C.1

THE HIGH AFFINITY-LOW CAPACITY ANDROGEN BINDING PROTEIN IN THE HEPATIC CYTOSOL OF STREPTOZOTOCIN DIABETIC WISTAR RATS

By

Marie R. Rodway

B.Sc. University of Alberta, 1981

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
IN
THE FACULTY OF GRADUATE STUDIES
FACULTY OF PHARMACEUTICAL SCIENCES
UNIVERSITY OF BRITISH COLUMBIA

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

February, 1986

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Pharmaceutical Sciences

The University of British Columbia
1956 Main Mall
Vancouver, Canada
V6T 1Y3

Date March 4 / 86

ABSTRACT

Recently several groups have identified and partially characterized a high affinity-low capacity androgen binding protein (HALC ABP) in the hepatic cytosol of rats, rabbits and humans. In order to further characterize and to identify the physiological control mechanisms of this protein we did a series of studies in Wistar rats. The synthetic androgen methyltrienolone (R1881) was used as the ligand in binding studies. Precipitation with 50% ammonium sulfate resulted in a 2 to 3 fold purification, but did not eliminate the glucocorticoid receptor to which R1881 also binds. Frozen storage, at -80°C has no apparent effect on the hepatic HALC ABP. Studies on sex and age differences showed that R1881 binding was present in mature females and immature rats at less than one-half the concentration found in mature males. Neonatal castration of males appears to decrease or eliminate the HALC ABP in mature rats. No evidence of binding of R1881 to androgen binding proteins in blood was seen.

The HALC ABP binding capacity was significantly reduced from control levels in 4 and 10 day streptozotocin (STZ, 60 mg/kg, intravenous) diabetic male Wistar rats; there are no apparent changes in Kd or in steroid specificity.

After induction of diabetes with STZ, serum insulin levels are significantly decreased in 24 hours. Testosterone serum levels and triiodothyronine serum levels are significantly decreased 3 days after STZ injection. Dampening of the normal peaks of growth hormone secretion in male rats is evident 18 hours after STZ injection, and continues progressively. These changes correspond to the decrease in binding capacity of the hepatic HALC ABP. If the HALC ABP were regulated by one of these hormones, restoration of their serum levels should have restored the binding capacity of the HALC ABP. Partial restoration of the binding capacity of the prostatic androgen receptor was possible with testosterone or insulin in STZ diabetic rats. Attempted restoration to control levels of the hepatic HALC ABP by treatment of 4 day STZ diabetics with the following hormones was unsuccessful: insulin (protamine zinc insulin, 10 U/kg s.c. daily, or Toronto insulin 15 U/kg s.c. twice daily); testosterone enanthate (1 mg/kg s.c. daily); triiodothyronine (30 μg/kg s.c. daily); or ovine growth hormone (by minipump 0.02 U/hr for 4 days, s.c. 30 μg/dose for 7 daily doses, vena cava catheter 30 μg/dose for 7 daily doses, and tail vein injection 30 μg/dose, for 4 daily doses).

It is concluded that the HALC ABP is not regulated by insulin levels, by testosterone levels, by T_3 levels, or by GH levels or the GH male secretory pattern. Stress

appears to cause a decrease in the binding capacity of the HALC ABP in adult male Wistar rats.

Gail D. Bellward, Ph.D.

Supervisor

TABLE OF CONTENTS

	PAGE
ABSTRACT	ii
TABLE OF CONTENTS	v
LIST OF TABLES	xi
LIST OF FIGURES	xiv
LIST OF ABBREVIATIONS	xv
ACKNOWLEDGEMENTS	xvii
INTRODUCTION	
I. Tissues in which androgen receptors have been studied	1
II. Androgen binding proteins in liver	4
III. Androgen receptors in diabetic rats	7
IV. Changes in hormone levels and secretion in diabetic rats	
1. Insulin	7
2. Testosterone	8
3. Triiodothyronine and thyroxine	13
4. Growth hormone	14
V. Objectives	14
METHODS AND MATERIALS	
I. Chemicals	18
II. Care and surgery of animals	19
III. Treatment of animals	20

IV. Preparation of cytosol for binding assays

1. Preparation of whole cytosol..... 22

2. Fractionation of whole cytosol..... 23

3. Freezing of ammonium sulfate pellet..... 23

4. Determination of protein concentration..... 23

V. Radioligand exchange binding studies

1. Incubation of cytosol..... 24

2. Separation of bound and free steroid fractions 25

VI. Analysis of radioactivity..... 25

VII. Statistics..... 26

RESULTS

I. The effects of ammonium sulfate fractionation of cytosol on the hepatic high affinity-low capacity androgen binding protein

1. The effect of ammonium sulfate fractionation on the Kd and binding capacity values of the high affinity-low capacity androgen binding protein 27

2. The presence of the glucocorticoid receptor in the 50 % ammonium sulfate fractionated hepatic cytosol of the adult male rat..... 29

3. The effect of 50 % ammonium sulfate fractionation on the optimum incubation time for the high affinity-low capacity androgen binding protein assay..... 33

II. The effect of the perfusate volume on the results of the high affinity-low capacity androgen binding protein assay..... 33

III. The effect of freezing on the high affinity-low capacity androgen binding protein..... 38

IV. Age and sex differences in the presence of the hepatic cytosolic high affinity-low capacity androgen binding protein in rats

1. In mature female rats..... 41
2. In immature male and female rats..... 43
3. In castrated male rats..... 43

V. Presence of the high affinity-low capacity androgen binding protein in the hepatic cytosol of genetically diabetic BioBreeding strain rats.... 46

VI. Effects of streptozotocin induced diabetes on the Kd values, binding capacity, temperature stability, and steroid specificity of the high affinity-low capacity androgen binding protein

1. High affinity-low capacity androgen binding protein binding studies in control, 4 and 10 day streptozotocin diabetic rats..... 48
2. Temperature stability of the high affinity-low capacity androgen binding protein..... 58

3. Effect of the induction of streptozotocin diabetes on the specificity of the high affinity-low capacity androgen binding protein..... 58

VII. Effect of hormone replacement on the hepatic cytosolic high affinity-low capacity androgen binding protein

1. Effects of once daily administration of protamine zinc insulin and twice daily administration of Toronto insulin..... 59
2. Effects of testosterone enanthate treatment or testosterone enanthate with protamine zinc insulin treatment..... 66
3. Effects of triiodothyronine treatment..... 66
4. Effects of growth hormone replacement
   a. Effects of continous infusion of ovine growth hormone by osmotic minipump..... 69
   b. Effects of multiple subcutaneous ovine growth hormone doses..... 69
   c. Effects of multiple doses of ovine growth hormone using a *vena cava* catheter..... 72
   d. Effects of ether *versus* halothane general anaesthesia in control rats..... 74
   e. Effects of multiple tail vein injections of ovine growth hormone..... 74

f. Effects of multiple tail vein injections of ovine growth hormone, with daily injections of testosterone enanthate, subcutaneously, and daily injections of protamine zinc insulin, subcutaneously..... 77

DISCUSSION

I. Ammonium sulfate fractionation..... 79

II. Inclusion of triamcinolone acetonide in the hepatic partially purified cytosolic high affinity-low capacity androgen binding protein assay..... 81

III. Artifactual results from blood contamination.... 82

IV. Kd and binding capacity values of the hepatic high affinity-low capacity androgen binding protein..... 83

V. Specificity of the hepatic high affinity-low capacity androgen binding protein..... 80

VI. Sex and age differences in the hepatic high affinity-low capacity androgen binding protein.. 87

VII. Effects of diabetes on the hepatic and prostatic high affinity-low capacity androgen binding protien..... 90

VIII. Non-specific effects of streptozotocin..... 92

IX. Use of ovine growth hormone to restore hepatic high affinity-low capacity androgen binding protein binding capacity..... 94

X. Effect of stress on the hepatic high affinity-low capacity androgen binding protein.. 94

XI. Subcellular location of the high affinity-low capacity androgen binding protein..... 95

XII. Possible function of the hepatic high affinity-low capacity androgen binding protein..... 97

FUTURE EXPERIMENTS..... 98

SUMMARY..... 103

BIBLIOGRAPHY..... 106

LIST OF TABLES

I. The effect of ammonium sulfate fractionation of whole hepatic cytosol on the high affinity-low capacity androgen binding protein assay.... 28

II. The effect of incubation times from 1.5 to 30 hours on the Kd and binding capacity values of the hepatic cytosolic high affinity-low capacity androgen binding protein..... 34

III. The effect of different liver perfusion volumes used prior to high affinity-low capacity androgen binding protein assays done in whole and 50 % ammonium sulfate fractionated hepatic cytosol.. 37

IV. The effect of increasing amounts of triamcinolone acetonide in assays of male and female rat hepatic cytosolic high affinity-low capacity androgen binding protein..... 42

V. The hepatic high affinity-low capacity androgen binding protein content of mature and immature male and female rats..... 44

VI. The effect of castration on the presence of the hepatic cytosolic high affinity-low capacity androgen binding protein in male rats..... 45

VII. Binding parameters in BioBreeding strain diabetic and BioBreeding strain non-diabetic male rats compared to control adult male rats..... 47

VIII. The effect of 4 and 10 day streptozotocin diabetes

on the binding parameters of the hepatic cytosolic high affinity-low capacity androgen binding protein..... 55

IX. (1,2)The effect of 4 and 10 day streptozotocin diabetes on the temperature stability of the hepatic cytosolic high affinity-low capacity androgen binding protien of adult male rats..... 56

X. The effect of 10 day streptozotocin diabetes on the hepatic cytosolic high affinity-low capacity androgen binding protein of male rats... 60

XI. The effect of treatment with regular (Toronto) and protamine zinc insulin on the hepatic cytosolic high affinity-low capacity androgen binding protein of 4 day streptozotocin diabetic male rats..... 65

XII. The effect of treatment of 4 day streptozotocin diabetic male rats with testosterone enanthate, or testosterone enanthate with protamine zinc insulin on the hepatic cytosolic high affinity-low capacity androgen binding protein..... 67

XIII. The effect of treatment with triiodothyronine on the hepatic cytosolic high affinity-low capacity androgen binding protein of 4 day streptozotocin diabetic male rats..... 68

