

# Effects of Acute and Chronic Counterregulatory Hormone Infusions on Glucose Tolerance and Insulin Sensitivity in Diabetic Dogs

MARK W. SLEEMAN, MICHAEL J. CHRISTOPHER, IVA K. MARTIN, GLENN M. WARD, FRANK P. ALFORD, AND JAMES D. BEST

**The effects of elevated EPI and CORT levels on  $K_G$ ,  $S_I$ , and  $S_G$  were studied in dogs with alloxan-induced diabetes. Conscious dogs received SAL, EPI 20  $\text{ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for 30 min (short EPI) or 72 h (long EPI), or CORT 200  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for 60 min (short CORT) or 72 h (long CORT) before assessment of glucose metabolism by rapid sampling for glucose and insulin levels after 300 mg/kg i.v. glucose and exogenous insulin infusion designed to simulate the normal secretory pattern. With EPI infusion,  $K_G$  fell acutely from  $2.9 \pm 0.4$  to  $2.0 \pm 0.2\%/ \text{min}$  (SAL vs. short EPI,  $P < 0.05$ ), but rose to  $3.4 \pm 0.4\%/ \text{min}$  during long EPI. Minimal-model analysis of the glucose response with the insulin data as input showed that  $S_I$  decreased acutely from  $4.7 \pm 1.8$  to  $2.5 \pm 0.6 \times 10^{-5} \text{min}^{-1}/\text{pM}$  (SAL vs. short EPI,  $P < 0.05$ ), but rose to  $4.5 \pm 2.5 \times 10^{-5} \text{min}^{-1}/\text{pM}$  during long EPI. The effects of EPI on  $S_G$  paralleled the results for  $K_G$  and  $S_I$ , with acute decline from  $3.9 \pm 0.4$  to  $2.1 \pm 0.4 \times 10^{-2} \text{min}^{-1}$  (SAL vs. short EPI,  $P < 0.05$ ) and recovery to  $3.3 \pm 0.3 \times 10^{-2} \text{min}^{-1}$  during long EPI. During CORT infusion,  $K_G$  tended to fall (SAL  $2.9 \pm 0.4$  vs. short CORT  $2.5 \pm 0.5$  vs. long CORT  $2.2 \pm 0.5\%/ \text{min}$ ). This decline was related to a fall of  $S_I$  (SAL  $4.7 \pm 1.8$  vs. short CORT  $2.7 \pm 1.8$  vs. long CORT  $1.2 \pm 0.7 \times 10^{-5} \text{min}^{-1}/\text{pM}$ ,  $P < 0.05$  long CORT vs. SAL), whereas  $S_G$  levels were similar for the three groups. These results indicate that, in the absence of any compensatory change of insulin secretion, adaptation to the metabolic effects of long-term hormone elevation**

**occurs for EPI but not CORT, which has a sustained effect on  $S_I$ . Therefore, CORT may be more important than EPI as a contributor to long-term stress-induced hyperglycemia in people with type I diabetes. *Diabetes* 41:1446–52, 1992**

The adrenal gland plays an important role in the acute physiological response to stress, with release of EPI from the medulla and CORT from the cortex. Both hormones have hyperglycemic actions, although they act via different signal-transduction systems and alter glucose metabolism by different mechanisms (1,2). Acute infusion of EPI suppresses insulin-mediated glucose disposal and enhances hepatic glucose production (3–5). There are also direct effects on insulin secretion, which depend on the balance between  $\beta$ -adrenergic stimulatory and  $\alpha$ -adrenergic inhibitory actions of EPI (6). The short-term effects of glucocorticoids on in vivo glucose metabolism have not been studied as extensively. In the first few hours, the predominant action is probably to impair insulin-mediated glucose disposal (7) with little direct action on hepatic glucose production (2) or on insulin secretion (8).

Patients with type I diabetes, who therefore have no endogenous insulin secretion, have an exaggerated hyperglycemic response to both EPI and CORT (9). In the case of acute EPI, the increased glycemia observed in type 1 diabetes is the result of reduced insulin secretion, because blockage of insulin secretion by somatostatin in nondiabetic subjects produced a similar increase in the glycemic response (10). The insulin secretory response to hyperglycemia is thus a major factor limiting the glycemic response to acute elevation of stress hormones. Another factor that restrains the hyperglycemic response is the ability of glucose to mediate its own disposal by mass action (11). It is obvious that this latter factor will be relatively more important when endogenous insulin secretion is absent, as in type I diabetes.

From the University of Melbourne, Department of Medicine, St. Vincent's Hospital, Fitzroy, Victoria, Australia.

Address correspondence and reprint requests to James D. Best, MD, University of Melbourne, Department of Medicine, St Vincent's Hospital, Fitzroy, Victoria 3065, Australia.

Received for publication 9 September 1991 and accepted in revised form 3 February 1992.

EPI, epinephrine; NE, norepinephrine; CORT, cortisol;  $K_G$ , glucose tolerance;  $S_I$ , insulin sensitivity;  $S_G$ , glucose-mediated glucose disposal; SAL, saline; IDDM or type 1 diabetes, insulin-dependent diabetes mellitus; IVGTT, i.v. glucose-tolerance test; NEFA, nonesterified fatty acid; ANOVA, analysis of variance; d.f., degrees of freedom; NIMGU, non-insulin-mediated glucose uptake.

With elevation of stress hormone levels for several days rather than a few hours, there are adaptatory changes that act further to limit the degree of hyperglycemia, but there also are some sustained hormonal effects. In a previous study, we showed that the action of EPI to inhibit insulin-stimulated glucose disposal is attenuated during long-term EPI infusion. On the other hand, we also found a sustained impairment of  $S_G$  (12). When there is prolonged exposure to corticosteroids, the main adaptatory response is enhancement of insulin secretion (13). We have reported sustained insulin resistance after infusion of hydrocortisone for 72 h (14), but other studies have found that the hyperinsulinemia was sufficient to restore normal glucose disposal after an oral glucose load (15).

