glucose tolerance test (OGTT), the IVGTT provided a more specific evaluation of the effect of Cs on the ability of the rats to clear glucose. The rats were fasted overnight and then, while under ether sedation, given 0.5 g glucose/kg body wt i.v. in a 50% solution. Blood was collected from the tail vein at 1, 2, 5, 10, 15, 25, 40, and 60 min. Blood glucose levels were determined with the use of Glucometer 2 (Miles, Elkhart, IN). To determine the rate of removal of glucose, a least-squares line was calculated from

TABLE 1
Blood cyclosporin (Cs) concentrations

Dose (mg · kg^{-1} · day^{-1})	*n*	Cs concentration (ng/ml)
1	15	153 ± 19*†
5	4	608 ± 37*†
10	5	1116 ± 164†‡§
25	4	4572 ± 351*‡§

Male Sprague-Dawley rats were treated with Cs via intramuscular injection for 14 days. Blood samples were drawn on days 7, 10, and 14 and assayed for Cs by high-performance liquid chromatography. Values are means ± SE of 3-day samples of each rat within group.
*Significantly different ($P < .05$) from 10 mg · kg^{-1} · day^{-1} Cs.
†Significantly different from 25 mg · kg^{-1} · day^{-1} Cs.
‡Significantly different from 1 mg · kg^{-1} · day^{-1} Cs.
§Significantly different from 5 mg · kg^{-1} · day^{-1} Cs.

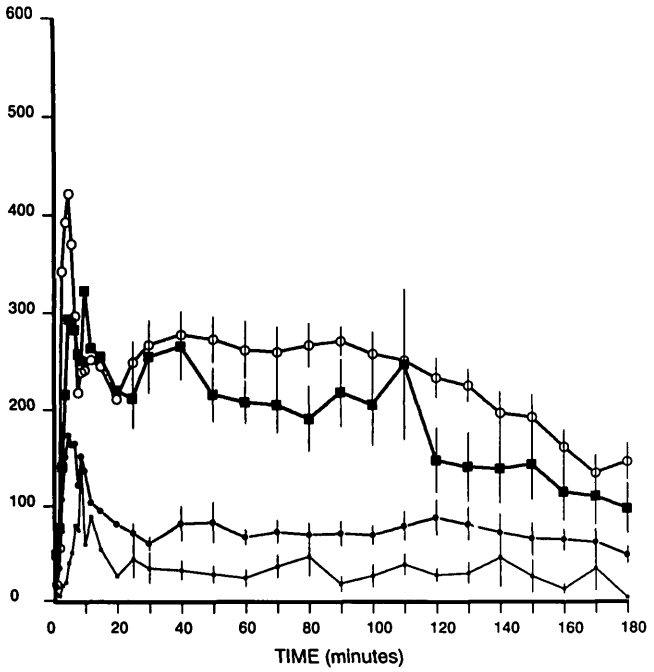

FIG. 1. Dynamic mean insulin release (IR, ng · min^{-1} · 300 g^{-1} body wt) in response to constant 3-h stimulus of glucose at 200 mg/dl. ■, IR curve of 8 pancreases from rats that received no cyclosporin (Cs); ○, IR curve of 8 pancreases from rats that received daily injection of Cs at 1 mg/kg for 14 days; ●, IR curve of 8 pancreases from rats that received daily injection of Cs at 5 mg/kg for 14 days; ▪, IR curve of 7 pancreases from rats that received daily injection of Cs at 25 mg/kg for 14 days. Standard error bars are omitted for 1st 20-min period for legibility, but SE values are included in Table 2. Data are summarized by treatment group in Table 2.

the natural log values of the peak glucose concentration (min 1 or 2) to min 60, and the slope was calculated.

Blood Cs measurement. Two milliliters of blood were drawn on days 7, 10, and 14 of Cs treatment to determine nadir blood levels. Blood was collected via cardiac puncture with EGTA-coated vacutainers ~24 h after the last injection. Samples were then assayed for Cs by the high-performance liquid chromatography method of Kabra and Wall (18).