XIV. The effect of infusion of ovine growth hormone by minipump on the hepatic cytosolic high affinity-low capacity androgen binding protein of

streptozotocin diabetic male rats..... 70

XV. The effect of multiple subcutaneous doses of ovine growth hormone on the hepatic cytosolic high affinity-low capacity androgen binding protein of 4 day streptozotocin diabetic male rats..... 71

XVI. The effect of multiple intravenous doses of ovine growth hormone by vena cava catheter on the hepatic cytosolic high affinity-low capacity androgen binding protein of streptozotocin diabetic male Wistar rats..... 73

XVII. The effect of halothane versus ether anaesthesia on the hepatic cytosolic high affinity-low capacity androgen binding protein of adult male rats..... 75

XVIII. The effect of multiple intraveneous doses of ovine growth hormone by the tail vein on the hepatic cytosolic high affinity-low capacity androgen binding protein of 4 day streptozotocin diabetic male rats..... 76

XIX. The effect of subcutaneous administration of testosterone enanthate and protamine zinc insulin with multiple tail vein injections of ovine growth hormone on the hepatic cytosolic high affinity-low capacity androgen binding protein of 4 day streptozotocin diabetic male rats..... 78

LIST OF FIGURES

PAGE

1. Saturation curves and Scatchard plot for binding studies of the hepatic cytosolic high affinity-low capacity androgen binding protein in 50 % ammonium sulfate fractionated cytosol with and without triamcinolone acetonide..... 30

2. Scatchard plot of the hepatic cytosolic high affinity-low capacity androgen binding protein from frozen 50 % ammonium sulfate fractionation pellet.. 39

3. Saturation curves for binding studies of hepatic cytosolic high affinity-low capacity androgen binding protein from control, 4 and 10 day streptozotocin diabetic adult male rats..... 49

4. Scatchard plot for binding studies of the hepatic cytosolic high affinity-low capacity androgen binding protein in control, 4 and 10 day streptozotocin diabetic adult male rats..... 53

5. Specificity studies of the high affinity-low capacity androgen binding protein from control and 10 day streptozotocin diabetic adult male rats..... 62

LIST OF ABBREVIATIONS

ABP	androgen binding protein
androstenedione	Δ^4androstene-3,17-dione
ANOVA	analysis of variance
AR	androgen receptors
C	centigrade
cm	centimeter
cyproterone acetate	6-cholor-1,2-dihydro-(1β,2β)-3'H-cyclopropa-(1,2)pregna-1,4,6-triene-3,20-dione
DHT	5α or β-androst-2-en,17β-ol
estradiol	1,3,5-estratriene-3,17β-diol acid
F	free
fmol	femto moles
g	gram
GH	growth hormone
^{3}[H]	tritiated
HALC ABP	high affinity-low capacity androgen binding protein
IC_{50}	concentration required to displace 50 % of the specific binding
Kd	equilibrium dissociation constant
kg	kilogram
L	litre
mg	milligrams per millilitre

mibolerone	(7α,17α-dimethyl[17α-methyl^{3}H]19-nortestosterone
μ	micro
mL	millilitre
mmol	millimoles
ng	nano gram
nM	nanomolar
oGH	ovine growth hormone
PZI	protamine zinc insulin
progesterone	Δ^{4}pregnene-3,20-dione
R1881	methyltrienolone; 17β-hydroxy-17methylestra-4,9,11-trien-3-one
s.c.	subcutaneously
STZ	streptozotocin; 2-deoxy-2-((methy-nitrosoamine)carbonyl)-amino(-D-glucopyranose)
TEDM	Tris base, EDTA, dithiothreitol, sodium molybdate, glycerol
testosterone	4-androsten-3-one-17β-ol
triamcinolone acetonide	9α-fluoro-11β,16α,17,21-tetra-ol-pregna-1,4-diene-3,20-dione cyclic 16,17 acetal with acetone
U	units

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Dr. G.D. Bellward, and my committee, Dr. B.D. Roufogalis, Dr. K.M. MacLeod, and Dr. R.J. Ensom. I would also like to thank Bruce Allen for listening to me complain. Most especially I would like to thank Dr. Geoffrey Sunahara, who showed me the value of consistency.

INTRODUCTION

I. Tissues in which androgen receptors have been studied

Initial investigation of androgen receptors (AR) was in tissue previously shown to be androgen dependent. Changes in size or function of a tissue after castration and reversal of these changes with testosterone replacement was indicative of androgen dependency. Tissue in which AR have been found are: the lung, comb and wattle of the cock (Dube et al, 1975), mouse kidney (Pajunen et al,1982), hamster sebaceous glands (Adachi and Kano, 1972), the hypothalamus of male mice, hamsters, guinea pigs, rabbits, and cats (Kato, 1975), rat skin (Eppenderberg and Hsia, 1972), rat lacrimal gland (Ota et al, 1985) rat prostate (Kreig and Voigt, 1976 and Traish et al, 1981), rat sertoli cells (Sanborn et al, 1984), rat ventral and dorsal prostate, epididymus, testis, seminal vesicle and an androgen dependent tumor of the prostate (Wilson and French, 1979), rat bulbocavernosus, levator ani and skeletal muscle (Kreig, 1975, and Kreig and Voigt, 1976), rat heart (Kreig et al, 1978), rat hypothalamus (Kato, 1975), the anterior pituitary of mature and immature male rats (Kato, 1975, and Thieulant, 1975), the uterus of immature rats (Giannopoulous, 1973), female rhesus monkey heart (McGill et al, 1980), male and female baboon heart (McGill and Sheridan, 1981), in human

sexual and non-sexual skin, striated muscle and kidney (Deslypere et al, 1980), human skin fibroblasts (Keenan et al, 1975), human myometrial tissue and mammary cancer tissue (Poortman et al, 1975, Bryan et al, 1984), human prostate (Mainwaring and Milroy et al 1973, Deslypere et al, 1980 Traish et al, 1981), human benign prostatic hypertrophy (Rosen et al,1975), and prostatic cancer (Snochowski et al, 1977, Hawkins et al, 1981, and Kirdani et al, 1985). From the above listing, it is evident that the tissues which have attracted most interest are classical sex hormone target tissues.

Several groups have studied AR isolated from different tissue in 1 animal to determine tissue specificity. Mainwaring and Irving (1973) found that AR in the prostate, seminal vesicles and epididymus of male Sprague-Dawley rats had identical steroid specificity, identical isoelectic points (5.8), and all had a sedimentation coefficient of 8 S. The AR of prostate, seminal vesicles and epididymus of guinea pigs had the same characteristics. Kreig and Voigt (1976) studied the cytosolic AR of the adult male Wistar rat prostate, bulbocavernosus/levator ani and skeletal muscle and found Kd's of 1.5, 0.7, and 2.4 nM, respectively, using [^{3}H]5α-dihydrotestosterone (DHT). They found that all three tissues contained AR from which [^{3}H]5α-DHT was displaced by 50 to 100 fold molar excess of 5α-DHT, testosterone and 19-nortestosterone, and by 1000 fold molar

excess of cyproterone acetate and estradiol; but was not displaced by 1000 fold molar excess of cortisol. Wilson and French (1976) produced evidence that AR in different tissues of the rat were the same. The AR of Sprague-Dawley and Osborn-Mendel rat testis, epididymus, and prostate were identical. Although not explicitly stated it appears that AR in both strains of rats were also identical. They found Kd values of 0.2 to 0.5 nM using 5α-DHT and testosterone; a higher affinity for 5α-DHT than for testosterone; optimum receptor binding at pH = 8; changes in the sedimentation coefficient occured when using ammonium sulfate, but no changes in the binding properties of the receptors; and similar rates of association and dissociation of [^{3}H]5α-DHT and [^{3}H]testosterone for AR of all three tissues. In these studies no tissue specificity has been seen.

If no difference in the AR exists, there must be another explanation for difference in tissue sensitivity to different androgenic steroids. It has been found that tissue sensitivity to natural and synthetic androgens depends on the presence and abundance of the AR, the chemical structure of the androgen administered, and the presence and abundance of specific androgen metabolizing enzymes. The chemical structure of the androgen will determine which enzymes can metabolize it. Enzymes such as 5α-reductase and 17β-hydroxysteroid dehydrogenase (which

metabolize the androgen) determine structure, and therefore affinity, of the product (Kreig and Voigt, 1976, Deslypere et al, 1980, and Bergink et al, 1985).

II. Androgen binding proteins in liver

Androgen binding proteins (ABP) which have properties of receptor proteins (saturable, low capacity and high affinity binding of androgens) have been studied in the liver of rabbits, rats and humans. Because physiological function has not been determined, we will refer to this androgen binding protein as "HALC ABP", unless the author of the referenced paper has identified it as the hepatic AR. The hepatic cytosolic AR of female New Zealand white rabbits has been studied by Eisenfeld et al, and Sheets et al, 1985. They found a Kd of 0.3-0.9 nM and a binding capacity of 79 fmol/mg. Methyltrienolone (R1881), 5α-DHT, testosterone, and estradiol, in descending order of affinity, bound to the AR. Progesterone and diethylstilbesterol did not bind. This protein was present in immature females and in adult males in concentrations about one-half that seen in mature female rabbits.

Human hepatic cytosolic AR have been studied by Aten and Eisenfeld, 1983, and by Bannister et al, 1985b. Aten and Eisenfeld reported a Kd of 1-2 nM, and a binding capacity of 30-50 fmol/mg protein. The sex of the patients

from which the biopsies were taken was not reported. Bannister et al, 1985b, reported results of nucleosolic and cytoplasmic AR binding studies. The cytosolic AR had a Kd value of 1.42 ± 0.30 nM and a binding capacity of 103 ± 31 fmol/mg protein. The order of affinity for steroids was: mibolerone > 5α-DHT > cyproterone acetate > testosterone > progesterone > estradiol. A total of 8 patients were biopsied, 2 were females.

Reports concerning ABP in rat liver cytosol appeared first in the early 1970's (Milin and Roy, 1973, Roy et al, 1974, and Gustafsson, 1975). The interpretation of these studies was complicated by the metabolism of ligands, transformation of the ABP, and the binding of ligands to an estrogen-androgen binding protein present in the liver of male rats (Gustafsson et al, 1975, Kyakumoto et al, 1984, Levinson and Decker, 1985, and Sunahara et al, 1985). These complications led to inaccurate estimations by Scatchard analysis of Kd values and binding capacities.