When endogenous insulin secretion is absent, a major adaptatory response to both acute and prolonged elevation of stress hormones is lost. Other compensatory mechanisms are required to prevent an uncontrolled glycemic response to stress hormone elevation. This study compares the effects of short-term and long-term infusion of EPI and CORT on glucose tolerance and its determinants in dogs with loss of  $\beta$ -cell function due to alloxan-induced diabetes. The homeostatic response to stress hormone elevation in this animal model is relevant to the effects of stress on glucose metabolism in patients with IDDM.

#### RESEARCH DESIGN AND METHODS

The studies were carried out, with the permission of the Experimental Medical and Surgical Research Ethics Committee, at St Vincent's Hospital, on dogs of mixed breed, 20–30 kg body weight. An indwelling venous catheter was placed under anesthesia as described previously (12,14). Diabetes was reliably induced in 24-h fasted animals by i.v. administration of alloxan monohydrate (Sigma, St. Louis, MO) at the relatively low dose of 25 mg/kg. Dogs all had fasting blood glucose  $>20$  mM within 1 wk of alloxan administration. Animals were monitored daily, but left in a relatively uncontrolled hyperglycemic state for the initial few days, with gradual control of glycemia being achieved by daily s.c. injection of porcine insulin (mixture of Actrapid MC and Protaphane MC insulins, CSL-Novo, Sydney, Australia) over  $\sim 2$  wk. Daily records were kept of fasting glucose levels and amount of insulin administered.

Dogs were studied after an 18-h fast with a minimum of 1 wk between successive experiments in the same animal. The standard IVGTT was performed on all animals before alloxan administration (prealloxan studies). After alloxan, the now diabetic animals were studied with a modified IVGTT protocol (postalloxan studies) with the following infusions: 1) 0.9% SAL infusion (for 30 min before and during IVGTT) with added insulin infusion to maintain basal glucose levels ( $n = 7$ ); 2) SAL infusion (as above) with a variable insulin infusion to mimic the normal insulin response pattern observed during an IVGTT protocol ( $n = 7$ ); 3) short-term EPI infusion ( $20 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  [ $110 \text{ pm} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ] for 30 min before and during the IVGTT) with variable insulin infusion ( $n = 6$ ); 4) long-term EPI infusion ( $20 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for 72 h plus

30 min before and during the IVGTT) with variable insulin infusion ( $n = 6$ ); 5) short-term CORT infusion ( $200 \text{ } \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  for 60 min before and during the IVGTT) with variable insulin infusion ( $n = 6$ ); and 6) long-term CORT infusion ( $200 \text{ } \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  [ $550 \text{ nm} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ] for 72 h plus 60 min before and during the IVGTT) with variable insulin infusion ( $n = 6$ ).

To lower the fasting glucose levels to basal values between 5 and 8 mM, we infused insulin at a rate of up to  $0.6 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ . Euglycemia then was maintained by an infusion rate of  $0.3 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for 30–60 min before the IVGTT. The pattern of supplemental insulin infused into diabetic dogs during the IVGTT was as follows: 0–4 min,  $0.3 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ; 4–5 min,  $12 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ; 5–6 min,  $6 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ; 6–8 min,  $0.3 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ; 8–16 min,  $1.2 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ; 16–50 min,  $0.6 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ; and 50–180 min,  $0.3 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ . A similar modification of the IVGTT with infusion of exogenous insulin to simulate the normal dynamic insulin response to i.v. glucose has been used previously in patients with IDDM (16). In this study, insulin was infused as neutral porcine insulin (Actrapid MC) in haemaccel 10% (1/20 saline dilution of degraded gelatine polypeptide, Hoechst Australia Ltd).

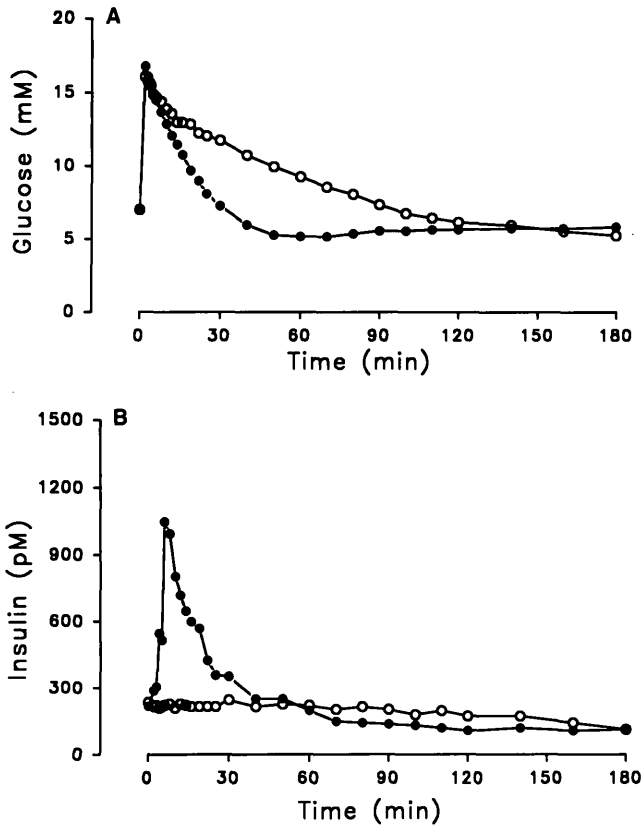
For blood sampling, a catheter (Jelco, Criticon, Tampa, FL) was placed in the cephalic vein of one foreleg, and an additional infusion catheter (Intracath, Deseret Medical, Sandy, UT) placed in the opposite cephalic vein. At least 30 min elapsed before infusions of either EPI (Adrenaline Injection B.P., Boots, Sydney, Australia, in 0.9% SAL containing 0.5 g/ml of ascorbic acid), CORT (Solu-Cortef, Upjohn Pty Ltd, Sydney, Australia, in 0.9% SAL), or SAL were commenced. Samples of blood were obtained for measurement of plasma concentrations of glucose, insulin, glucagon, catecholamines, CORT, and NEFA before the IVGTT ( $-10$  and  $-1$  min). A glucose load of 300 mg/kg was then injected i.v. at time 0, and additional blood samples were obtained at frequent intervals over 180 min as described previously (12,14).