Statistics. Duncan's multiple-comparison test or Student's *t* test was used to determine significant differences between groups, as appropriate. Significance was set at $P < .05$.

RESULTS

Table 1 shows the distribution of blood Cs levels (ng/ml) in rats treated with doses of 1, 5, 10, or 25 mg · kg^{-1} · day^{-1}. There is a linear increase in plasma Cs levels as the Cs dose is increased from 1 to 10 mg · kg^{-1} · day^{-1}: for each 1-mg · kg^{-1} · day^{-1} dose increase, there is a rise in blood Cs levels of ~110–130 ng/ml. However, between the 10- and 25-mg · kg^{-1} · day^{-1} doses, the increase in blood Cs levels is ~220–250 ng/ml for each milligram increase in Cs administration. This suggests that binding (e.g., Cs binding by erythrocytes), excretion, and/or degradative mechanisms are saturated at a blood level >1000–1200 ng/ml.

Figure 1 shows the dynamic insulin secretory response of the in vitro perfused pancreas as a function of Cs dose. The data are reported as micrograms of insulin secreted per 300 g body wt to normalize for small differences in body weight between animals (19). There is an effect of Cs on the "triphasic" insulin secretory response (17). When compared with controls, neither a normal rising second phase nor a normal declining third phase occurred. At a very low dose (1 mg · kg^{-1} · day^{-1}), there is no effect on the dynamic secretory response. At the 5- and 25-mg · kg^{-1} · day^{-1} doses, total amounts of insulin secreted are increasingly depressed. Table 2 reports insulin secretion by the perfusions shown in Fig. 1 for each of various periods as indicated. It shows that at the 5-mg · kg^{-1} · day^{-1} dose, total secretion decreased to ~40% of that of controls (13.3 ± 2.2 vs. 33.3 ± 4.0 μg). Clearly, at the 25-mg · kg^{-1} · day^{-1} dose, the secretory response is even more severely limited, with the total release <20% of that of controls. It is evident that both first and second phases of insulin release are being affected in a Cs dose–dependent manner. The amount of insulin released in the first 10 min, which primarily reflects the release of prestored insulin (14), i.e., independent from de novo synthesis, is severely compromised over the range of Cs doses, just as is total release.

When pancreases from nontreated animals were perfused with Cs in the perfusate at 1000 ng/ml, total release was not significantly altered compared with controls (data not shown). Thus, Cs has no acute direct toxic effect on the endocrine pancreas.

As seen in Table 3, there is a dose-related decline in the IC of perfused pancreases from rats treated with 1, 5, 10,

TABLE 2
Insulin release in response to constant 3-h glucose stimulus (200 mg/dl) by perfused pancreases from control rats and rats treated with cyclosporin for 14 days

Time	Group A (n = 8)	Group B (n = 8)	Group C (n = 8)	Group D (n = 7)
0–10 min	2.2 ± 0.4*†	2.6 ± 0.2*†	1.2 ± 0.2‡§	0.5 ± 0.2‡§
11–20 min	2.4 ± 0.4*†	2.3 ± 0.3*†	0.9 ± 0.2‡§	0.5 ± 0.2‡§
21–60 min	9.2 ± 1.1*†	10.8 ± 1.0*†	2.9 ± 0.5‡§	1.2 ± 0.4‡§
0–1 h	13.8 ± 1.5*†	15.8 ± 1.3*†	5.0 ± 0.8‡§	2.1 ± 0.7‡§
1–2 h	12.2 ± 2.3*†	15.6 ± 1.2*†	4.4 ± 0.8‡§	1.8 ± 0.5‡§
2–3 h	7.4 ± 1.9†	10.7 ± 1.2*†	3.9 ± 0.8§	1.4 ± 0.6‡§
0–3 h	33.3 ± 4.0*†	42.1 ± 3.4*†	13.3 ± 2.2‡§	5.4 ± 1.6‡§

Values are μg · 300 g^{-1} body wt · time^{-1}. Group A, control rats; group B, 1 mg · kg^{-1} · day^{-1}; group C, 5 mg · kg^{-1} · day^{-1}; group D, 25 mg · kg^{-1} · day^{-1}.
*Significantly different ($P < .05$) from group C.
†Significantly different from group D.
‡Significantly different from group A.
§Significantly different from group B.