In order to overcome these difficulties, molybdate, R1881, and triamcinolone acetonide were incorporated into the ABP assay. Sodium molybdate was effective in preventing the transformation of the ABP (reviewed by Dahmer et al, 1984). One of the most important changes to the hepatic ABP assay was the introduction of synthetic androgens used as radiolabeled and competing steroids in radioligand exchange binding studies. They were minimally metabolized and did

not bind to the sex steroid binding globulin in blood. R1881 was introduced first and is most widely used. It is minimally metabolized in rat prostatic (Bonne and Raynaud, 1975 and 1976), and hepatic cytosol (Decker and Levinson, 1983, Levinson and Decker, 1985, and Turocy et al, 1985). The use of R1881 and of mibolerone in ABP assays revealed two binding sites, with different affinities for R1881 and therefore different Kd values (Kyakumoto et al, 1984, Decker and Levinson, 1985, Sunahara et al, 1985, and Bannister et al, 1985a). Previous work done using R1881 in prostatic cytosol also showed two binding sites. In the prostate the second binding site was the progestin receptor (Zava et al, 1979). Levinson and Decker, 1985, demonstrated the presence of the glucocorticoid receptor and absence of a progestin receptor in rat hepatic cytosol, so it is believed that the second binding protein of R1881 in hepatic cytosol is the glucocorticoid receptor. Triamcinolone acetonide, a synthetic glucocorticoid, and cortisol have been used in 100-1000 fold molar excess concentrations in order to eliminate the binding of radiolabeled and competitor ligand to the progestin receptor in prostate cytosol, and the glucocorticoid receptor in hepatic cytosol (Zava et al, 1979, Eisenfeld et al, 1983, and Aten et al, 1983). Saturation curves of specific R1881 binding and Scatchard analysis suggest 1 R1881 binding site, so calculation of the Kd and maximum binding capacity

has been simplified to linear regression analysis of Scatchard plots.

Studies done to determine the necessity of both triamcinolone actetonide and cortisol in the incubation of the HALC ABP assay showed that 100 fold molar excess was adequate to eliminate the interference of the glucocorticoid receptor in the assay of the hepatic cytosolic HALC ABP in male adult Wistar rats (Sunahara, 1984).

As a result of the above modifications to the hepatic ABP assay, several papers have been published which describe initial characterization studies of the rat hepatic ABP (Kyakumoto et al, 1984, Levinson and Decker, 1985, Sunahara et al, 1985, and Bannister et al, 1985a).

III. Androgen receptors in diabetic rats

In 1980, Tesone et al examined the effect of streptozotocin (STZ, 65 mg/kg), 14 days after induction of diabetes on AR in prostatic cytosol. A significant decrease in number of AR, partially reversible with testosterone (500 μg/rat subcutaneously (s.c.) daily) or protamine zinc insulin (PZI, 2 U/rat s.c. daily) treatment was seen. Association constants (Ka) did not change significantly. The association contant for the control group was 0.13 ± 0.01 nM; for the diabetic group was 0.17 ± 0.02 nM; for the insulin treated diabetic group was 0.10 ± 0.02 nM; and for

the testosterone treated diabetic group was 0.12 ± 0.01 nM. Binding capacity values were 94.2 ± 11.3 fmol/mg protein for the control group; 12.6 ± 3.0 fmol/mg protien for the diabetic group, 18.3 ± 2.4 fmol/mg protein for the insulin treated diabetic group, and 41.1 ± 3.1 fmol/mg protein for the testosterone treated diabetic group. Hense, the sensitivity of the prostatic AR to testosterone, and to a lesser extent, insulin, has been demonstrated.

IV. Changes in hormone levels and secretion in diabetic rats

1. Insulin

Induction of diabetes with STZ causes a wide range of hormonal changes. The initial and most obvious change is the change in the amount of available insulin in the pancreas. STZ induces diabetes by causing degranulation and necrosis of β pancreatic cells. In mature male Wistar rats made diabetic with a dose of 65 mg/kg STZ, large amounts of insulin were released into the blood at around seven hours post injection. Excess insulin was still present in the blood at 10 hours post injection, as the blood glucose levels remained depressed. At 24 hours post injection pancreatic insulin content was about 5 % of the normal value, serum immunoreactive insulin was at fasting levels,

and serum glucose was 300-400 mg/100 mL, control serum glucose is under 100 mg/100 mL (Junod, et al, 1967). Serum insulin in male 4 day STZ diabetic rats was significantly reduced from control values, 25.9 ± 3.7, and 14.1 ± 1.6 μUnits/mL, respectively (Warren et al, 1983).

2. Testosterone

Decreases in serum and testicular levels of testosterone in STZ diabetic rats have been well documented. Howland and Zebrowski (1976) studied STZ (70 mg/kg or 65 mg/kg) diabetes in Sprague-Dawley rats. They found a significant reduction in serum concentration of testosterone (control 4.33 ± 0.89, and STZ diabetic 1.08 ± 0.10 ng/mL), three weeks after induction of diabetes with STZ.

Tesone et al, 1976, studied the effects of STZ (50 mg/kg) diabetes on the serum and testicular levels of testosterone of adult male Wistar rats and saw a significant decrease in serum testosterone, which could be partially restored with insulin treatment, and a significant decrease in testicular testosterone, which could be restored with insulin treatment. Serum levels of testosterone in control, diabetic, and diabetic treated with PZI (0.5 U/rat s.c. daily) were, respectively, 486 ± 19, 171 ± 9, and 290 ± 15 ng/100 mL, measured 15 days after induction of diabetes, or 15 days after initiation of insulin treatment.

Testicular levels of testosterone for control, diabetic and insulin treated diabetic were, respectively, 131 ± 10, 30 ± 5, and 111 ± 7 ng/g of testis.

Paz et al, 1978, studied serum and testicular levels of testosterone in male albino rats made diabetic with STZ (15 mg/rat) and found significant decreases in serum testosterone in diabetic compared to control rats at 6 to 7 weeks after induction of diabetes , which was partially restored with insulin treatment. They found serum levels in control, diabetic, and diabetic treated with 2 IU of PZI s.c. daily, of, respectively, 2.4 ± 0.4, 0.9 ± 0.3, and 1.1 ± 0.2 ng/mL. Testicular levels of testosterone were also significantly reduced and partially restored with insulin treatment. Values for control, STZ, and STZ treated with PZI were, respectively, 110 ± 16, 38 ± 8, and 49 ± 4 ng/g of testis.

Baxter et al, 1981, studied the effect of STZ (100 mg/kg) diabetes in mature male Wistar rats. He found a significant reduction in serum levels of testosterone 1 week after induction of diabetes with diabetes and found that the reduction was insulin reversible. Serum levels of testosterone for control, diabetic, and diabetic treated with 10-12 U daily of Monocomponent Actrapid and Lente insulin administered twice daily, were respectively, 9.0 ± 1.3, 3.9 ± 0.8, and 7.7 ± 1.1 nM.

Murray et al, 1981, studied serum testosterone levels

in adult male Wistars rats made diabetic with 65 mg/kg STZ. They found significant reductions from control at 4 weeks after injection of STZ. Control levels were 4.11 ± 0.65 ng/mL, levels at 2, 3, and 4 weeks after injection of STZ, were, respectively, of 2.41 ± 0.64, 3.49 ± 0.9, and 1.41 ± 0.58 ng/mL. Rats treated with 1-2 U/100g PZI had levels of 3.71 ± 0.27 ng/mL which was not different from control levels.

In 1980 Tesone et al measured serum testosterone levels in adult male Wistar control, diabetic (65 mg/kg STZ), and insulin (2 IU PZI s.c. daily) treated diabetic rats and found a significant reduction partially reversible with insulin treatment in STZ diabetics. Measurements were made 28 days after induction of diabetes. Values were 540 ± 64, 238 ± 37, and 358 ± 18 ng/100 mL, respectively. Testicular testosterone levels were significantly reduced in diabetics and reversed with insulin. In the same order values were 154 ± 13, 41 ± 5, and 142 ± 9 ng/g of testis.

Skeet et al, 1984, showed serum testosterone levels in adult male Wistars of 1.8 ± 1.0, and a significant decrease in STZ (60 mg/kg) diabetics to 1.0 ± 0.03 mg/mL, measured 3 days after induction of diabetes with STZ.

Other models of diabetes also show significant reduction in serum testosterone and testicular levels of testosterone. Howland and Zebrowski (1976) showed a

significant reduction in serum testosterone in alloxan (15 mg/100 g) diabetes. Values in control and diabetic rats were, respectively, 4.33 ± 0.89, and 1.54 ± 0.34 ng/mL. Warren et al, 1983, showed decreased serum testosterone levels in genetically diabetic (BioBreeding) rats as compared to internal controls, or external, Wistar, controls. Internal controls to BioBreeding rats also have serum testosterone levels significantly reduced from Wistar control levels. Values were 1.16 ± 0.44, 2.99 ± 0.42, and 6.4 ± 1.25 ng/mL, for diabetic BioBreeding, non-diabetic BioBreeding, and Wistar control male rats, respectively. Serum testosterone levels in BioBreeding rats were measured 3 days after stopping insulin treatment.

In all studies, diabetes, from STZ, alloxan or genetically determined, resulted in a significant decrease in serum and testicular levels of testosterone. Insulin administered in a dose of 3 to 12 U/day/rat appeared to completely reverse the decreased serum testosterone levels; 2 U daily, however, only partially restored serum testosterone levels. Complete restoration of testicular content of testosterone has been reported with as little as 0.5 U/rat daily, and with 2 U/rat daily; however, 2 U/rat/day has also been reported to only partially restore testicular amounts of testosterone. The restoration of serum and testicular amounts of testosterone does not appear to be determined by the amount of STZ used to induce

diabetes in the rat. Decreased biosynthesis of testosterone may also occur in chemically diabetic rats. In Instituto de Fisiologia strain white rats which had 95 % of the pancreas removed, Foglia et al, 1969, have shown decreased testosterone biosynthesis. Percentage of ^{14}C progesterone transformed to testosterone was over 3.5 % in control rats and 0 to 3.5 % in rats with fasting blood glucose over 120 mg/mL. Decreases in serum and testicular testosterone levels, partially reversible with insulin treatment occur in STZ diabetic male rats. Significant decreases in testosterone synthesis occurs in pancreatectomized diabetic male rats and may occur in other models of diabetes.