In the long-term studies, EPI or CORT were infused via the indwelling i.v. catheter by a small infusion pump (Mill Hill Infusor model 1001, Muirhead, Kent, UK) carried by the dog in the pocket of its jacket. After 72-h infusion, the infusion protocol and IVGTT were performed as described for the short-term studies.

#### Laboratory, computer, and statistical analyses.

Plasma glucose was measured with an automatic analyzer (YSI, Yellow Springs, OH) by a glucose-oxidase method. Plasma insulin, glucagon, catecholamines, NEFA, and CORT levels were assayed as described previously (12,14). In all diabetic animals, nonspecific plasma binding of radiolabeled insulin was the same as in plasma samples taken before induction of diabetes.

Computer analysis of the glucose and insulin data was based on the minimal model of insulin action (17) and used the modeling program CONSAM/SAAM (18) on a MicroVax computer. Published values of parameters were used as initial estimates (11,12,14). Analysis of IVGTT data from animals before alloxan administration was performed as described previously (19,20). After alloxan, the endogenous insulin response to i.v. glucose



**FIG. 1.** Comparison of mean plasma glucose (A) and insulin (B) profiles, during IVGTT in 6 dogs with basal insulin infusion (○) or with dynamic insulin infusion (●) after induction of diabetes with alloxan. After basal blood sampling (-10 and -1 min), glucose (300 mg/kg) was injected over 30 s beginning at time 0 min; 26 additional blood samples were collected over 180 min. Basal insulin infusion consists of exogenous insulin infusion designed to maintain basal glucose levels and left at a constant rate for the duration of the experiment (protocol 1). Dynamic insulin infusion consists of exogenous insulin infusion designed to simulate normal insulin secretory pattern and produce normal glucose decay rate (protocol 2).

injection was absent. With continuation of the constant basal insulin infusion, the decline in plasma glucose should be exponential, and the rate of decline should be determined by  $S_G$  (11). This parameter was estimated by nonlinear regression analysis of the glucose data from infusion protocol 1, by use of all values above basal from

2 min (Fig. 1). For protocols 2 to 6, the plasma insulin data was modeled for optimal fit by adjustment of the insulin distribution space and the insulin disappearance rate (19) and by use of multiple time interrupts, a feature of the SAAM program that allows discontinuous fitting of the data (18). The subsequently modeled insulin profile from exogenous infusion closely resembled a modeled normal insulin secretory profile. This insulin profile then was used as input to calculate from modeling of the glucose data  $S_I$  ( $\text{min}^{-1}/\text{pM}$ ), and  $S_G$  ( $\text{min}^{-1}$ ). This method of data analysis has been described previously in a study of patients with IDDM (16), and performance was based on goodness of fit, analysis of residual errors, and the precision of parameter estimates (19).

A measure of overall glucose tolerance, which is independent of the model analysis, was obtained for each experiment by calculating the least-squares slope of the log absolute glucose concentration ( $K_G$ ) between 10 and 40 min after the glucose bolus. Statistical analysis was performed using paired Student's *t* tests or two-way ANOVA for repeated measures for comparisons of treatment groups. Correlations were assessed by simple regression analysis. Values are reported as means  $\pm$  SE.

**RESULTS**

**Effect of alloxan administration on basal levels of metabolites and hormones.** There was no significant difference between fasting plasma glucose levels in the groups of diabetic animals for each study (SAL,  $10.7 \pm 1.4$  mM; short EPI,  $9.3 \pm 1.1$  mM; long EPI,  $12.1 \pm 0.5$  mM; short CORT,  $10.7 \pm 1.0$  mM; and long CORT,  $11.7 \pm 2.0$  mM) or in the amount of insulin administered to each animal in the 7 days preceding the studies (SAL,  $43 \pm 0.3$  U; short EPI,  $44 \pm 0.8$  U; long EPI,  $44 \pm 0.8$  U; short CORT,  $41 \pm 0.3$  U; and long CORT,  $43 \pm 1.5$  U of s.c. administered porcine insulin per day). No extra insulin was administered s.c. during any of the studies.

Plasma concentrations of metabolites and hormones immediately before the IVGTT are shown in Table 1. Plasma glucose levels before the IVGTT remain significantly elevated in the diabetic animals compared with prealloxan levels, despite significantly elevated plasma insulin levels from the insulin infusion.

**TABLE 1**  
Plasma concentrations of metabolites and hormones immediately before IVGTT

	Prealloxan SAL	Postalloxan				
		SAL	Short-term EPI	Long-term EPI	Short-term CORT	Long-term CORT
EPI (pM)	202 $\pm$ 44	279 $\pm$ 55	1573 $\pm$ 218*	1884 $\pm$ 535†	431 $\pm$ 295	295 $\pm$ 131
Glucose (mM)	5.4 $\pm$ 0.1†	7.0 $\pm$ 0.4	7.4 $\pm$ 0.7	7.2 $\pm$ 0.4	6.6 $\pm$ 0.5	6.9 $\pm$ 0.4
Insulin (pM)	90 $\pm$ 24*	216 $\pm$ 54	162 $\pm$ 30	228 $\pm$ 48	162 $\pm$ 18	246 $\pm$ 48
Glucagon (ng/L)	74 $\pm$ 8	64 $\pm$ 23	59 $\pm$ 14	35 $\pm$ 15	31 $\pm$ 9	48 $\pm$ 18
CORT (nM)	50 $\pm$ 8	48 $\pm$ 10	40 $\pm$ 8	68 $\pm$ 15	140 $\pm$ 8*	250 $\pm$ 41*‡
NE (nM)	1.6 $\pm$ 0.9	0.86 $\pm$ 0.19	0.92 $\pm$ 0.19	1.0 $\pm$ 0.23	0.97 $\pm$ 0.45	0.68 $\pm$ 0.25
NEFA (mM)	0.35 $\pm$ 0.06	0.34 $\pm$ 0.05	0.78 $\pm$ 0.1†	0.24 $\pm$ 0.04*	0.58 $\pm$ 0.14	0.80 $\pm$ 0.07†

