HEPATIC NUCLEAR FACTOR-3 BETA AND C/EBP BIND TO INSULIN RESPONSE SEQUENCES (IRSs) IN INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-1 (IGFBP-1) AND PHOSPHOENOLPYRUVATE CARBOXYKINASE (PEPCK) GENES. I Unterman, R Costa*, and R Lawson*. Depts of Medicine and Biochemistry, Univ of IL College of Medicine and VA West Side Medical Centers, Chicago, IL.

Insulin rapidly inverts hepatic IGFBP-1 and PEPCK gene transcription. We recently identified a 22 bp 5'-flank sequence which mediates effects of insulin on IGFBP-1 promoter activity and resembles the IRS in the PEPCK gene (Endocrinology, In Press). To determine whether related proteins may bind to IGFBP-1 and PEPCK insulin response sequences, we performed gel shift and supershift studies with oligonucleotide probes, specific antisera and recombinant peptides.

Computer analysis revealed that the IGFBP-1 IRS is similar (10/12 bases) to a consensus sequence for HNF-3 binding. Gel shift studies showed that IGFBP-1 and PEPCK IRSs partially compete with each other for binding to nuclear proteins prepared from H4IIE hepatoma cells; and that an oligomer containing a high affinity HNF-3 binding site competes with both IGFBP-1 and PEPCK probes. Antiserum against HNF-3 beta (but not alpha or gamma) supershifted ~40% of complexes formed with the IGFBP-1 probe and studies with recombinant protein confirmed that the DNA binding domain of HNF-3 binds competitively to both IGFBP-1 and PEPCK IRSs. Of note, recombinant C/EBP (provided by S. McKnight) also binds to both IGFBP-1 and PEPCK IRSs but not to the HNF-3 oligo or an unrelated probe, while CTF/NF-1 (by R. Tanese), which also binds to specific AT-rich, sequences, did not bind to either IRS. Summary: these results indicate that IGFBP-1 and PEPCK IRSs are fonctionally related and may interact with HNF-3 and/or C/EBP-related DNA binding proteins. Interactions between HNF-3 and C/EBP-related proteins may contribute to the regulation of IGFBP-1, PEPCK and other hepatic genes controlled by insulin.

ACUTE EFFECTS OF CORTISOL ON HEPATIC GLYCOGEN SYNTHESIS IN AWAKE RATS. Emil D. Engels, Gary C. Cline, Jennifer M. Otis, Patrick H. McNulty, Gerald I. Shulman. Dept. of Int. Med., Yale Med School, New Haven, CT.

In order to examine the acute effects of hypercortisolemia on hepatic glycogen synthesis either cortisol or saline was administered to fasted, chronically catheterized, awake rats. At t=0 min the cortisol group (n=6) received a prime (1mg)-continuous 20 ug/(kg-min)] infusion of cortisol whereas the control group (n=16) received a saline infusion. At the same time a hyperglycemic clamp, using [1-^{13}C] glucose, was initiated where plasma glucose was maintained at ~180 mg/dl. After 180 min, the animals were sacrificed, the liver tissue was analyzed for glycogen content, glycogen synthase (GS) activity and the ^{13}C percent labeling in C1 glycogen. The relative flux through the direct pathway of glycogen synthesis was assessed by the ratio of the ^{13}C percent labeling in the C1 position of glycogen relative to that in C1 plasma glucose.

Group	Mean gluc (mg/dl)	Mean ins (ng/ml)	Net syn rate (umol/g/min)	GS activity (umol/g/min)	% Direct pathway
CONTROL	177.2±1.3	17.4±0.6	0.26±0.03	0.13±0.03	78±6
CORTISOL	178.4±1.5	15.9±1.2	0.70±0.07*	0.43±2.08*	56±1*

Despite similar concentrations of mixed venous plasma insulin and glucose, net hepatic glycogen synthesis was stimulated 2.7 fold in the cortisol treated group. This increase in net hepatic glycogen synthesis was due mostly to a stimulation of the indirect (gluconeogenic) pathway. Cortisol also caused a 3.3 fold stimulation in glycogen synthase activity. Conclusions: 1) Acute administration of cortisol stimulates net hepatic glycogen synthesis independent of changes in insulin concentration. 2) This increase in glycogen synthesis can be attributed to both a stimulation of the gluconeogenic pathway as well as to activation of glycogen synthase.