3. Triiodothyronine and thyroxine

Jennings (1984) induced diabetes with STZ (65 mg/kg) in mature male Sprague-Dawley rats. He showed a decrease in serum levels of thyroxine (control, 3.7 $\pm$ 0.4 µg/dL, 3 days post STZ injection-earliest time of significant difference-2.1 $\pm$ 0.4 µg/dL) and triiodothyronine (control, 35 $\pm$ 3 ng/dL, 2 days post STZ injection-earliest time of significant difference-24 $\pm$ 4 ng/dL). Using a isolated perfused rat liver, he showed a progressive decrease in hepatic triiodothyronine production (from 56 $\pm$ 11 to 12 $\pm$ 4 ng/hour after 5 days). The reduction in production was first significantly different after 2 days. The

reduction in triiodothyronine production was primarily due to reduced thyroxine to triiodothyronine conversion (3.27 ± 0.53 to 0.75 ± 0.21 %, on the 5th day of diabetes). Diabetic rats treated with insulin (2.5 U/100 g, Lente), produced serum thyroxine and triiodothyronine levels equal to controls. Insulin treatment also restored conversion of thyroxine to triiodothyronine, and triiodothyronine production to control levels, in the isolated perfused rat liver. Treatment of diabetic rats with thyroxine (1 µg/100 g daily) restores serum thyroxine levels and thyroxine to triiodothyronine conversion, and therefore serum triiodothyronine levels.

Pittman et al, 1981, showed a significant decrease in serum thyroxine and triiodothyronine levels in mature male Sprague-Dawley rats made diabetic with 50 mg/kg STZ, but not until 8 days after induction of STZ diabetes. A significant reduction of thyroxine to triiodothyronine conversion in liver homogenates was seen after 3 days of STZ diabetes.

Mitsuma and Nogimori, 1982, studied the effects of STZ (60 mg/kg) diabetes on the hypothalamic-pituitary-thyroid axis in adult male diabetic Wistar rats. Immunoreactive thyrotropin releasing hormone in the hypothalamus was reduced after 4 weeks of diabetes. Insulin treatment (2 U zinc insulin i.m. daily), partially restored this. Values for control, diabetic, and insulin treated diabetic are, respectively, 4.4 ± 0.2, 3.2 ± 0.2, 3.7 ± 0.3

ng/hypothalamus. Basal thyrotropin levels were significantly reduced at the first measurement after induction of diabetes, which was 2 weeks. Amounts were partially restored with insulin treatment, values for control, diabetic, and insulin treated diabetic are, respectively 260 ± 22, 160 ± 14, 196 ± 13 ng/mL. Responses of immunoreactive thyrotropin releasing hormone and plasma thyrotropin responses to cold were significantly reduced 2 weeks after the induction of diabetes, and this was partially reversed with insulin treatment. Plasma thyroxine, triiodothyronine, and 3,3'-diiodothyronine were significantly decreased (respectively, 3.6 ± 0.3 μg/dL, 35 ± 4 ng/dL, 2.3 ± 0.4 ng/dL) from control amounts (5.0 ± 0.4 μg/dL, 53 ± 5 ng/dL, and 3.4 ± 0.6 ng/dL), at two weeks after induction of diabetes with STZ, which was the first time point in this study. Insulin treatment restored plasma levels to control values (thyroxine 3.8 ± 3 μg/dL, triiodothyronine 48 ± 4 ng/dL, and 3,3'-diiodothyronine 3.0 ± 0.6 ng/dL). The reduction in triiodothyronine and thyroxine and in thyroxine to triiodothyronine conversion clearly occurs, and is insulin reversible. How soon these decreases become physiologically relevant after STZ induction of diabetes is unknown, although they are measurable in the perfused rat liver at 2 days after STZ injection.

4. Growth hormone

The pattern of GH release was changed, and the mean 8 hour plasma level of GH was decreased in 15 day STZ (70 mg/kg) male diabetic Wistar rats (Ortiz-Caro et al, 1984). Normal mature male rats exhibit an ultradian pattern of release (Tannenbaum, 1976) which was dampened by STZ diabetes so that the pattern more closely resembles the more constant feminine pattern of release . This change can be seen 18 hours after injection and continues progressively. A decrease in pituitary GH was also seen. Reduced pulses of GH may be mediated by somatostatin (Tannebaum, 1981). Insulin (15 U/kg twice a day for 12 days) restored the normal release pattern (Ortiz-Caro et al, 1984). The continuous presence of testosterone is required to maintain the low GH baseline levels in adult male rats. Studies done with neonatally castrated male and female rats suggest an imprinting of the pattern of GH release by neonatal testosterone surges (Jansson, et al 1984). In male rats STZ diabetes feminizes GH release patterns.

V. Objectives

The long standing interest of this laboratory has been in hepatic drug metabolism; in particular, in the sex differences of some hepatic drug and steroid metabolizing

presence of androgens in the male rat. Androgens are believed to act by binding with a cytoplasmic receptor. If the difference in these enzyme activities is due to local, as opposed to central, activity of androgen, one would expect an AR in the liver. An hepatic androgen binding protein with the characteristics of a receptor protein has been demonstrated (Kyakumoto et al, 1984, Levinson and Decker, 1985, Sunahara et al, 1985, and Bannister et al, 1985). If the sex difference depends on the hepatic AR, and therefore local action of androgens, one would expect age and sex dependency of the AR that correlates with age and sex dependency of the sex dependent enzyme activities.

It is well known that feminization of sex dependent enzyme activities occurs in male rats which are made diabetic. Sunahara et al, 1985, demonstrated a change in the hepatic receptor-like binding protein in the STZ (60 mg/kg) induced diabetic male Wistar rat. Changes in patterns of secretion or absolute amounts of hormones secreted are also known to occur in acute diabetes; the hepatic HALC ABP may be regulated by one or more of these hormones.

In order to further characterize and investigate the regulation of the HALC ABP, and to determine if there was a correlation between sex dependent enzyme activities and the HALC ABP under different physiological conditions, studies using 50 % ammonium sulfate fractionated cytosol were done to: 1) re-examine age and sex specificity of the

were done to: 1) re-examine age and sex specificity of the HALC ABP; 2) determine the reason for the apparent decrease in the binding capacity of the HALC ABP in STZ diabetic males; 3) and to attempt restoration of this decreased binding capacity of the HALC ABP.

Specifically, the objectives of this study were: 1) to study the presence of the hepatic HALC ABP in mature and immature male and female Wistar rats, in mature castrated males, in neonatally castrated males, and in neonatally castrated testosterone treated male rats; 2) to study the effect of diabetes induced by STZ on the temperature stability, binding specificity, and Kd and binding capacity values, determined from Scatchard analysis, of the hepatic HALC ABP; and 3) to attempt to reverse the changes seen in the HALC ABP of STZ diabetic male rats by treatment with insulin, testosterone, triiodothyronine and oGH.

MATERIALS AND METHODS

I. Chemicals

The following chemicals were purchased from Sigma Chemical Company (St. Louis, MO): Tris (hydroxymethyl)aminomethane (Trizma®-base), ethylenediamine-tetraacetic acid, DL-dithiothreitol, sodium molybdate, activated charcoal, bovine serum albumin, Brillant blue G 250, streptozotocin (STZ), citric acid (free acid and trisodium salt), testosterone enanthate, triamcinolone acetonide, 5α-DHT, 5β-DHT, androstenedione, estradiol, diethylstilbesterol, progesterone, and triiodothyronine.

Radioactively labeled [17α-methyl-^{3}H] methyltrienolone (R1881, specific activity 86 or 87 Ci/mmol) and R1881 were purchased from New England Nuclear, under licenced agreement with Roussel-Uclaf (Romainville, France). Biofluor® was purchased from New England Nuclear. Ovine growth hormone (oGH) was provided by Dr. S. Raiti (National Hormone and Pituitary Program, Baltimore Maryland). Dextran T70® was purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). Glycerol was purchased from British Drug House Chemicals. Ammonium sulfate, ultrapure, was obtained from ICN Biomedical. PZI and Toronto insulin, beef and pork, (100

U/mL) from Connaught Laboratories (Willowdale, Ontario) were used. Silica gel thin layer chromatography sheets (20 x 20 cm) with fluorscent indicator were purchased from Eastman Kodak Co. (Rochester NY). Osmotic pumps were purchased from Alzet Corporation (Palo Alto, California). Solvents and other reagents used were of reagent grade. Radiochemical purity of [^{3}H]R1881 was verified by thin layer chromatography. Two solvent systems were used, benzene:ethylacetate (8:2), and choloroform:ethanol (98:2). Checks were performed on each new shipment and periodically thereafter. Radiochemical purity of 97% or above was considered acceptable.

II. Care and surgery of animals

Adult male (240-390 g), adult female (200-260 g), and pregnant female Wistar rats were purchased from Canada Breeding Laboratories (La Prairie, Quebec). Animals were housed, 4 to a cage, in plastic cages on corncob bedding, Lobund Grade (Paxton Processing Ltd. Paxton, Il. Lobund grade bedding is preservative free), at 22°C, 14 hours light (0600-2000), 10 hours dark. Purina Lab Chow and tap water were freely available. Adult male Wistar rats were made diabetic under ether anaesthesia by a single tail vein injection of STZ, administered in 0.1 M citrate buffer, pH = 4.5, at a dose of 60 mg/kg. Experiments were done using

both 4 and 10 day post-injection diabetic rats. All rats injected with STZ, with the exception of those treated with insulin, had blood glucose levels of 0.5 g/100 mL or greater, as determined by TesTape® (Eli Lilly and Company Ltd., Toronto, Ontario) urine measurements. Neonatal castrations were performed by the abdominal route using ice anaesthesia (Gustafsson and Stenberg, 1974). Minipumps were implanted under ether anaesthesia subcutaneously into the dorsal region. Vena cava catheters were implanted by the abdominal route under ether or halothane anaesthesia, as described in the results. Local anaesthetic (lidocaine) and topical antibiotic (Cicatrin ®, each g contains: neomycin sulphate BP 3300 IU, Zinc Bacitracin BP 250 I.U., L-cysteine 2 mg,. DL-threonine 1 mg, and glycine 10 mg) were used at the incision and site of catheter skin entry.