Values are means  $\pm$  SE for 6 animals (SAL, *n* = 7).  
\**P* < 0.01 vs. postalloxan SAL infusion.  
†*P* < 0.05 vs. postalloxan SAL infusion.  
‡*P* < 0.05 vs. corresponding short-term infusion.

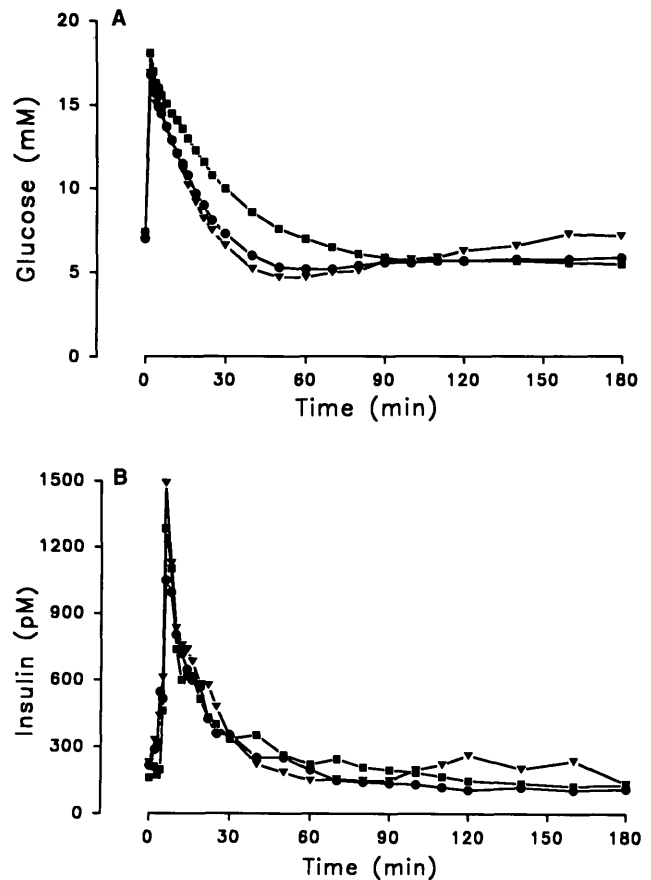
**Effects of hormone infusion on basal levels of metabolites and hormones.** No significant difference was observed between study groups in plasma glucose levels immediately before the glucose bolus (Table 1). Plasma levels of EPI during SAL infusion studies were  $279 \pm 55$  pM immediately before and  $480 \pm 82$  pM 180 min after the glucose bolus, indicating that the dogs were not stressed during the study. Pre-IVGTT plasma glucose, glucagon, CORT, and NE concentrations during either short EPI or long EPI studies were not significantly different from control (SAL) levels. NEFA levels were elevated significantly for short EPI compared with both SAL and long EPI administration ( $P < 0.05$  in both cases). Both short CORT and long CORT did not significantly alter pre-IVGTT plasma glucose, glucagon, EPI, or NE concentrations. Long CORT but not short CORT significantly increased plasma NEFA ( $P < 0.05$ ).

**Basal and dynamic insulin-infusion protocol.** The effect of alloxan administration and subsequent impairment of  $\beta$ -cell function on insulin responsiveness can be seen in Fig. 1. With only basal insulin infusion, the dynamic insulin response to glucose is lost and plasma glucose decline is impaired. From these data, the effect of incremental glucose to promote its own disposal at basal insulin can be calculated (11). Exogenous administration of insulin to simulate the normal dynamic response produces a profile (Figs. 2 and 3) that is quite reproducible across the different hormone treatment groups. (Two-way ANOVA,  $df = 1,26$ ,  $F < 1.0$ , NS for all comparisons). Minimal-model analysis of  $S_G$  from this insulin-replacement regimen gave a value of  $3.9 \pm 0.5$  compared with  $2.9 \pm 0.5 \times 10^{-2} \text{ min}^{-1}$  for  $S_G$  calculated directly from the basal insulin-infusion protocol. The difference between these two values was not statistically significant ( $P = 0.24$ ).

**Effect of hormone infusion on glucose disposal.** After i.v. glucose, the rate of decline of glucose level was slowed by short EPI administration (Fig. 2), but returned to the same rate as SAL after long EPI infusion (2-way ANOVA, SAL vs. short EPI,  $d.f. = 1,26$ ,  $F = 4.29$ ,  $P < 0.001$ ; SAL vs. long EPI,  $d.f. = 1,26$ ,  $F = 0.55$ , NS).  $K_G$  declined from  $2.9 \pm 0.4$  (SAL) to  $2.0 \pm 0.2\%/min$  (short EPI;  $t = 2.4$ ,  $P < 0.05$ ) and later rose to  $3.4 \pm 0.4\%/min$  (long EPI; Fig. 4, Table 2).

After short CORT infusion, the rate of decline of glucose was not significantly different from SAL infusion (Fig. 3), but was significantly impaired after long CORT (two-way ANOVA,  $d.f. = 1,26$ ,  $F = 2.5$ ,  $P < 0.001$ ). However, there was not a significant difference between the glucose curves for short CORT and long CORT.  $K_G$  values fell from  $2.9 \pm 0.4$  with SAL to  $2.5 \pm 0.5\%/min$  with short CORT and to  $2.2 \pm 0.5\%/min$  with long CORT, but these differences were not statistically significant.