INSULIN REGULATION OF RENAL PROTEIN SYNTHESIS AND DEGRADATION IN VIVO. E Cersosimo*, R Judd*, M Persson*, and JM Miles, Mayo Clinic & Foundation, Rochester, MN.

Very little information is available regarding the regulation of protein metabolism in the kidney. We placed sampling catheters in the left renal vein (RV) and femoral artery (FA) of dogs together with a nonobstructive infusion catheter in the left renal artery 10d before study. On the study day, indocyanine green (ICG) was infused intrarenally to measure plasma flow and ^{3}H phenylalanine (Phe) was infused peripherally. Serial RV and FA samples were obtained before and during a 2h intrarenal infusion of either insulin (INS, n=6) or saline (SAL, n=6). Left RV insulin concentration doubled during INS from 41±8 to 92±23 pM (p<0.05), but did not change during SAL. FA insulin and glucose concentrations were ~50 pmol/L and ~5.4mmol/L, respectively, and did not change in either group. Arterial Phe concentration and systemic Phe appearance (Ra) were ~65μmol/L and ~3.0 μmol.kg^{-1}.min^{-1} respectively and did not change during INS or SAL. Left renal Phe release decreased by 50% in INS (from 0.17±0.02 to 0.08±0.02 μmol.kg^{-1}.min^{-1}, p<0.05), but did not change in SAL (0.11±0.01 vs 0.13±0.01 μmol.kg^{-1}.min^{-1}, p=NS). Phe uptake did not change in either group (0.14±0.01 vs 0.16±0.02 and 0.11±0.01 vs 0.11±0.02 μmol/kg.min, in INS and SAL, respectively). These data indicate that the kidney accounts for ~10% of systemic Phe Ra. Physiological hyperinsulinemia inhibits renal proteolysis but does not affect renal protein synthesis. Thus insulin regulation of protein metabolism in the kidney is similar to that in skeletal muscle, but different from that in liver, where insulin increases protein synthesis.

INCRETIN HORMONES REGULATE GLUCOSE-DEPENDENT INSULIN SECRETION IN THE RIN 1046-38 CELLS: MECHANISMS OF ACTION C. Montrose-Rafizadeh, J. Egan and J. Roth*. NIA, NIH and Geriatric Medicine, JHU, Baltimore, MD

Novel therapeutic agents that could normalize beta cell response to glucose would be of considerable benefit in the treatment of non insulin-dependent diabetes mellitus. Glucagon-like peptide-1 (GLP) and glucose insulinotropic peptide (GIP) are known incretin hormones which enhance insulin secretion but only in the presence of elevated blood glucose. In this study we used a rat insulinoma cell line (RIN 1046-38) to study the mechanism underlying the interaction of incretins and glucose. In these cells GLP stimulated insulin secretion with half maximal concentration of 3.3×10^{-11} M with GLP being two order of magnitude more potent than GIP. The two hormones have additive effects and both stimulate insulin secretion by decreasing the required concentration of glucose to produce half maximum insulin secretion and by increasing the maximal amount of insulin secreted. GLP acts in a glucose-dependent manner to recruit more cells to secrete insulin as well as enhancing insulin secretion by individual cells. The glucose requirement for GLP-1 action can be replaced by cell membrane depolarization, suggesting that a rise of intracellular Ca^{2+} may be an early step required for GLP action.

THE VENTROMEDIAL HYPOTHALAMUS PLAYS A CRITICAL ROLE IN TRIGGERING COUNTERREGULATORY RESPONSES TO HYPOGLYCEMIA. WF Ng*, MJ Dunag,* RS Sherwin,** MA Borg,* GI Shulman. Department of Medicine, Yale University, New Haven, CT.

