Effect of the Immunosuppressant FK 506 on Insulin Release From Adult Rat Islets of Langerhans

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CLINICAL introduction of cyclosporine A (CyA) was a critical development that improved the success rate of organ transplantation.[1] CyA has also been useful in the elucidation and treatment of type I diabetes as an autoimmune disease in animals[2] and humans.[3] Despite the impressive achievements obtained with the use of CyA, nephrotoxicity has limited its use in an autoimmune disease such as diabetes. Additionally, CyA decreases insulin synthesis and secretion from several species[4-7] and decreases insulin release from human islets in vitro,[8] although in vivo glucose tolerance and insulin dynamics may be normal in patients taking the drug.[9] FK 506 is a new immunosuppressive agent that is more potent on a weight basis than CyA[10] and has recently been used successfully in humans undergoing organ transplantations.[11] Since the drug has important potential applications in the fields of islet, whole pancreas transplantation, and immune intervention for type I diabetes, we looked at the effects of FK 506 on insulin secretion from adult rat islets of Langerhans.

METHODS

We used freshly isolated islets obtained by collagenase digestion of the pancreas from adult male Wistar rats.[12] Insulin was measured by standard radioimmunoassay using a charcoal and dextran separation method.[13] After isolation, islets were washed five times in basal (2.8 mmol/L glucose) containing buffer.

We measured insulin released by basal (2.8 or 5.6 mmol/L) or stimulatory (8.3 to 22 mmol/L) glucose during a 90-minute static incubation of groups of five freshly isolated islets incubated in the presence or absence of FK 506 (provided by Dr Raman Venkatarmanan, University of Pittsburgh). Some studies were performed using CyA (kindly provided by Sandoz Research Institute, East Hanover, NJ). For all experiments a modified Krebs buffer was used that contained (in mmol/L): 120 NaCl, 5 KCl, 25 $NaHCO_3$, 2.5 $CaCl_2$, and 1.1 $MgCl_2$, equilibrated with 95% O_2/% CO_2, and supplemented with 5 mg/mL bovine serum albumin, pH 7.4, 37°C.

Statistics

Results are expressed as mean ± SEM. The statistical significance of insulin release data was analyzed by Student's *t* test. Data with multiple groups were analyzed using a one-way analysis of variance (ANOVA) and the Wilcoxon ranked sums test. Conclusions drawn from parametric and nonparametric analyses were the same. We used a stepwise multiple comparisons procedure to assess FK 506 dose-response results.[14]

RESULTS

Figure 1 summarizes the effects of acute exposure of islets to various concentrations of either FK 506 or CyA on glucose-induced insulin secretion. In all experiments 16.7 mmol/L glucose evoked a three to sevenfold rise in insulin secretion compared with basal ($P < .01$). The glucose-stimulated insulin release was inhibited approximately 35% by 100 ng/mL FK 506 ($P < .05$). This concentration was inhibitory in every experiment.