**Effect of hormone infusion on determinants of glucose tolerance.** EPI caused an acute decline in  $S_I$  from  $4.7 \pm 1.8$  (SAL) to  $2.5 \pm 0.7 \times 10^{-5} \text{ min}^{-1}/\text{pM}$  (short EPI,  $t = 2.1$ ,  $P < 0.05$ ) but by 72 h of infusion  $S_I$  rose to  $4.5 \pm 2.5 \times 10^{-5} \text{ min}^{-1}/\text{pM}$  (Fig. 4, Table 2).  $S_G$  decreased during EPI infusion from  $3.9 \pm 0.4$  (SAL) to  $2.1 \pm 0.4 \times 10^{-2} \text{ min}^{-1}$  (short EPI,  $t = 3.1$ ,  $P < 0.05$ ), but by 72 h of infusion,  $S_G$  rose to  $3.3 \pm 0.3 \times 10^{-2}$



**FIG. 2.** Comparison of mean plasma glucose (A) and insulin (B) profiles, during IVGTTs in 6 diabetic dogs after infusion of 0.9% SAL (●), short-term EPI  $20 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for 30 min (■), and long-term EPI  $20 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for 72 h (▼); (protocols 2, 3, and 4). Plasma insulin profile results from exogenous insulin infusion aimed at simulating the normal endogenous insulin secretory pattern.

$\text{min}^{-1}$ . With cortisol infusion, there was a stepwise decline in  $S_I$  from  $4.7 \pm 1.8$  (SAL) to  $2.7 \pm 1.8 \times 10^{-5} \text{ min}^{-1}/\text{pM}$  (short CORT) and then to  $1.2 \pm 0.7 \times 10^{-5} \text{ min}^{-1}/\text{pM}$  (long CORT,  $P < 0.05$  vs. SAL).  $S_G$  was not affected by either short CORT or long CORT.

The changes of  $S_I$  during the short-term and long-term infusions of EPI and CORT varied inversely with the changes of plasma NEFA levels ( $r = 0.484$ ,  $P < 0.025$ ).

## DISCUSSION

This study used an animal model of IDDM to examine short-term and long-term stress hormone effects on glucose metabolism in the absence of compensatory changes of endogenous insulin secretion. EPI impaired glucose disposal acutely, but after prolonged exposure to elevated EPI, homeostasis prevailed, and glucose tolerance was normal. This result was unexpected because previous studies in nondiabetic dogs showed that impairment of glucose tolerance by EPI was sustained after 72 h (12). In contrast to the evanescent effects of EPI, the trend to reduction of glucose tolerance during acute elevation of CORT was sustained at 72 h and resembled the response to short- and long-term hypercortisolemia reported previously in normal dogs (14).

The alloxan-induced diabetic dog has been used

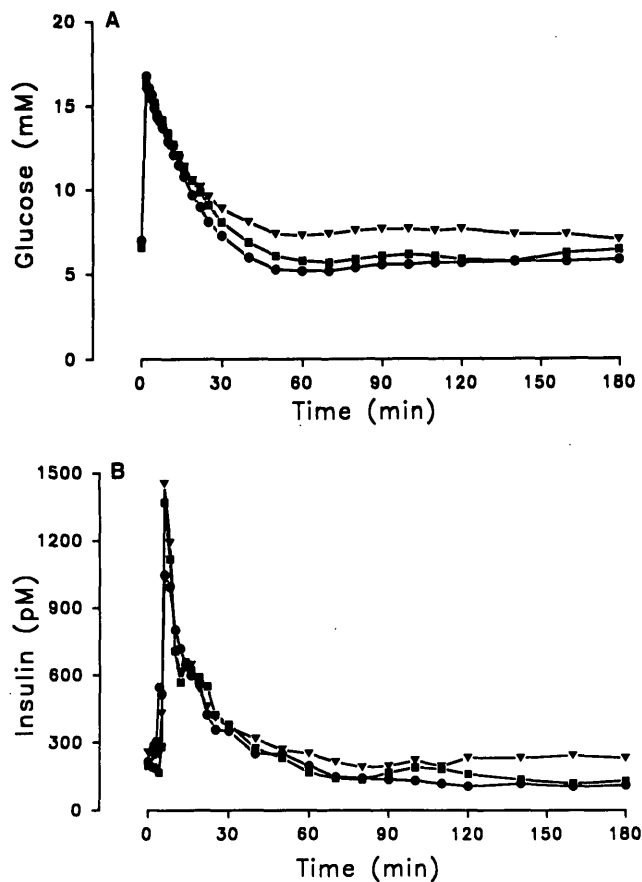


FIG. 3. Comparison of mean plasma glucose (A) and insulin (B) profiles, during IVGTTs in 6 diabetic dogs, after infusion of SAL (●), short-term CORT  $200 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  for 60 min (■), and long-term CORT  $200 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  for 72 h (▼); (protocols 2, 5, and 6). Plasma insulin profile results from exogenous insulin infusion aimed at simulating normal endogenous secretory pattern.

extensively as a model for IDDM. The absence of an endogenous insulin response to i.v. glucose was confirmed in studies of i.v. glucose tolerance, with basal insulin infusion only. The dogs were treated with daily

insulin to achieve moderate glycemic control and were insulin resistant compared with their prediabetic state. Previous studies have shown that insulin resistance in this diabetic model is strongly related to glycemic control (21), and a similar situation has been documented for IDDM in humans (22). Although initially only moderate control of glycemia was attained in the diabetic animals, just before and during the IVGTT, the animals were very well insulinized by the exogenous insulin infusion and had near-normal glucose profiles.