Although the role of individual counterregulatory hormones in hypoglycemia correction has been studied extensively, the mechanisms that link glucopenia with activation of the counterregulatory system are poorly understood. An important role for the CNS, particularly the ventromedial hypothalamic nuclei (VMH) has been proposed. To test this hypothesis, we performed hypoglycemic clamp studies in conscious Sprague-Dawley rats with bilateral VMH lesions produced by local ibotenic acid injection 2 weeks earlier. Rats with lesions in the lateral hypothalamic area (LHA), frontal lobe (FL), sham-operated (stereotaxic needle placement into hypothalamus without injection) and naive animals served as control groups. Each clamp study had two phases: for the first hour plasma glucose was fixed by a variable glucose infusion at euglycemia (~ 5.9 mM). Thereafter, for an additional 90 min., glucose was allowed to fall and then clamped at either (a) mild (~ 3.0 mM) or (b) more severe hypoglycemic levels (~ 2.5 mM). The table below shows glucagon (GN), epinephrine (EPI), and norepinephrine (NOR) levels during mild and severe hypoglycemia in VMH and naive control animals:

	Naive		VMH	
Hypoglycemia:	Mild	Severe	Mild	Severe
	n=6	n=5	n=6	n=5
GN (ng/L)	450±45	860±157	217±24*	230±39*
EPI (nM)	28.2±1.4	60.9±6.6	12.2±1.1*	12.5±2.1*
NOR (nM)	8.7±1.4	36.5±4.3	3.0±0.1*	3.1±0.1*

* p<0.05 vs. naive rats. As shown above, counterregulatory hormone responses in the VMH-lesioned rats (vs. naive controls) were markedly inhibited and failed to rise as hypoglycemia progressed. Glucagon and catecholamine responses of LHA-, FL-lesioned, and sham operated animals were virtually identical to the naive rats, indicating that the differences observed were not due to the lesioning procedure itself. Conclusion: the VMH plays a crucial role in triggering the release of glucagon and catecholamines during hypoglycemia.

NITRIC OXIDE BLOCKADE PREVENTS ENDOTHELIN-1 INDUCED INSULIN RELEASE BUT NOT ENDOTHELIN-1 INDUCED HYPOGLYCEMIA. RS Zimmerman and M Maymind*, Akron Ochsner Medical Institutions, New Orleans, LA.

We have previously demonstrated that endothelin-1 (ET-1) increases insulin (INS) and lowers blood glucose (GLU, Midwest AFCR 1993). Because ET-1 has been shown to increase nitric oxide (NO) and because NO has been shown to stimulate INS release we hypothesized that blocking production of NO with N-MET-Arginine (NMA, 100 mg/kg I.V.) 15 minutes before starting ET-1 infusion at 75 ng/kg/min I.V. in inactin (100 mg/kg i.p.) anesthetized rats (n=6) would prevent ET-1 induced INS release. NMA alone (100 mg/kg I.V.) was infused in a second group of rats (n=6). Saline (SAL) was infused for 45 minutes in a third group of rats (n=6) and ET-1 at 75 ng/kg/min was infused for 30 minutes in a fourth group of rats (n=6).

	Glucose				Insulin			
TIME(MIN)	0	15	30	45	0	15	30	45
NMA/ET-1	95±2	80±4*	67±3*	66±5*	5.8±0.1	4.3±0.8	2.9±1.1*	2.2±0.4*
NMA/SAL	87±7	71±6*	67±4*	68±3*	5.7±1.0	4.4±1.7	3.0±0.7*	4.0±1.0
SAL/SAL	89±6	87±5	86±5	88±5	5.8±1.2	5.8±1.2	5.8±1.1	5.2±1.3
SAL/ET-1	86±4	85±4	73±4*	81±3*	3.6±0.6	3.6±0.6	5.6±0.7	6.0±0.6*

p<0.05 compared to values at T=0

The present study demonstrates 1) ET-1 increases INS and decreases GLU, 2) NO blockade with NMA prevents ET-1 induced insulin release, 3) NO blockade with NMA does not affect ET-1 induced hypoglycemia, 4) NMA alone decreases glucose despite its effect on lowering insulin. These findings suggest that ET-1 increases insulin by stimulating NO production but ET-1 induced hypoglycemia is at least partially independent of its effects on insulin release.