III. Treatment of animals

The replacement doses of insulin were: PZI, 10 U/kg; Toronto, 15 U/kg (Ortiz-Caro et al, 1984). PZI was administered once a day; Toronto insulin, twice a day. Experiments were done 24 or 12 hours, respectively, following the last insulin dose. The replacement dose of testosterone enanthate used in STZ diabetic animals was 2.5 μmol/kg in corn oil. This dose was chosen because it has been shown to produce physiological serum levels in

castrated rats (Sunahara, 1984). Experiments were done 12 or 24 hours after the last dose of testosterone enanthate. Triiodothyronine was administered in a dose of 30 µg/kg daily (Tahaliani, 1983). The dose of growth hormone secreted by minipump was 0.02 U/hour, for four days. The dose of growth hormone in the 7 daily s.c. dose experiment, the 7 daily doses admininstered by vena cava catheter, and the 4 daily tail vein injections was 30 µg per rat per dose, administered in saline (pH 8.5).

IV. Preparation of cytosol for binding assays

1. Preparation of whole cytosol

Animals were killed by stunning, cervical dislocation, then decapitation. Livers were perfused with ice cold TEDM buffer (0.05 M Tris-base; 1.5 mM ethylenediamine tetraacetic acid disodium; 0.5 mM dithiothreitol, 10% glycerol; 20 mM sodium molybdate), and were dissected, weighed, minced, then homogenized, 1:15 (w/v) with fresh TEDM buffer, at 4°C using a precooled Potter-Elvejheim homogenizer with teflon pestle. The crude homogenate was centrifuged at 10,000 x g for 10 minutes at 4°C in an International Equipment Company, model B-20 centrifuge. This crude cytosol was centrifuged at 105,000 x g for 65 minutes at 4°C in Beckman model L5-50, or L2-65B ultracentrifuges. Whole cytosol was diluted by

one-half with TEDM buffer to produce a protein concentration of 1-3 mg/mL, and was then incubated (see below, V, 1).

2. Fractionation of whole cytosol

Whole cytosol prepared as above was fractionated with ammonium sulfate according to the following procedure. Saturated ammonium sulfate solution was added drop by drop over half an hour to whole cytosol which was stirring on ice. This was centrifuged at 12,000 x g for 30 minutes. The pellet was resuspended with TEDM buffer, and incubated in the same manner as the whole cytosol (see below, part V, 1).

3. Freezing of the 50 % ammonium sulfate fractionated cytosolic pellet

Pellets from fractionated cytosol were quick frozen in acetone-dry ice, then stored at -80°C for periods up to 60 days, in order to determine the stability of the HALC ABP to freezing. Pellets were resuspended in a volume of ice cold TEDM buffer equal to the volume of original cytosol immediately before the incubation was started.

4. Protein concentration determination

Protein concentration in whole and fractionated cytosol

was determined by the method of Bradford (1976), using a Perkin Elmer Model 124 double beam spectrophotometer. The standard curve was generated using bovine serum albumin in concentrations of 0.10 to 0.40 mg/mL.

V. Radioligand exchange binding studies

1. Incubation of cytosol

Cytosol was incubated according to the method of Sunahara (1984). For Scatchard analysis a 500 µL aliquot of cytosol (0.5-1.0 mg protein/mL) was incubated with increasing concentrations of [^{3}H]R1881 in the presence and absence of 100 fold molar excess R1881, and with 100 fold molar excess of triamcinolone acetonide. This mixture was incubated at 4°C for 3 hours. In specificity studies, a 500 µL aliquot of cytosol (0.5-1.0 mg protein/mL) was incubated with 1 nM [^{3}H]R1881 and 0.1 to 1000 fold molar excess of R1881, as well as 100 fold molar excess of 1 nM triamcinolone acetonide. When R1881 was used as the competitor, lower concentrations of R1881 (0.0001 to 1000 fold molar excess) were used to generate a complete displacement curve.

2. Separation of bound and free steroid fractions

Free steroid was removed from bound using 2.5% dextran-coated charcoal in TEDM buffer as described by Beato and Feigelson (1972) and Clark and Peck (1980). This contained 2.5% charcoal in TEDM buffer pH 7.5, with 0.25% Dextran T70®. Aliquots of 500 µL were added to each incubation tube, tubes were vortexed lightly and centrifuged at 1500 x g for 10 minutes at 4°C. Five hundred µL of the supernant was added to 10 mL Biofluor®. Liquid scintillation counting was done in a Mark III liquid scintillation counter Model 6880 (Searle Analytical Inc., Des Plaines, Il 60018). Tritium was quenched 35 to 47%. Data was recorded onto a microcomputer diskette using an Apple II-plus disc-operating system (Apple Computer Inc., Cupertino, CA 95014), either connected directly to the Mark III by a serial card interface or entered manually.

VI. Analysis of radioactivity

Free ligand concentration was determined by subtracting counts due to specific binding of [^{3}H]R1881 from total radioactivity added to each tube.
Bound radioactivity was the radioactivity in incubation tubes with radiolabeled ligand and triamcinolone acetonide only; non-specific bound was determined from the

radioactivity in incubation tubes with radiolabeled R1881, 100 fold molar excess ligand and triamcinolone acetonide. Specific bound was determined by subtracting non-specific bound from bound. Binding was expressed as fmol/mg protein, and was analysed using linear Scatchard plots (specific bound/free versus specific bound; Scatchard, 1949). Binding kinetics were expressed as apparent dissociation constant of equilibrium (K_d; nM) and apparent maximum number of binding sites or binding capacity (B_{max}; fmol/mg of protein).

VII. Statistics

ANOVA and Newman-Keul's Range test were used to determine significance of differences. The null hypothesis was assumed and the level of significance was $P < 0.05$.

RESULTS

I. The effects of ammonium sulfate fractionation of cytosol on the hepatic high affinity-low capacity androgen binding protein

1. The effect of ammonium sulfate fractionation on the Kd and binding capacity values of the high affinity-low capacity androgen binding protein

Ammonium sulfate fractionation was attempted in order to partially purify the HALC ABP. This was desirable to decrease non-specific binding and therefore to more readily see changes in the amount of HALC ABP in different physiological states such as diabetes. Preliminary experiments in which 50 % ammonium sulfate fractionation was used reliably resulted in linear Scatchard plots (r^2 = 0.80 or above) so to determine the percentage of ammonium sulfate required for purification, experiments using a range of concentrations were done. Also a two step procedure was used in which an initial precipitation with 33 % ammonium sulfate was discarded, and a second pellet, prepared by precipitation with either a further 17 or 32 % ammonium sulfate (final amount of ammonium sulfate 50 or 65 %), was resuspended and used in the incubation. Results from 33, 50, 65 % and from the two step procedure are in Table I.

Table I

THE EFFECT OF AMMONIUM SULFATE FRACTIONATION OF WHOLE HEPATIC CYTOSOL ON THE HIGH AFFINITY-LOW CAPACITY ANDROGEN BINDING PROTEIN ASSAY

Standard assay conditions were used except that different amounts of ammonium sulfate were used to partially purify the hepatic cytosol.

% Fractionation	Kd (nM)	Binding capacity (fmol/mg)	
Whole Cytosol	0.32 ± 0.04	5.86 ± 0.67	(17)
33	1.01 *	82.91 *	2/6 **
50	0.30 ± 0.02	10.37 ± 2.34	5/6 **
65	0.33 ± 0.07	6.40 ± 1.15	6/6 **
33-50	0.13	8.55	1/4 **
33-65	0.13 ± 0.07	4.30 ± 0.37	3/4 **

* Indicates values significantly different from all other values at the $P < 0.05$ level using ANOVA and Newman-Keul's Range test

** the denominator represents the number of assays done, the numerator, the number of linear Scatchard plots obtained

( ) = N

From the table it can be seen that 33 % fractionation did not reliably produce linear Scatchard plots, and produced a Kd significantly different from the Kd of the HALC ABP previously shown to have high androgen specificity in the whole cytosol. The two step fractionation procedure proved disappointing as it was rarely produced linear Scatchard plots. Fifty to sixty-five percent ammonium sulfate fractionation produced linear Scatchard plots reliably, and there were no major differences in Kd values compared to unfractionated cytosol. The decision to continue with a 50 % fractionation was based on this and other preliminary data collected, in which 50 % ammonium sulfate fractionation had been used, and had produced reliable and consistent results. Fifty percent ammonium sulfate fractionation resulted in a 2 to 3 fold purification based on increased binding capacity (tables I and III).

2. The presence of the glucocorticoid receptor in the 50 % ammonium sulfate fractionated hepatic cytosol of the adult male rat

Three experiments were done in which the necessity of adding triamcinolone acetonide to partially purified cytosol was examined. This was to determine whether or not the glucocorticoid receptor co-purified with the HALC ABP. A representative saturation binding curve and Scatchard plot

Figure 1

SATURATION CURVES AND SCATCHARD PLOTS FOR BINDING STUDIES OF THE HEPATIC CYTOSOLIC HIGH AFFINITY-LOW CAPACITY ANDROGEN BINDING PROTEIN IN 50 % AMMONIUM SULFATE FRACTIONATED CYTOSOL WITH AND WITHOUT TRIAMCINOLONE ACETONIDE

Adult (240-390 g) male rat hepatic cytosol was prepared and fractionated with 50 % ammonium sulfate as described in the Methods section and was incubated with varying concentrations of [^{3}H]R1881 (0.05-5 nM) with or without 100 fold molar excess R1881, and with (a) or without (b) 100 fold molar excess triamcinolone acetonide, for 3 hours at 4°C.