Because endogenous secretion was absent and infused insulin was matched in all the studies, the variables determining  $K_G$  were restricted to  $S_1$  and  $S_G$ . Of course, the roles of these parameters in determining  $K_G$  will depend on the amount of insulin present. In these studies, a high insulin profile was used to maintain near-normal glucose tolerance, as recommended by Finegood et al. (23). This experimental design would be expected to increase the importance of  $S_1$  relative to  $S_G$ .

The diabetic dogs were already insulin resistant, but there was a further significant decline of  $S_1$  during short-term elevation of EPI. Despite the absence of endogenous insulin secretion,  $S_1$  was restored to the basal—although still insulin-resistant—level during long-term EPI infusion. This adaptatory response has been documented in both animals and humans when insulin secretion is normal (12,24). This study's results show that adaptation is not dependent on  $\beta$ -cell function and puts more emphasis on desensitization of  $\beta$ -adrenergic receptors (25) as a probable, although as yet unproven, mechanism. The trend for changes of  $S_1$  during short- and long-term CORT infusion was also very similar to that reported in nondiabetic dogs (14). Fivefold elevation of plasma CORT for 72 h reduced  $S_1$  to 25% of the control level, indicating a sustained and major effect of hypercortisolemia. Some studies reported reversibility of corticosteroid-induced insulin resistance (15,26), but this effect appears to require both a longer time and the presence of normal endogenous insulin secretion (27). In

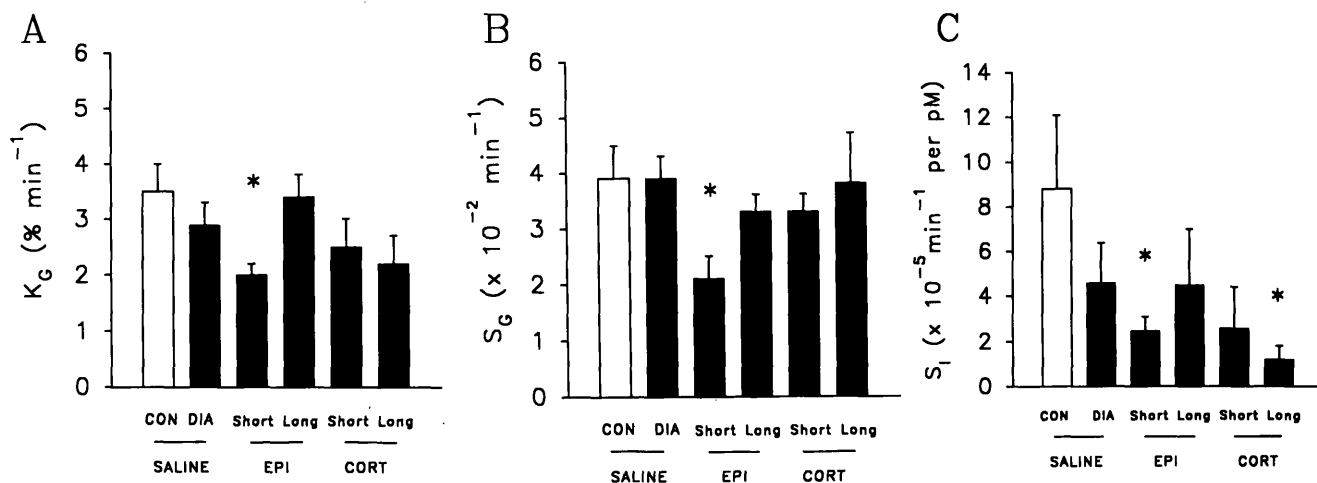


FIG. 4. Estimates (means  $\pm$  SE) from analysis of IVGTT data of  $K_G$  (A),  $S_G$  (B), and  $S_1$  (C) in 6 dogs (SAL,  $n = 7$ ). Data are shown for control (CON, □) and diabetic animals (DIA, ■) during SAL infusion. In diabetic animals, effects of 30-min (short EPI) and 72-h (long EPI) EPI infusions and 60-min (short CORT) and 72-h (long CORT) CORT infusions are compared with SAL infusions results. \* $P < 0.05$  vs. SAL diabetic dog studies.

TABLE 2  
Parameters of glucose tolerance and its determinants

	Prealloxan SAL	Postalloxan				
		SAL	Short-term EPI	Long-term EPI	Short-term CORT	Long-term CORT
$K_G$ (%/min)	3.5 ± 0.5	2.9 ± 0.4	2.0 ± 0.2*†	3.4 ± 0.4	2.5 ± 0.5	2.2 ± 0.5
$S_G$ ( $\times 10^{-2}$ min $^{-1}$ )	3.9 ± 0.6	3.9 ± 0.4	2.1 ± 0.4*†	3.3 ± 0.3	3.3 ± 0.3	3.8 ± 1.1
$S_I$ ( $\times 10^{-5}$ min $^{-1}$ /pM)	8.8 ± 3.3*	4.7 ± 1.8	2.5 ± 0.6*	4.5 ± 2.5	2.6 ± 1.8	1.2 ± 0.7*

Values are means ± SE for 6 animals (SAL,  $n = 7$ ).

\* $P < 0.05$  vs. postalloxan SAL infusion.

† $P < 0.05$  vs. corresponding long-term infusion.

both respects, the action of long-term hypercortisolemia contrasts with the more readily reversible effects of elevated EPI.

These effects of EPI and CORT on  $S_I$  varied inversely with the changes produced in plasma NEFA levels. With short-term EPI, NEFA levels more than doubled, but reverted to base line during long-term EPI infusion. This pattern supports the hypothesis that there is desensitization to the  $\beta$ -adrenergic stimulatory effect of EPI. With CORT infusion, NEFA levels tended to rise in the short term and more than doubled in the long term, mirroring the marked reduction of  $S_I$  produced by sustained elevation of CORT. The inverse relationship between NEFA levels and  $S_I$  could represent an important contribution of elevated NEFA to stress hormone-induced insulin resistance in people with IDDM.