TABLE 3
Insulin release (IR), insulin content (IC), IC at time zero ($IC_{t=0}$), and net insulinogenesis (IG) in control rats and rats treated with cyclosporin for 14 days

	Group A	Group B (n = 8)	Group C (n = 8)	Group D (n = 6)	Group E (n = 7)
IR	33.2 ± 4.0*†‡	42.1 ± 3.4*†‡	13.3 ± 2.2§‖	12.3 ± 1.4§‖	5.4 ± 1.6§‖
IC	87.2 ± 7.1†‡	95.9 ± 5.2*†‡	73.3 ± 4.8†‡§	47.5 ± 3.2*§‖	44.2 ± 4.8*§‖
$IC_{t=0}$	89.9 ± 9.9 (n = 11)	99.5 ± 13.1 (n = 7)	80.4 ± 9.0 (n = 8)	59.2 ± 4.7 (n = 9)	59.0 ± 5.2 (n = 4)
IG	31.0 ± 6.4*†‡	41.4 ± 5.9*†‡	6.3 ± 5.7§‖	0.5 ± 2.7§‖	−9.6 ± 4.5§‖

Values are μg insulin/300 g body wt. Group A, control rats; group B, 1 mg · kg^{-1} · day^{-1}; group C, 5 mg · kg^{-1} · day^{-1}; group D, 10 mg · kg^{-1} · day^{-1}; group E, 25 mg · kg^{-1} · day^{-1}.
*Significantly different ($P < .05$) from group C.
†Significantly different from group D.
‡Significantly different from group E.
§Significantly different from group A.
‖Significantly different from group B.

or 25 mg · kg^{-1} · day^{-1} Cs. Net 3-h IG was significantly depressed in pancreases from rats treated with 5, 10, or 25 mg · kg^{-1} · day^{-1} Cs. Even at the relatively low dose of 5 mg · kg^{-1} · day^{-1} Cs, net IG was only one-fifth of that seen in the controls (6.3 ± 5.7 vs. 31.0 ± 6.4 μg). Each of the two higher doses completely inhibited IG during the perfusion time.

There is substantial evidence documenting the reversibility of Cs toxicity on the pancreas (2,4,20,21). To demonstrate reversibility in our experimental model, we compared a group of rats treated with 10 mg · kg^{-1} · day^{-1} Cs for 2 wk followed by a 2-wk recovery with a group treated at the same dose without a recovery period. Figure 2 shows the restoration of the normal triphasic secretory response to control levels after the 2-wk recovery. Total release over the 3-h perfusion (37.8 ± 4.9 μg) was not significantly different from control animal total release (33.3 ± 4.0 μg; Table 4).

To assess the effects of Cs on glucose metabolism in vivo, IVGTTs were performed. As seen in Table 5, there is again a dose response when the k value is expressed as a function of Cs dose. It is interesting that at the 5-mg · kg^{-1} · day^{-1} dose, where in vitro insulin secretion is severely decreased, there is no apparent effect on the intact animal. Thus, there appears to be a different sensitivity to toxicity on the secretory response of the isolated perfused pancreas and the ability of the animal to adequately tolerate a glucose load in vivo. Although there is a definite visual decline in the rate of glucose removal as Cs dose is increased, the only group significantly different from control animals was the 25-mg · kg^{-1} · day^{-1} group (slope 0.80 ± 0.16 vs. 1.81 ± 0.06). Note, however, that the inherent variability in this method of measuring blood glucose is much greater than that in the glucose oxidase method.