In one experiment 10 ng/mL was also clearly inhibitory

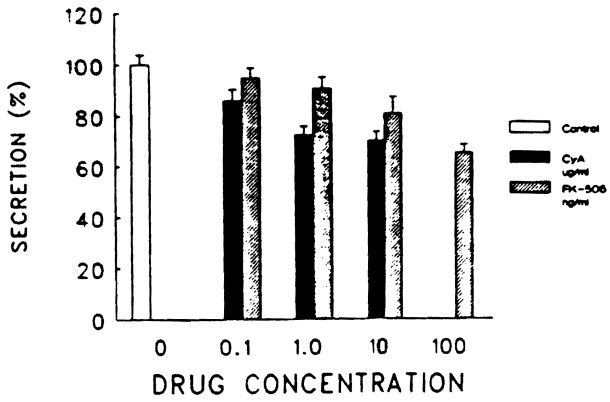

Fig 1. Acute Effect of FK 506 on 16.7 mmol/L glucose-induced insulin release. In this and subsequent figures insulin release was measured at the end of a 90-minute incubation in all conditions. Values are expressed as the mean ± SEM (36 observations). In this and subsequent figures, 100% secretion corresponds to insulin measured in 16.7 mmol/L glucose after subtraction of basal release. Basal insulin release (2.8 mmol/L glucose-induced) was 650 ± 5.8 pg/islet/90 min, and stimulated release (16.7 mM glucose induced) was 2200.8 ± 20.9 pg/islet/90 min ($P < .01$). A concentration of 100 ng/mL FK 506 produced an average inhibition of 35% ($P < .05$). This concentration was inhibitory in every experiment. In one experiment, a concentration of 10 ng/mL was also significantly inhibitory. Comparable data from islets incubated with CyA are shown in filled bars. Stimulated release was 1556.0 ± 10.2 pg/islet/90 min ($P < .01$) and CyA (1 and 10 μg/mL) produced a 30% inhibition of this release ($P < .05$).

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A.C.B. was supported by a grant from the Brazilian foundation CAPNS (Grant # 1662/89); A.A.G. was supported by CNPq, Sao Paolo, Brazil.

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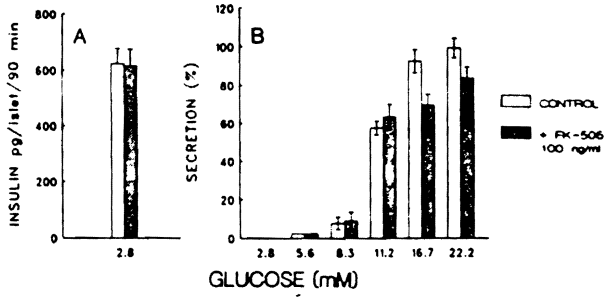

Fig 2. Effect of FK 506 on basal insulin release **(A)** and glucose dose-response **(B)**. Insulin release in 2.8 mmol/L glucose was 624.8 ± 53.0 pg/islet/90 min in the control and 616.5 ± 58.3 pg/islet/90 min in FK 506-treated islets (n = 60 observations) (P = .92). Data in B were normalized to the basal and expressed as a percent of maximal secretion in each condition (n = 18 observations at each glucose concentration, three separate experiments).

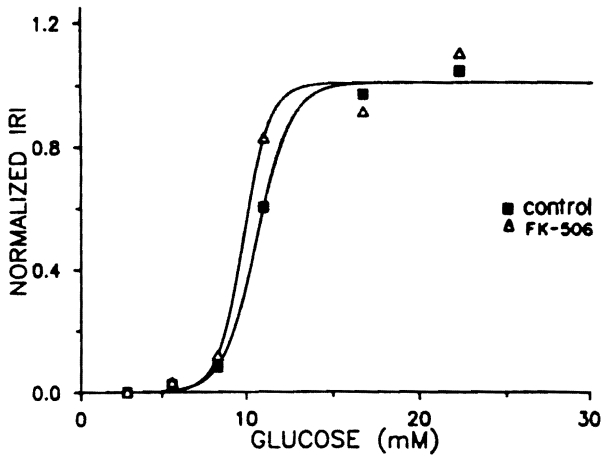

Fig 3. Effect of FK 506 on the glucose dose-response slope and 50% maximum insulin secretion. To examine the effects of 100 ng/mL FK 506 on the slope of the glucose dose-response curve and the 50% maximum secretion, we fitted the data points from Fig 2B using the Boltzmann equation (see text). The data were normalized to the maximum values in each condition. FK 506 produced a small shift in the 50% maximum along the x axis, the slope of glucose sensing was increased.

(P < .05). CyA at concentrations of 1 and 10 μg/mL produced an approximately 30% inhibition of insulin release (P < .05). The effect of 1 ng/mL FK 506 was not additive with 1 or 10 μg/mL CyA to further suppress insulin release (data not shown).