Classically, impairment of glucose disposal by EPI is restricted to insulin-stimulated glucose disposal (3–5). However, more recent studies in both dogs (12) and humans (L.A. Morrow, G.S. Morganroth, W.H. Herman, R.N. Bergman, J.B. Halter, unpublished observations) examined the action of EPI on  $S_G$ , which was the other variable determining glucose tolerance in this study. During short-term EPI infusion,  $S_G$  was significantly reduced, contributing to the decline of glucose tolerance. However, just as occurred with  $S_I$ ,  $S_G$  was restored to normal values during long-term EPI infusion, ensuring that glucose tolerance was also normal. By comparison, CORT had little effect on  $S_G$  short or long term. In fact, maintenance of normal  $S_G$  during long-term hypercortisolemia prevented significant deterioration of glucose tolerance, despite quite marked insulin resistance. The acute decline and subsequent recovery of  $S_G$  during long-term EPI infusion in diabetic dogs contrasts with the sustained impairment of  $S_G$  previously reported in nondiabetic dogs (12).

Recovery of  $S_G$  in diabetic dogs given exogenous insulin injection and lack of recovery in nondiabetic dogs with endogenous insulin secretion (12) suggests that the difference may be attributable to an ability of prolonged EPI infusion to impair endogenous insulin secretion. There are other examples of impaired insulin secretion leading to lower  $S_G$  values. Significant reductions of  $S_G$  were reported in islet-autotransplanted dogs (28), nondiabetic subjects treated with somatostatin (29), patients with IDDM in remission (23), and patients with IDDM moderately well controlled with insulin therapy (16). All these results are consistent with an important role for the

degree of insulinization as a determinant of  $S_G$ . From this study, a question that arises on this interpretation of glucose effectiveness is why  $S_G$  was not impaired by induction of alloxan diabetes. The explanation is likely to be that the dogs were very well insulinized both before and during the IVGTT studies. Thus, although  $S_G$  represents the ability of glucose, independent of any increase of insulin concentration above basal level, to promote its own disposal, insulin appears to have at least a permissive effect on this important determinant of glucose tolerance.

The influence of EPI and CORT on  $S_G$  emphasizes the distinction between  $S_G$  and NIMGU as measures of glucose disposal. Baron et al. (7,30) have shown that NIMGU is unaffected by EPI or hydrocortisone infusion over 4 h in nondiabetic subjects. However, NIMGU measures total glucose disposal at fixed hyperglycemia in the absence of insulin. This measurement would not be expected to equal  $S_G$ , which measures the change of glucose disposal in response to incremental glucose at basal insulin.

Another important difference between NIMGU and  $S_G$  is that  $S_G$  includes the effect of hyperglycemia to suppress hepatic glucose production, as well as to promote glucose uptake, whereas NIMGU measures glucose uptake only. Ader et al. (11) estimated that suppression of glucose production may account for >40% of the  $S_G$  value. Suppression of hepatic glucose output is also included in the minimal-model definition of  $S_I$  and so contributes to the changes in  $S_I$  found in this study. However, we are not able to estimate the relative contributions of hepatic glucose production and glucose disposal to the changes in either  $S_G$  or  $S_I$ . The results represent overall ability to dispose of a glucose load, whether by suppression of hepatic glucose production or by enhancement of tissue glucose uptake.

$S_I$  including the hepatic glucose production component, was measured in the absence of  $\beta$ -cell function with exogenous insulin. The insulin profiles were identical between the control and the short- and long-term stress hormone infusions, so that portal insulin levels also would have been the same. In addition, there is evidence that peripheral rather than portal insulin levels influence hepatic glucose production (31). Consistent with this finding, estimates of  $S_I$  with exogenous insulin were similar to tolbutamide-modified protocols that involve only endogenous insulin (23,32). Consequently, valid comparisons

of  $S_1$  can be made between control and stress hormone-infused dogs, despite the use of exogenous insulin.

This study demonstrates desensitization to the metabolic effects of EPI, even when the adaptatory response does not involve insulin secretion. There was restoration of initially impaired values for both  $S_G$  and  $S_1$  during long-term EPI infusion. There was no such downregulation of the insulin resistance induced by hydrocortisone, which therefore is likely to be more important than EPI as a contributor to long-term stress-induced hyperglycemia in type I diabetes.

#### ACKNOWLEDGMENTS

The work was supported by the National Health and Medical Research Council of Australia, the Kellion Diabetes Foundation of Australia, and St. Vincent's Hospital Research Committee, Melbourne, Australia.

We thank the staff of the Endocrine Laboratory and the Experimental Medical and Surgical Unit at St. Vincent's Hospital for assistance with hormone assays and care of the experimental animals, respectively. We also thank June Papadopoulos and Margaret Harris for secretarial assistance. We acknowledge the advice and assistance of Richard Bergman, PhD, and Raymond Boston, PhD, in establishment of the minimal-model method of data analysis in our laboratories.

