PARADOXICAL DISSOCIATION BETWEEN GLUCONEOGENIC EFFECTS AND GLUCOCORTICOID-RECEPTOR BINDING BY GONADAL STEROIDS

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Estradiol and testosterone derivatives both decreased liver glycogen and impaired triamcinolone acetonide mediated gluconeogenesis in male, adrenalectomized fed rats. Neither gonadal steroid influenced binding of either glucocorticoid to its cytoplasmic receptor. Association of ^{3}H-estradiol to liver cytoplasmic binders remained unhampered in presence of one hundredfold excess of either cortisol or triamcinolone.

The equation: Steroid + Receptor $\rightleftharpoons$ S-R Complex $\longrightarrow$ Response currently sums up the mechanism of action of all five major classes of steroid hormones (1,2). An antagonist, perhaps expected to reverse the above process, would not only provide a new tool to probe the mechanism of hormone action but may also be endowed with considerable clinical significance. Although selected aspects of glucocorticoid action in isolated thymocytes can be blocked by 11-deoxycortisol (3), and induction of tyrosine transaminase activity can be inhibited by testosterone analogues *in vitro* (4), no such antagonism has hitherto been demonstrated *in vivo*. The present report adds a new dimension to the metabolic influence exerted by common sex steroids by demonstrating that various analogues of both estradiol and testosterone can selectively impair gluconeogenesis *in vivo* at a time when binding of a glucocorticoid to its receptor is not affected.

Male, Wistar rats (150-200 g) were bilaterally adrenalectomized 3-5 days prior to experimental manipulation. Animals were

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TABLE 1: ANTIGLUCONEOGENIC EFFECT OF COMMON SEX STEROIDS

Treatment°	Glycogen (mg%) -TA	Glycogen (mg%) +TA	TP Activity (μM kynurenine/g liver/h)	TT Activity (μg parahydroxyphenylpyruvate per mg liver / 10 min)
Water control	9.03 ± 0.85	18.60 ± 3.50	3.68 ± 0.24	3.00 ± 0.47
Estradiol				
2 mg	9.39 ± 0.96			
20 mg	2.37 ± 0.31	9.42 ± 1.19	3.00 ± 0.20	3.81 ± 0.26
50 mg	2.24 ± 0.89			
Testosterone				
2 mg	11.83 ± 1.66			
20 mg	2.12 ± 0.55	13.36 ± 1.11	2.71 ± 0.34	4.61 ± 0.66
50 mg	2.66 ± 0.42			

°Triamcinolone acetonide (1 mg) was given as a single, intraperitoneal injection along with the test compound 4 h before assay. Each value is the mean ± the standard error of five separate determinations.

injected, intraperitoneally, with various doses of the desired test material 4 h prior to sacrifice. Classical procedures were used for determination of liver tyrosine transaminase (TT), tryptophan pyrrolase (TP) and glycogen levels (5,6). For binding studies, liver cytosol (0.5 ml) was incubated (1 h, 2-4°C) with 3×10^{-8} M/ml of either ^{3}H-triamcinolone acetonide or ^{3}H-estradiol alone, or in presence of 3×10^{-6} M/ml of a non-radioactive competitor of choice. After addition of 50 mg of a carbon suspension (Sigma C-5260) in 0.5 ml of 0.01 M Tris-HCl pH 7.4, further incubation (10 min), and centrifugation (5000 g, 5 min), 0.2 ml aliquots were mixed with 10 ml Unisolve (Kochlight, G.B.) and counted in Packard Tricarb Scintillation Spectrometer. Protein was assayed by the Biuret method. Non-radioactive steroids were purchased from Steroloids, N.Y. 1,2,4 ^{3}H-triamcinolone acetonide (16 Ci/mM; batch ZT 1423) was obtained from Schwarz Mann, N.J., and 1,2,^{3}H-estradiol (90 Ci/mM; lot 975) was a product of Amersham, G.B.

Data in table 1 show that both estradiol and testosterone effectively decreased liver glycogen and inhibited ($p < 0.01$) triamcinolone acetonide (TA) mediated gluconeogenesis in adrenalectomized , fed rats. Whereas 2 mg of either steroid did not lower liver glycogen, depletion with 20 mg ($p < 0.005$) was as pronounced as with 50 mg. Furthermore, liver TT or TP activities were not influenced by either steroid at the 20 mg (100 mg/kg) dose level. Thus, the antigluconeogenic activity of the naturally occurring, as well as of various other derivatives (not shown), sex steroids would appear to be selective and must be borne in mind in clinical prescription of oestro-progestative hormones.

Data in table 2 show that even in 100 fold excess neither gonadal steroid was able to significantly diminish

TABLE 2: BINDING OF ADRENOCORTICAL STEROIDS TO LIVER CYTOSOLIC RECEPTOR.

Competing steroid (3×10^{-6} M/ml)	Active steroid (3×10^{-8} M/ml) ^{3}H-triamcinolone acetonide	^{3}H-estradiol
	CPM/ mg protein	
None	1887	4795
Triamcinolone acetonide	61	5459
Estradiol	1195	2117
Testosterone	1420	5814
Cortisol	110	6117

Each value is a mean of three separate determinations. Radioactivity obtained in presence of 1000 fold excess of cold, homologous steroid was substracted from all values to account for non specific binding.

^{3}H-TA binding to its cytoplasmic receptor that was nearly totally abolished in presence of 100 fold excess of cold TA > cortisol. Thus, the antigluconeogenic effect (table 1) would appear to proceed by mechanisms other than antagonism at the level of cytoplasmic receptor occupancy. Similar results were obtained with six other derivatives of gonadal hormones or when ^{3}H-cortisol was used in place of ^{3}H-TA. Mammalian liver is endowed with high affinity estrogen receptors (7,8). Data in table 2 further show that binding of ^{3}H-estradiol to liver cytosol sites was not diminished by either TA, testosterone or cortisol. It remains to be seen whether the slight increase in ^{3}H-estradiol binding, in presence of various competitors , are in favour of some sort of receptor synergism.

The results described here are the first demonstration of an antiglucocorticoid action in vivo and add another parameter

to the known effects of sex steroids on liver metabolism (2). It is however difficult to say whether they argue for an actual repression of the operon controlling glycogen synthesis or are simply an expression of increased energy demand than can be met by endogenous synthesis. Since antigluconeogenic activity with 20 fold excess sex > gluco-steroid concentrations (table 1) could not be correlated with lack of diminution of TA-receptor binding in presence of even in 100 fold excess of sex steroids (table 2) the influence of estradiol may proceed via an independent mechanism involving either alteration in nuclear acceptor occupancy, another physiological locus, or binding to inhibitory moities of the heterogeneous, polymorphic receptor protein in the cell cytoplasm (9). However, there have been no claims that liver contains high affinity androgen binders (8), and it must be assumed that this steroid is metabolized to another active component in order to exert its antigluconeogenic effect; aromatization to estrogens is an obvious possibility. Finally, these studies lend support to conceptual search for synthetic molecules endowed with antiglucocorticoid activity at designated loci rather than derivatives with all or none action.

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