a: Scatchard analysis and, inset, saturation curve without triamcinolone acetonide

b: Scatchard analysis and, inset, saturation curve with triamcinolone acetonide

FIGURE 1a

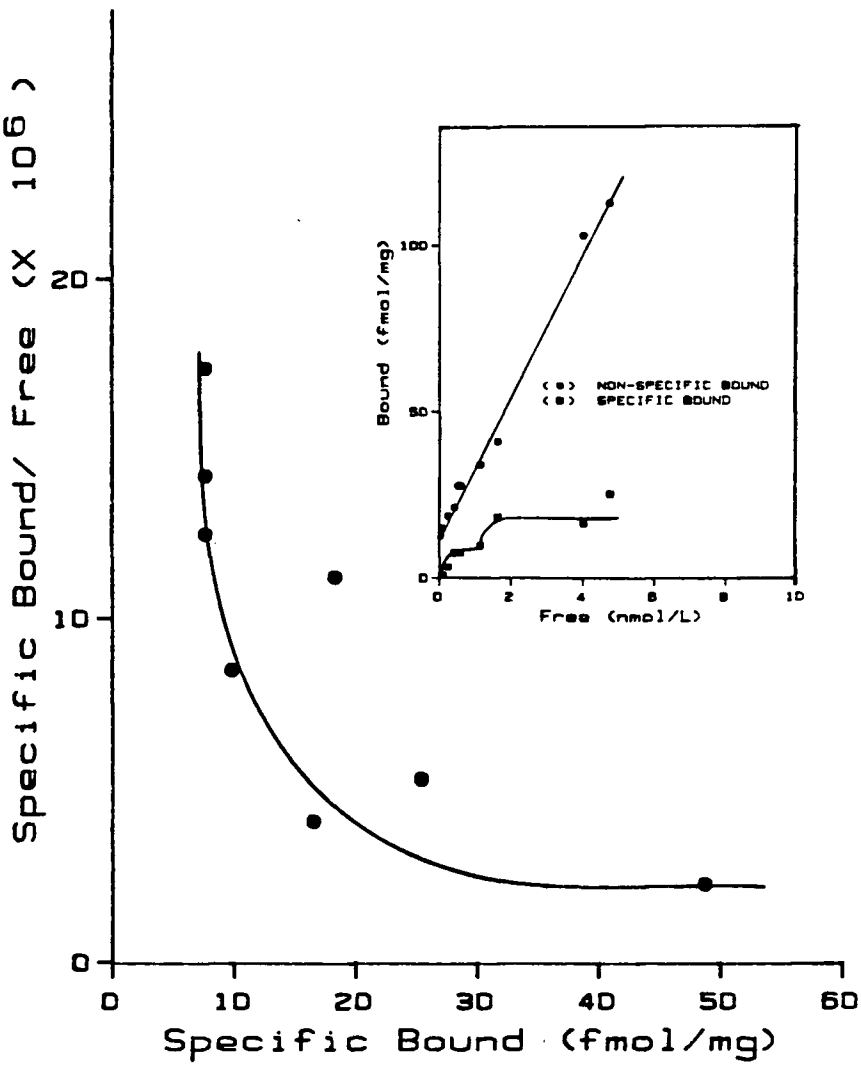
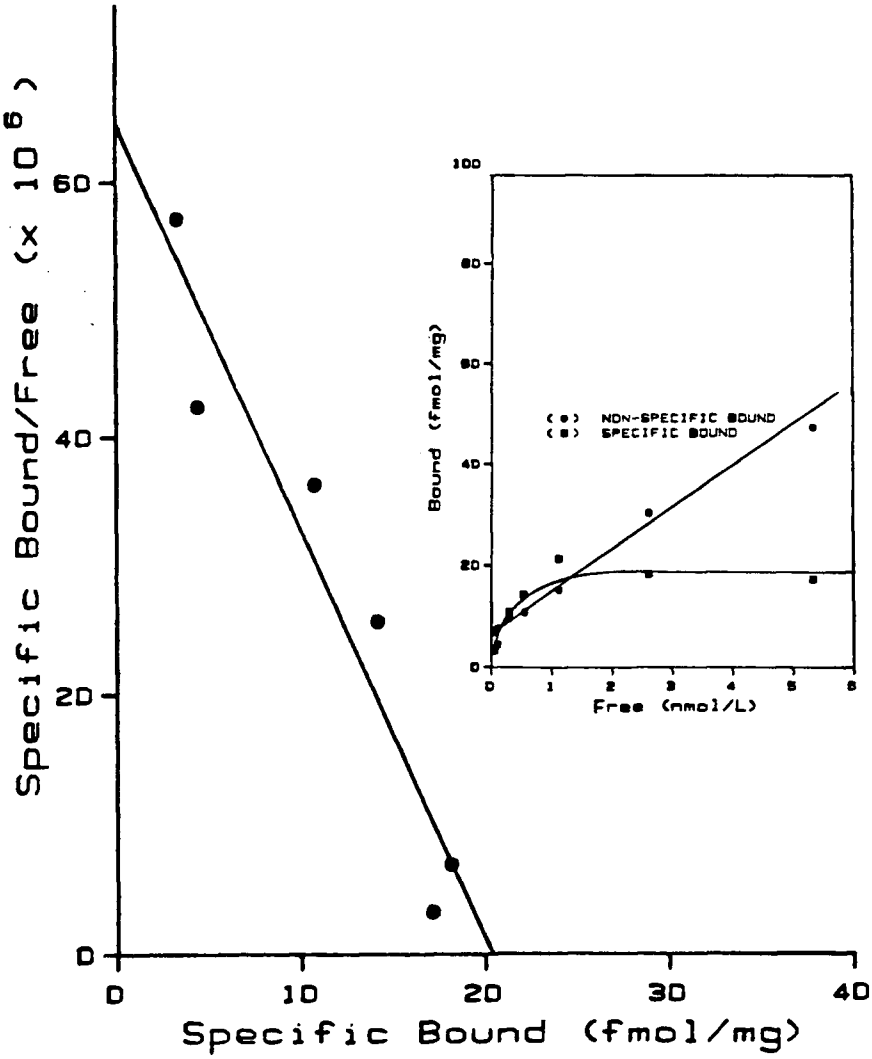

Specific Bound/ Free (X 106)
20
10
0
0
10
20
30
40
50
60
Specific Bound (fmol/mg)
Bound (fmol/mg)
100
50
0
0
2
4
6
8
10
Free (nmol/L)
(●) NON-SPECIFIC BOUND
(●) SPECIFIC BOUND

FIGURE 1 b


Specific Bound/Free (x 10 6)
60
40
20
0
0
10
20
30
40
Specific Bound (fmol/mg)
100
80
60
40
20
0
Bound (fmol/mg)
(●) NON-SPECIFIC BOUND
(■) SPECIFIC BOUND
0
1
2
3
4
5
6
Free (nmol/L)

is seen in figure 1, a and b. Evidence of 2 binding sites may be seen in both, therefore we continued the inclusion of triamcinolone in the incubation mixture.

3. The effect of 50 % ammonium sulfate fractionation on the optimum incubation time for the high affinity-low capacity androgen binding protein assay

In order to determine the optimum incubation time for the HALC ABP binding assay, experiments using whole and partially purified hepatic cytosol were conducted for time periods from 1.5 to 30 hours. A similar experiment was carried out with partially purfied hepatic cytosol from 10 day STZ diabetic rats. Results are shown in table II (1-3). Although there is some variation in the data, after 3 hours binding characteristics of the HALC ABP did not change significantly in whole or in fractionated hepatic cytosol.

II. The effect of the perfusate volume on the results of the high affinity-low capacity androgen binding protein assay

Early in our studies the suggestion that blood contamination of hepatic cytosol could produce artifactual results due to large amounts of non-specific polycyclic aromatic hydrocarbon binding proteins (Poellinger et al,

Table II

THE EFFECT OF INCUBATION TIMES FROM 1.5 TO 30 HOURS ON THE Kd AND BINDING CAPACITY VALUES OF THE HEPATIC CYTOSOLIC HIGH AFFINITY-LOW CAPACITY ANDROGEN BINDING PROTEIN

1. Whole cytosol from control rats

Standard assay conditions were used except that incubation times of 1.5 to 30 hours were used.

Time(hours)	Kd (nM)	Binding Capacity (fmol/mg)
1.5	1.07	6.67
3	0.17	1.97
6	0.09	2.43
9	0.23	2.76
12	0.16	2.64
15	0.16	2.29
18	0.09	7.10
21	0.12	3.43
24	0.35	5.25
30	0.08	2.23

Table II

2. 50 % ammonium sulfate fractionated cytosol from control rats

Time (hours)	Kd (nM)	Binding Capacity (fmol/mg)	N
1.5	0.46 ± 0.13	25.53 ± 6.61	5
3	0.30 ± 0.02	21.03 ± 1.49	43 *
6	0.29 ± 0.09	20.93 ± 7.43	5
9	0.42 ± 0.12	25.31 ± 12.50	5
18	0.23 ± 0.15	27.09 ± 6.04	2
21	0.17 ± 0.09	22.43 ± 1.56	2
24	0.10 ± 0.01	18.69 ± 3.86	2
30	0.23 ± 0.12	29.51 ± 11.78	2

Values are not significantly different from each other at ($P < 0.05$) using ANOVA and Newman-Keul's Range test.

* Untreated controls were assayed in experiments presented in the following tables: II, III, IV, V, VI, VII, and X.

Table II

3. 50 % ammonium sulfate fractionated cytosol from 10 day streptozotocin diabetic rats

Time (hours)	Kd (nM)	Binding Capacity (fmol/mg)
1.5	0.27	13.36
3	0.30	15.90
6	0.20	9.43
9	0.23	11.39
18	0.22	14.26
21	0.14	8.05
24	0.37	16.60
27	0.55	25.72
30	0.12	9.42

Table III

THE EFFECT OF DIFFERENT LIVER PERFUSION VOLUMES USED PRIOR TO HIGH AFFINITY-LOW CAPACITY ANDROGEN BINDING PROTEIN HALC ABP ASSAYS DONE IN WHOLE AND 50 % AMMONIUM SULFATE FRACTIONATED HEPATIC CYTOSOL

Livers were perfused with 10 or 100 mL of TEDM buffer, then standard assay conditions were used.