#### REFERENCES

- Smith U, Lager I: Insulin-antagonistic effects of counterregulatory hormones: clinical and mechanistic aspects. *Diabetes Metab Rev* 5:511–25, 1989
- Shamoon H, Hendler R, Sherwin RS: Synergistic interactions among antiinsulin hormones in the pathogenesis of stress hyperglycemia in humans. *J Clin Endocrinol Metab* 52:1235–41, 1981
- Rizza R, Haymond M, Cryer P, Gerich J: Differential effects of epinephrine on glucose production and disposal in man. *Am J Physiol* 237:E356–62, 1979
- Deibert DC, De Fronzo RA: Epinephrine-induced insulin resistance in man. *J Clin Invest* 65:717–21, 1980
- Gray DE, Lickley HLA, Vranic M: Physiologic effects of epinephrine glucose turnover and plasma free fatty acid concentrations mediated independently of glucagon. *Diabetes* 29:600–608, 1980
- Porte D: Sympathetic regulation of insulin secretion: its relation to diabetes mellitus. *Arch Intern Med* 123:252–60, 1969
- Baron AD, Wallace P, Brechtel G: In vivo regulation of non-insulin-mediated and insulin-mediated glucose uptake by cortisol. *Diabetes* 36:1230–37, 1987
- Kitabchi AE, Jones GM, Duckworth WC: Effect of hydrocortisone and corticotropin on glucose-induced insulin and proinsulin secretion in man. *J Clin Endocrinol Metab* 37:79–84, 1973
- Shamoon H, Hendler R, Sherwin RS: Altered responsiveness to cortisol, epinephrine and glucagon in insulin-infused juvenile-onset diabetes. *Diabetes* 29:284–91, 1980
- Berk MA, Clutter WE, Skor D, Shah SD, Gingerich RP, Parvin CA, Cryer PE: Enhanced glycemic responsiveness to epinephrine in insulin-dependent diabetes mellitus is the result of the inability to secrete insulin. *J Clin Invest* 75:1842–51, 1985
- Ader M, Pacini G, Yang Y, Bergman RN: Importance of glucose per se to intravenous glucose tolerance: comparison of the minimal model prediction with direct measurement. *Diabetes* 34:1092–102, 1985
- Martin IK, Weber KM, Boston RC, Alford FP, Best JD: Effects of epinephrine infusion on determinants of intravenous glucose tolerance in dogs. *Am J Physiol* 255:E668–73, 1988
- Beard JC, Halter JB, Best JD, Pfeifer MA, Porte D Jr: Dexamethasone-induced insulin resistance enhances B cell responsiveness to glucose level in normal men. *Am J Physiol* 247:E592–96, 1984
- Martin IK, Christopher MJ, Alford FP, Best JD: Distinct but nonadditive effects of epinephrine and cortisol on determinants of glucose tolerance in dogs. *Am J Physiol* 260:E148–53, 1991
- Stojanovska L, Rosella G, Proietto J: Evolution of dexamethasone-induced insulin resistance in rats. *Am J Physiol* 258:E748–56, 1990
- Ward GM, Weber KM, Walters JM, Aitken PM, Lee B, Best JD, Boston RC, Alford FP: A modified minimal model analysis of insulin sensitivity and glucose-mediated glucose disposal in insulin-dependent diabetes. *Metabolism* 40:4–9, 1991
- Bergman RN, Ider YZ, Bowden CR, Cobelli C: Quantitative estimation of insulin sensitivity. *Am J Physiol* 236:E667–77, 1979
- Berman M, Weiss MF: *SAAM Manual (Simulation, Analysis and Modeling). User's Guide*. Bethesda, MD, Natl. Inst. Health, 1978 (NIH publ. no. 78–180)
- Martin IK, Weber KM, Ward GM, Best JD, Boston RC: Application of the SAAM modeling program to minimal model analysis of intravenous glucose tolerance test data. *Comput Methods Programs Biomed* 33:193–203, 1990
- Weber KM, Martin IK, Best JD, Alford FP, Boston RC: Alternative method for minimal model analysis of intravenous glucose tolerance data. *Am J Physiol* 256:E524–35, 1989
- Caruso G, Proietto J, Calenti A, Alford F: Insulin resistance in alloxan-diabetic dogs: evidence for reversal following insulin therapy. *Diabetologia* 25:273–79, 1983
- Yki-Jarvinen H, Koivisto VA: Continuous subcutaneous insulin infusion therapy decreases insulin resistance in type 1 diabetes. *J Clin Endocrinol Metab* 58:659–66, 1984
- Finegood DT, Hramiak IM, Dupre J: A modified protocol for estimation of insulin sensitivity with the minimal model of glucose kinetics in patients with insulin dependent diabetes. *J Clin Endocrinol Metab* 70:1538–49, 1990
- Bessey PQ, Watters JM, Aoki TT, Wilmore DW: Combined hormonal infusion simulates the metabolic response to injury. *Ann Surg* 200:264–80, 1984
- Snavely MD, Mahan LC, O'Conner DT, Insel PA: Selective downregulation of adrenergic receptor subtypes in tissues from rats with pheochromocytoma. *Endocrinology* 113:354–61, 1983
- Olefsky JM, Kimmerling G: Effects of glucocorticoids on carbohydrate metabolism. *Am J Med Sci* 271:202–10, 1976
- Issekutz B Jr, Shaw W: Glucose turnover in the exercising dog with chemically induced diabetes and effect of methylprednisolone. *Diabetes* 24:915–21, 1975
- Finegood DT, Watnock GL, Kneteman NM, Rajotte RV: Insulin sensitivity and glucose effectiveness in long-term islet-autotransplanted dogs. *Diabetes* 38 (Suppl. 1):189–91, 1989
- Kahn SE, Klaff LJ, Schwartz MW, Beard JC, Bergman RN, Taborsky GJ Jr, Porte D Jr: Treatment with a somatostatin analog decreases pancreatic  $\beta$ -cell and whole body sensitivity to glucose. *J Clin Endocrinol Metab* 71:994–1002, 1990
- Baron AD, Wallace P, Olefsky JM: In vivo regulation of non-insulin-mediated and insulin-mediated glucose uptake by epinephrine. *J Clin Endocrinol Metab* 64:889–95, 1987
- Ader M, Bergmann RN: Peripheral effects of insulin dominate suppression of fasting hepatic glucose production. *Am J Physiol* 258:E1020–32, 1990
- Welch S, Gebhart SSP, Bergman RN, Phillips LS: Minimal model analysis of intravenous glucose tolerance test-derived insulin sensitivity in diabetic subjects. *J Clin Endocrinol Metab* 71:1508–18, 1990