Figure 2 illustrates the effects of acute exposure of the islets to 100 ng/mL FK 506 on basal-induced (2.8 mmol glucose) insulin release (Fig 2A) or the graded increase in insulin evoked by increasing glucose concentrations (Fig 2B). In this series of experiments, basal insulin release was 624.8 ± 53 pg/islet/90 min and, in the presence of FK 506, was 616.5 ± 58.3 pg/islet/90 min (P = .92). While FK 506 did not inhibit insulin release at lower glucose concentrations, it inhibited both 16.7 and 22 mmol/L glucose-induced insulin release significantly (P < .005 and P < .03, respectively). In order to examine the effects of FK 506 on the 50% maximal and slope of the insulin response to glucose, the data points from Fig 2B were normalized to the maximum response within each condition and were fitted using the Boltzmann equation: % = P(1)/(1 + exp) {P(2) × ([G]-P(3)} where [G]-glucose concentration, P(2) = glucose sensitivity, P(3) = 50% maximal, and P(1) is the maximal stimulation in either condition. FK 506, while inhibiting the maximal secretion induced by high glucose concentrations (Fig 2B), produced a small apparent shift in the glucose dose-response curve to the left from a 50% maximum of 10.6 mmol/L in the control to 9.8 mmol/L in the FK 506-treated islets. At the 50% maximum, the slope of glucose sensing was increased.

DISCUSSION

FK 506 is a potent new immunosuppressant agent. When used long-term IM in baboons, FK 506 had an apparent diabetogenic effect.[15] When the immunosuppression was changed at 4 days to an oral regimen, this was not observed in baboons undergoing renal transplantation[16] nor in cynomolgus monkeys undergoing pancreaticoduodenal allotransplantation.[17] Preliminary studies in human recipients of liver allografts do not show a marked effect of this drug on carbohydrate tolerance when compared with historical controls who were treated with CyA.[18] Thus far there have been few studies of the effect of this agent on islet cell function. FK 506 apparently does not alter morphology or interfere with insulin secretion from fetal islets of Langerhans.[19] Insulin secretion in neonatal and fetal islets is not comparable to the adult response,[20,21] and since transplantation frequently involves tissue obtained from adult donors, we focused our studies on the effect of acute exposure to the drug on basal and glucose-stimulated insulin release.

The present study shows that FK 506 does not inhibit basal insulin release. It does inhibit glucose-induced insulin release at the highest concentrations used (10 and 100 ng/mL). Our acute data are compatible with results that show a small inhibition after a 4-day culture of rat islets in the presence of FK 506.[22] These observations suggest that the inhibition is not cumulative at least during this time period. The amount of inhibition produced by FK 506 at high concentrations is similar to that produced by 1 and 10 μg/mL CyA. Our results with CyA are comparable to those reported by others.[6]

Of interest is the finding of an apparent shift in the glucose dose-response of insulin secretion in the presence of the drug. At lower glucose concentrations FK 506 does not inhibit insulin release and in fact some slight stimulation of secretion is observed. The inhibition of release observed at 16.7 and 22 mmol/L glucose represents a shift of the normal dose-response of the islets to the left (Fig 3). The mechanism of such a shift is not clear from the current studies. One possible explanation would be that FK 506

enhances calcium entry into the cells, which in high concentrations may be inhibitory in β cells as proposed by Draznin.[23] These observations could have important clinical correlates.

In summary, our studies and those of others indicate that inhibition of insulin release is apparent only at high concentrations of FK 506 and glucose. FK 506 appears to have considerable steroid sparing effect.[11] Thus, there may be fewer problems with the diabetogenic effect of this agent except when used IV, in very high concentrations, or when there are preexisting problems of carbohydrate metabolism. There are important species differences reported with side effects of this agent[24] and further studies in humans are needed. Since there are potentially important applications of FK 506 in the field of diabetes, additional studies of effects of this agent on peripheral insulin action, glucose metabolism, response to nonglucose secretagogues, effect on other islet hormones, and molecular mechanism of action on secretory cells and target cells are required.

ACKNOWLEDGMENTS

The authors acknowledge the technical assistance of Mr A. Heffess in some aspects of the work. Dr Venkatarmanan provided insights into the pharmacological properties of the agents used. We acknowledge the assistance provided by the Laboratory of Statistical and Mathematical Methodology, NIH. We thank Dr E. Rojas for his many helpful discussions, review of the manuscript, and ongoing support.

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