DISCUSSION

One advantage of using the organ-perfusion technique is its ability to more closely represent the in vivo response than other in vitro techniques, e.g., cell culture. The secretory response of the endocrine pancreas consists of three phases: the sharp peak and decline in the first 10–15 min (phase 1), followed by a more steady state or even increasing rate of release for the next 30–50 min (phase 2); in long-term perfusions, i.e., several hours, these first two phases are followed by a third phase characterized by a declining rate of insulin secretion (19). It has been demonstrated that phase 1 represents primarily the secretory process (stimulus-secretion coupling), because it consists of insulin that is already stored and released via exocytosis on stimulation. Phases 2 and 3, unlike phase 1, can be decreased in the presence of protein synthesis inhibitors (14), e.g., cycloheximide, and therefore are dependent on both the normal secretory and synthetic capabilities of the β-cells (synthesis-secretion coupling).

The data presented here show that all phases of IR are inhibited in pancreases from animals that have been treated with Cs at doses of 5, 10, or 25 mg · kg^{-1} · day^{-1}. To distinguish if only secretion or synthesis or both processes are affected by Cs inhibition, we examined the IC of pancreases

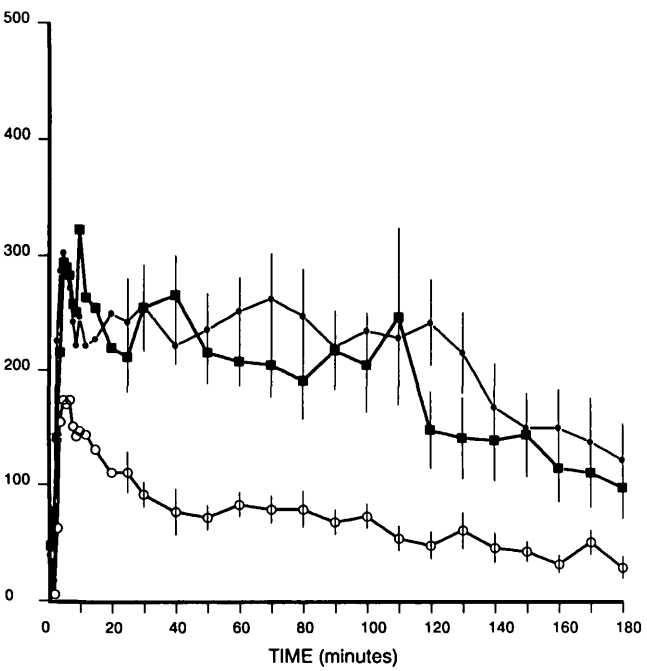

FIG. 2. Mean insulin release (IR, ng · min^{-1} · 300 g^{-1} body wt) in response to constant 3-h stimulus of glucose at 200 mg/dl. ■, IR curve of same 8 pancreases shown in Fig. 1 from rats that received no cyclosporin (Cs); ○, IR curve of 6 pancreases from rats that received daily injection of Cs at 10 mg/kg for 14 days; ●, IR curve of 5 pancreases from rats that received daily injection of Cs at 10 mg/kg for 14 days and then were allowed additional 14-day recovery period before perfusion. Standard error bars are omitted for 1st 20-min period for legibility. Data are summarized by treatment group in Table 4.

TABLE 4
Insulin release in response to constant 3-h glucose stimulus by perfused pancreases

Time	Group A (n = 8)	Group B (n = 6)	Group C (n = 4)
0–10 min	2.2 ± 0.4*	1.2 ± 0.2†‡	2.1 ± 0.3*
11–20 min	2.4 ± 0.4*	1.2 ± 0.1†‡	2.4 ± 0.3*
21–60 min	9.2 ± 1.1*	3.3 ± 0.5†‡	9.6 ± 1.1*
0–1 h	13.8 ± 1.5*	5.7 ± 0.7†‡	14.1 ± 1.5*
1–2 h	12.2 ± 2.3*	4.0 ± 0.5†‡	14.3 ± 1.8*
2–3 h	7.4 ± 1.9*	2.6 ± 0.4†‡	9.3 ± 1.9*
0–3 h	33.3 ± 4.0*	12.3 ± 1.4†‡	37.8 ± 4.9*