Type of Cytosol	Perfusion Volume (mL)	Kd (nM)	Binding Capacity (fmol/mg)	
whole	10	0.37	5.72	a
50 % ammonium sulfate fractionated	10	0.38	36.95	a
whole	100	0.31	4.93	b
50 % ammonium sulfate fractionated	100	0.05	3.95	b
whole	10	0.31	7.48	c
50 % ammonium sulfate fractionated	10	0.32	20.41	c
whole	100	0.49	10.16	d
50 % ammonium sulfate fractionated	100	0.37	29.41	d

Letters indicate samples taken from the same liver

1983). In order to investigate this possibility, experiments using livers perfused with 10 or 100 mL of TEDM buffer were done. Assays were performed on whole and partially purified cytosol. Results are presented in table III and show no major differences in Kd and binding capacity between samples homogenized from liver perfused with either 10 or 100 mL TEDM buffer. One 50 % ammonium sulfate fractionated sample showed a binding capacity much decreased from other ammonium sulfate purified cytosol volumes, however, this sample also exibited an unusual Kd value. Experiments in which blood was used in place of hepatic cytosol in the HALC ABP assay were also done. In 4 experiments no displacable binding was evident.

III. Effect of freezing on the high affinity-low capacity androgen binding protein

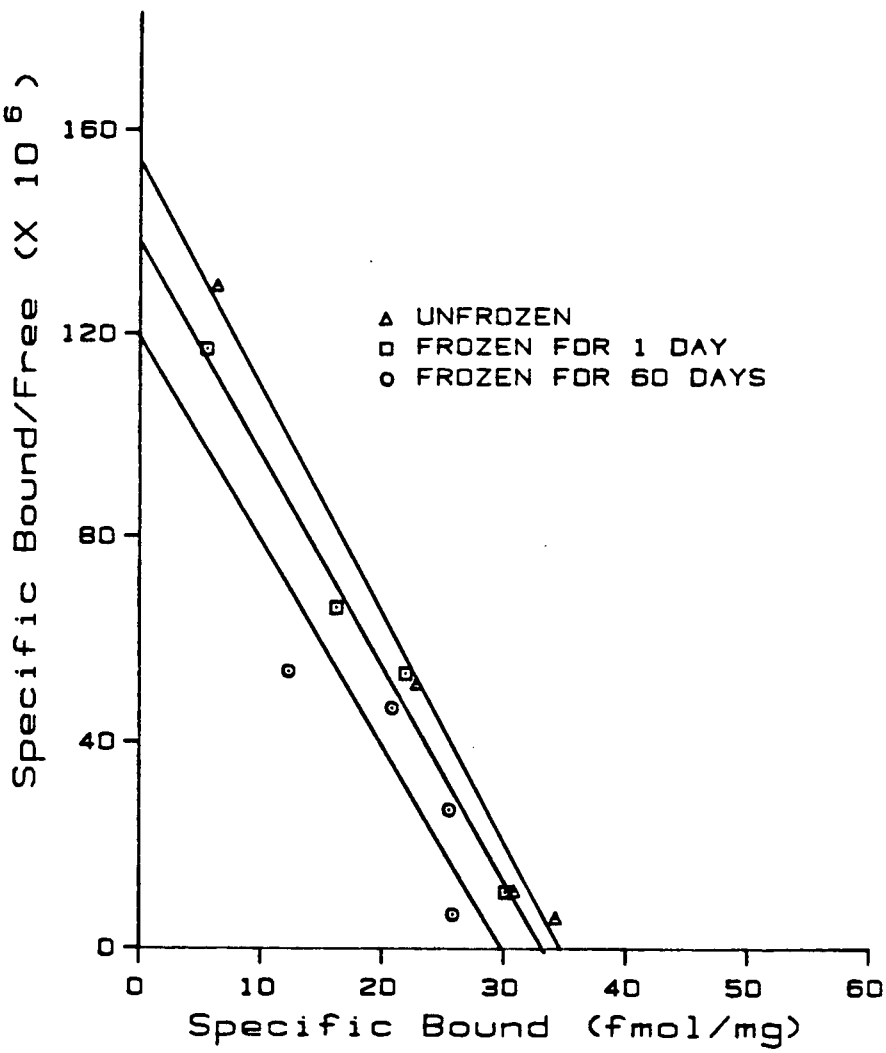
Preliminary studies were done to examine frozen storage of the HALC ABP. The HALC ABP could be preserved frozen at -80°C in the pellet prepared with 50 % ammonium sulfate. Three Scatchard plots constructed from data obtained from the partially purified hepatic cytosol of one animal, assayed at three time points: on the day of kill, and on one day and two months following are in figure 2. Kd values for 0, 1, and 60 days of storage are, respectively, 0.15, 0.15, and 0.25 nM; values for binding capacity at the same time

Figure 2

SCATCHARD PLOT OF THE HEPATIC CYTOSOLIC HIGH AFFINITY-LOW CAPACITY ANDROGEN BINDING PROTEIN FROM FROZEN 50 % AMMONIUM SULFATE PELLET

Adult (240-390 g) male rat hepatic cytosol fractionated with 50 % ammonium sulfate (0.05-1.0 mg/mL) was prepared to the ammonium sulfate pellet stage, as described in the Methods section. The ammonium sulfate pellet was quick frozen in dry ice-acetone, then stored at -80°C until use. Storage time was either 0, 1, or 60 days. The resuspended pellet was incubated with varying concentrations of tritiated R1881 (0.05-5 nM), with or without 100 fold molar excess R1881, and with 100 fold molar excess triamcinolone acetonide for 3 hours at 4°C.

FIGURE 2


Specific Bound/Free (X 10 6)
160
120
80
40
0
0
10
20
30
40
50
60
Specific Bound (fmol/mg)
UNFROZEN
FROZEN FOR 1 DAY
FROZEN FOR 60 DAYS

points are, respectively, 33.3, 30.6, and 30.0 fmol/mg protein. Essentially no changes in Kd or in binding capacity were seen in our assay.

IV. Age and sex differences in the presence of the hepatic cytosolic high affinity-low capacity androgen binding protein in rats

1. In mature female rats

The results of experiments done in mature female Wistar rats are in table IV. There was less than one-half the amount of hepatic cytosolic HALC ABP in female as in male rats, when 100 fold molar excess triamcinolone acetonide was used in both. However, this may not be a correct interpretation because when triamcinolone acetonide was increased to 500 fold molar excess, there was an apparent, although not statistically significant, decrease in binding capacity in females, but no change in the binding capacity of males. There is a significant difference in the Kd value between the HALC ABP of males and females with 100 versus 500 fold molar excess triamcinolone acetonide, and a significant difference between the Kd of the HALC ABP males versus females with 100 or 500 fold molar excess triamcinlone acetonide.

Table IV

THE EFFECT OF INCREASING AMOUNTS OF TRIAMCINOLONE ACETONIDE IN ASSAYS OF MALE AND FEMALE RAT HEPATIC CYTOSOLIC HIGH AFFINITY-LOW CAPACITY ANDROGEN BINDING PROTEIN

Standard assay conditions were used with male and female hepatic cytosol. Different amounts of triamcinolone acetonide were included in the incubation mixture.

Animal Model		Kd (nM)	Binding Capacity (fmol/mg)	N
sex	amount of triamcinolone molar excess			
male	100 fold	0.30 ± 0.02a	20.76 ± 1.49d	43
male	500 fold	0.48 ± 0.10b	16.65 ± 6.91d	5
male	1000 fold	0.62	19.08	1/2 *
female	0	0.37	16.00	1/8 *
female	100 fold	0.15 ± 0.03c	7.04 ± 0.94e	14
female	500 fold	0.21 ± 0.03a,c	3.00 ± 0.13e	8
female	1000 fold	0.04	2.05	1/6 *

Similar letters indicate no significant difference at $P < 0.05$ level using ANOVA and Newman-Keul's range test.

* the denominator indicates the number of hepatic HALC ABP assays done, the numerator indicates the number which produced linear Scatchard plots

2. In mature male and female rats

Experiments in which pools of liver from immature male and female rats were used showed equal binding capacity HALC ABP in both. This binding capacity is similar to the that of the mature female (see table V). Experiments using increasing amounts of triamcinolone acetonide were not done in immature male or female rats.

3. In castrated male rats

Table VI shows the results of HALC ABP assays done on castrated mature males one week following castration. There is no significant difference according to ANOVA and Newman-Keul's Range test in either Kd or binding capacity values between control, sham and castrated mature males.

Neonatal castrations were performed on male rats which were then allowed to grow to maturity. Although specific binding was still present in neonatally castrated rats, Scatchard plots were obtained for only 2 of 6 rats, and those had very low binding capacities (3.74 and 2.76 fmol/mg protein). Rats castrated neonatally, then treated with testosterone enanthate in a dose of 17 mmol/kg at day 8 (Sloop et al, 1983) showed low binding capacities (5.81 $\pm$ 2.04 fmol/mg), but consistent Scatchard plots.

Table V

THE HEPATIC HIGH AFFINITY-LOW CAPACITY ANDROGEN BINDING PROTEIN CONTENT OF MATURE AND IMMATURE MALE AND FEMALE RATS

Standard assay conditions were used with hepatic cytosol from Adult (240-390 g) male, adult (200-260 g) female and and immature (30-40 g) male and female rat.

Animal Model	Kd (nM)	Binding Capacity (fmol/mg)	N
mature male	0.30 ± 0.02a	20.76 ± 1.49c	43
immature male	0.10	9.45	pool 1
	0.15	5.40	pool 2
mature female	0.15 ± 0.03b	7.04 ± 0.94d	14
immature female	0.40	9.20	pool 1
	0.07	9.57	pool 2

Similar letters indicate no significant difference ($P < 0.05$) using ANOVA and Newman-Keul's range test

Table VI

THE EFFECT OF CASTRATION ON THE PRESENCE OF THE HEPATIC CYTOSOLIC HIGH AFFINITY-LOW CAPACITY ANDROGEN BINDING PROTEINS IN MALE RATS

Standard assay conditions were used with hepatic cytosol from castrated adult (240-390 g) male rats, sham castrated adult male rats, neonatally castrated adult male rats, and neonatally castrated testosteone treated (17 mmol/kg, day 8) adult male rats.

Animal Model	Kd (nM)	Binding Capacity (fmol/mg)	
male	0.30 ± 0.02	20.76 ± 1.49 (43)	
mature sham castrated male	0.18 ± 0.04	10.90 ± 4.10 (4)	
mature castrated male	0.33 ± 0.08	11.85 ± 2.73 (6)	
neonatally castrated male	0.77	3.74	2/6 *
neonatally castrated male	0.10	2.76	
neonatally castrated testosterone treated male	0.06 ± 0.02	5.81 ± 2.04	3/3 *

( ) = N

* the denominator represents total number of assays done, the numerator, the number of assays resulting in linear Scatchard plots

ANOVA and Newman-Keul's range test showed no significant difference between male and castrated male Kd and binding capacities. Statistics were not done on neonatal castrated rats because of the low N value.