Values are $\mu g \cdot 300\ g^{-1}$ body wt · $time^{-1}$. Glucose stimulus was 200 mg/dl. Group A, control rats; group B, rats treated with cyclosporin for 14 days at 10 mg · kg^{-1} · day^{-1}; group C, rats treated for 14 days at 10 mg · kg^{-1} · day^{-1} followed by 14 days of recovery.
*Significantly different ($P < .05$) from group B.
†Significantly different from group A.
‡Significantly different from group C.

both before and after perfusion with a glucose stimulus. The IC of pancreases perfused for 3 h with a 200-mg/dl glucose stimulus decreased as a function of Cs dose. If the secretory process per se were the only defective element in glucose-induced insulin secretion, no Cs effect on pancreatic IC after perfusion would be expected. By also measuring the IC of pancreases from identically treated rats without perfusion ($IC_{t=0}$), net IG can be calculated as IG = IR + IC − $IC_{t=0}$. This term represents the ability of the pancreas to replenish stores lost to secretion. In both the control and 1-mg · kg^{-1} · day^{-1} groups, net IG was virtually identical to the amount of IR; thus, these pancreases were fully capable of maintaining intracellular insulin stores. However, at the three highest Cs doses, there is a mismatch of IR and IG. This could only occur if the amount of insulin synthesized is less than the amount secreted, insulin degradation occurred, or a combination of both. If net degradation occurred, the amount released during perfusion plus the IC after perfusion must be significantly lower than the IC of nonperfused pancreases (IR + IC < $IC_{t=0}$). We could not demonstrate this relationship at any dose, even though net IG at the 25-mg · kg^{-1} · day^{-1} dose was a negative number. Therefore, the decline in IG could only be due to significantly impaired insulin synthesis. The three highest doses were all significantly lower than controls in their ability to replenish the insulin that was secreted during perfusion.

Yoon et al. (22) reported decreased mRNA production from β-cells isolated from pancreases of animals undergoing Cs treatment, which is consistent with our observations. There have been no studies that examine specific secretory events in β-cells in the presence of Cs, but the dynamic and insulinogenic responses we have demonstrated clearly show both depressed synthetic and secretory function.

This action of Cs is especially relevant in the clinical setting, due to its widespread use in immunosuppressive therapy. Therefore, it is vital to investigate the effects of the drug on the whole animal and determine the extent of toxicity in vivo and in vitro. We evaluated animals with an IVGTT instead of an OGTT to avoid the uncontrolled variability on insulin secretion due to gastrointestinal absorption factors. The only group of rats with a significantly lower rate of glucose removal (compared with controls) occurred at 25 mg · kg^{-1} · day^{-1}. At this dose, the ability to clear glucose from the blood is severely impaired. In addition, the k value for the 10-mg · kg^{-1} · day^{-1} group (1.52 ± 0.11), although not significant, is highly abnormal. In practice, this is considered evidence of a borderline diabetic state. It is interesting that at the 5-mg · kg^{-1} · day^{-1} dose, the slope of the IVGTT is no different from that of control animals, yet the ability of the pancreas to synthesize and secrete insulin is very depressed. In practice, it is this in vivo response that is of importance to the patient undergoing Cs therapy. The blood Cs levels that accompany the affected groups (1116 ± 164 and 4572 ± 351 ng/ml) are high and certainly should not be used in a transplantation patient. Nonetheless, it is likely that glucose intolerance may develop at even lower Cs levels in patients whose pancreatic function is already borderline, e.g., those with non-insulin-dependent diabetes. This substantiates the observation by many transplantation physicians of conversion of patients with non-insulin-dependent to insulin-dependent diabetes with the onset of Cs therapy when corticosteroid dosages are held constant. Because there is a high individual variability in blood levels of patients treated wtih identical dosages of Cs, it is imperative that blood levels be carefully monitored to avoid such a decline in glucose tolerance.

In the case of the pancreatic transplantation patient, special consideration is necessary, because Cs toxicity may be superimposed on an organ already compromised by rejection and the effects of ischemia incurred during the transplantation procedure. Basadonna et al. (23) have shown that an adverse effect of Cs on islet autograft function does not occur when Cs therapy is delayed 10 days postoperatively. When autografted animals were given Cs on day 5, however, there was a higher graft failure rate and a lower fasting insulin output. There is evidence that Cs may play a role in inhibiting wound healing at the level of the endothelial cell via interference of prostaglandin formation (24). It follows that there may be a cumulative effect of wound healing and graft function inhibition in the case of the transplanted pancreas or islet graft. Van Schilfgaarde et al. (25) report an interference with segmental pancreatic graft function in the presence of Cs treatment. They propose that this inhibition is due in part to the incomplete endocrine reserve capacity of the organ, perhaps explaining why other investigators do not see the toxicity of Cs in the intact transplanted pancreas (6,7).

TABLE 5
Glucose tolerance tests

Cs dose (mg · kg^{-1} · day^{-1})	n	Slope
0	10	1.81 ± 0.06
1	9	1.76 ± 0.13
5	9	1.80 ± 0.14
10	8	1.52 ± 0.11
25	8	0.80 ± 0.16*

Male Sprague-Dawley rats were treated with cyclosporin (Cs) for 14 days at 0, 1, 5, 10, or 25 mg · kg^{-1} · day^{-1}. Rats were injected with a 50% glucose solution (0.5 g/kg i.v.), and blood glucose measurements were taken at min 1, 2, 5, 10, 15, 25, 50, and 60. Slope of log glucose vs. time was calculated from least-squared line from peak value to min 60 for each rat tested. Data are mean slopes ± SE for each group.
*Significantly different ($P < .05$) from control rats.

In agreement with other studies (2,4,20,21), we demonstrated the reversibility of Cs toxicity on the endocrine pancreas. The significantly reduced triphasic secretory response of the perfused pancreas from rats treated with 10 mg · kg^{-1} · day^{-1} Cs for 2 wk was completely reversed when the rats were allowed a 2-wk recovery. Because the second and third phases of the dynamic response are comprised of both stored and newly synthesized insulin (19), it can be assumed that both the secretory and synthetic capabilities were restored during the recovery period. Note, however, that at very high doses, reversal time may be substantially longer than 2 wk (20). It has been demonstrated that Cs accumulates in the pancreas, yielding high localized tissue concentrations (20,26). Thus, long-term Cs administration, even at lower doses, could result in a progressive accumulation and slower recovery times.

In a recent summary of clinical transplantation at Huddinge Hospital (27), there were very encouraging results regarding pancreatic graft survival. Their protocol consists of immunosuppression via azathioprine, prednisolone, and rabbit antithymocyte globulin at the time of surgery, followed by Cs therapy 2 wk later. However, they also report an impairment of glucose tolerance in patients receiving Cs. It is evident, therefore, that there is a delicate balance between immunosuppressive efficiency and graft toxicity in the case of pancreas transplantation. There are several factors that will probably contribute to the successful outcome of the graft, including very careful dose maintenance, the time allowed before onset of Cs treatment, and the islet reserve capacity of the transplanted tissue. In the setting of pancreatic transplantation, the effect of warm and cold ischemia combined with rejection episodes may result in an allograft with marginally effective islet cell mass.

In summary, this study demonstrates the profound inhibitory effect of Cs on insulin synthesis-secretion coupling in the normal pancreas. Fortunately, within 14 days of ceasing injection of the relatively high concentration of Cs (10 mg · kg^{-1} · day^{-1}), this inhibitory effect appears to be totally reversible. Nonetheless, this study demonstrates the profound inhibitory effect of Cs on the endocrine pancreas. If this effect were superimposed on clinical conditions of marginal pancreatic reserve, such as may occur in human pancreatic transplantation or immunosuppression of borderline diabetic patients, the effects might be substantial and warrant close monitoring of glucose tolerance in Cs-treated patients.

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