V. Presence of the high affinity-low capacity androgen binding protein in the hepatic cytosol of genetically diabetic, BioBreeding strain, rats

During this study it was planned that genetically diabetic rats (BioBreeding strain) would be compared with STZ induced diabetics. Limited access due to colony problems resulted in the assay of only three male BioBreeding strain diabetics and five male BioBreeding non-overtly diabetic littermate controls. Presence of diabetes was determined by the presence of glucosuria. Results from these experiments are in table VII. No differences were seen in Kd values. The binding capacity of non-diabetic and diabetic BioBreeding strain rats were significantly reduced from Wistar control values. No significant difference was seen in binding capacity between diabetic and non-diabetic BioBreeding strain rats. It would be worthwhile to increase numbers of animals in order to verify whether an increased number would prove the apparent difference significant.

Table VII

BINDING PARAMETERS IN BIOBREEDING STRAIN DIABETIC, AND BIOBREEDING STRAIN NON-DIABETIC MALE RATS COMPARED TO CONTROL ADULT MALE RATS

Standard assay conditions were used with hepatic cytosol from Adult male BioBreeding strain diabetic (280-320 g), BioBreeding-strain non-diabetic (390-440 g) and Wistar control rat (240-390 g)

Animal Model	Kd (nM)	Binding Capacity (fmol/mg)
Wistar control	0.30 ± 0.02	20.76 ± 1.49 a (43)
BioBreeding strain internal control	0.36 ± 0.04	11.57 ± 2.23 b (5)
BioBreeding strain diabetic	0.49 ± 0.32	4.58 ± 0.73 b (3)

( ) = N

Similar letters indicate no significant difference at the $P < 0.05$ level using ANOVA and Newman-Keul's Range test

VI. Effects of streptozotocin induced diabetes on the Kd values, binding capacity, temperature stability, and steroid specificity of the high affinity-low capacity androgen binding protein

1. High affinity-low capacity androgen binding protein binding studies in control, 4 and 10 day streptozotocin diabetic rats

Preliminary studies in our laboratory (Sunahara, 1984) showed that a decrease in binding capacity of the HALC ABP was seen in the liver of rats four days after induction of diabetes with STZ. In order to determine if there were other changes in the HALC ABP the following series of studies were done. Binding studies using partially purified hepatic cytosol from control, 4 and 10 day STZ diabetic rats were done. Results of these experiments may be seen in table VIII and figures 3 a-c, and 4. No significant differences in Kd values were seen. However, there was a significant decrease in the binding capacity between either untreated control or citrated treated control rats and 4 or 10 day STZ diabetics.

Figure 3

SATURATION CURVES FOR BINDING STUDIES OF HEPATIC CYTOSOLIC HIGH AFFINITY-LOW CAPACITY FROM CONTROL, 4 AND 10 DAY STREPTOZOTOCIN DIABETIC ADULT MALE RATS

Adult (240-390 g) male rat hepatic cytosol, fractionated with 50 % ammonium sulfate (0.5-1.0 mg/mL) from (a) control, (b) 4 and (c) 10 day STZ diabetic rats was prepared as described in the Methods section and was incubated with varying concentrations of tritiated R1881 (0.05-5 nM), with or without 100 fold molar excess R1881, and with 100 fold molar excess triamcinolone acetonide for 3 hours at 4°C.

FIGURE 3 a

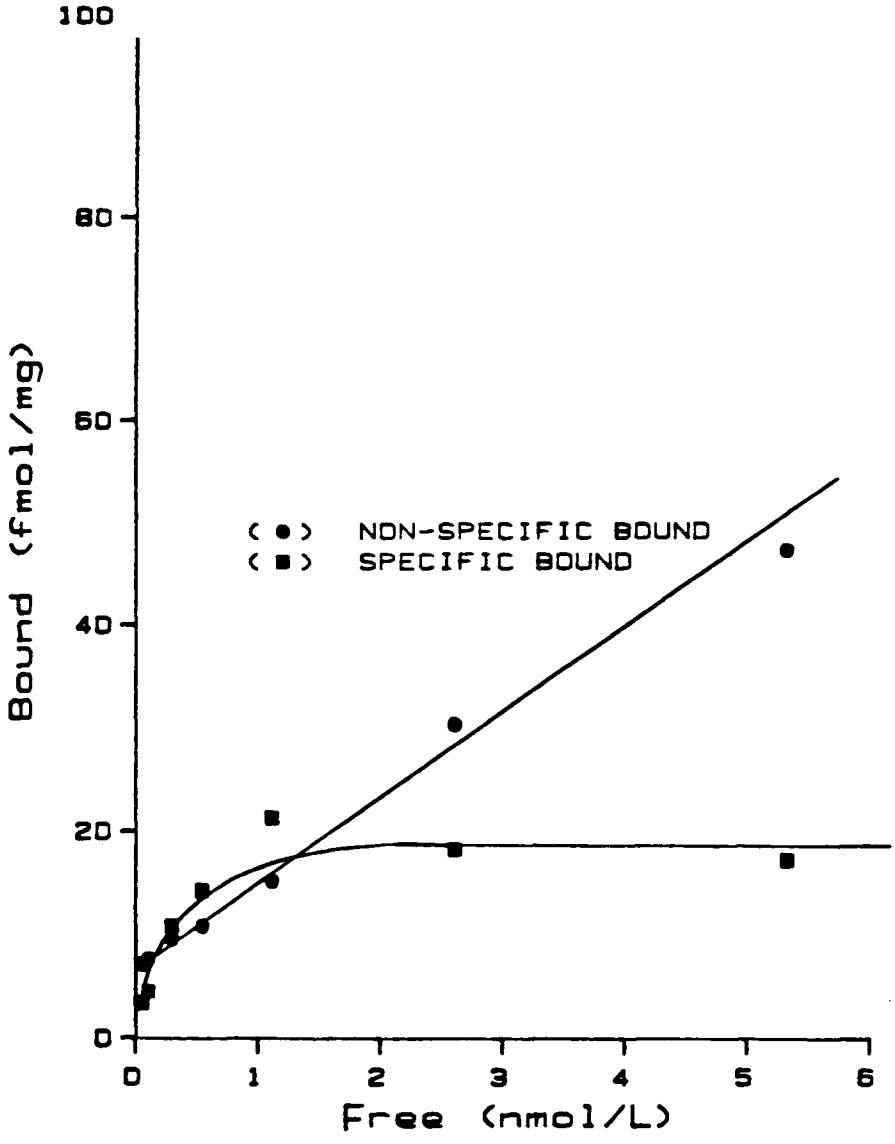

100
80
60
40
20
0
Bound (fmol/mg)
(●) NON-SPECIFIC BOUND
(■) SPECIFIC BOUND
0
1
2
3
4
5
6
Free (nmol/L)

FIGURE 3 b

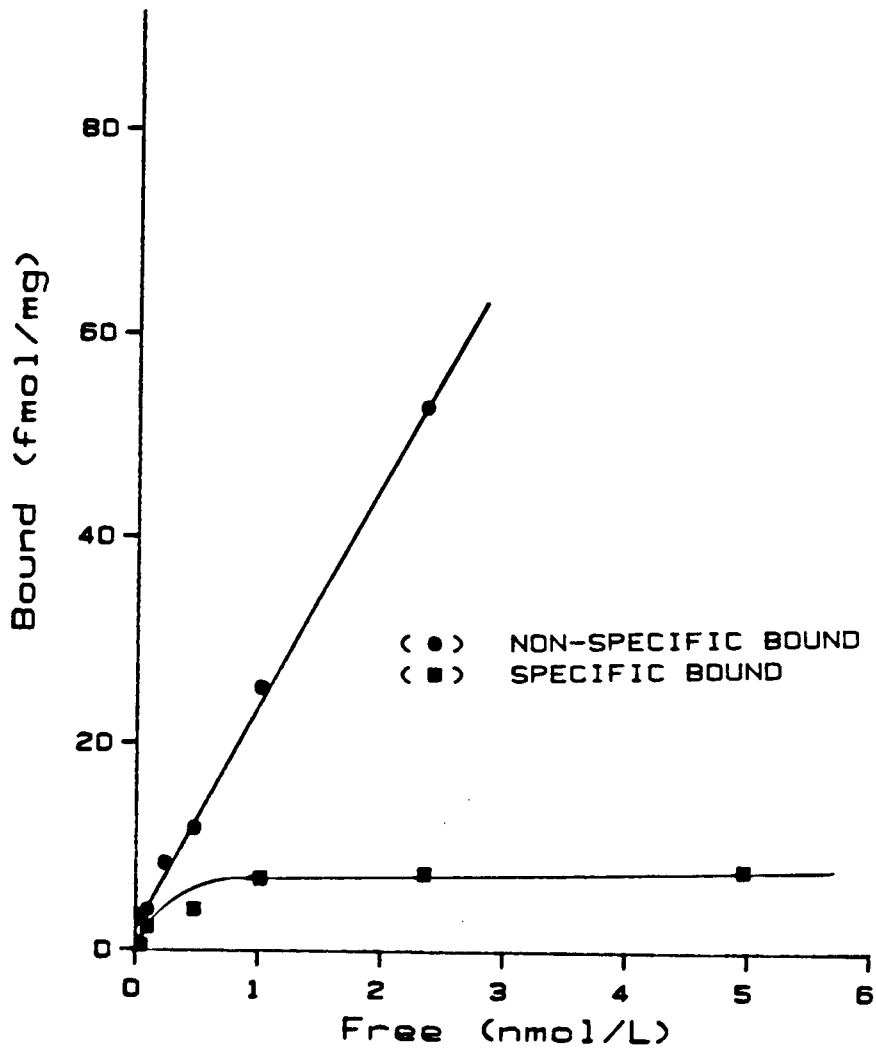

Bound (fmol/mg)
80
60
40
20
0
0
1
2
3
4
5
6
Free (nmol/L)
(●) NON-SPECIFIC BOUND
(■) SPECIFIC BOUND

FIGURE 3 c

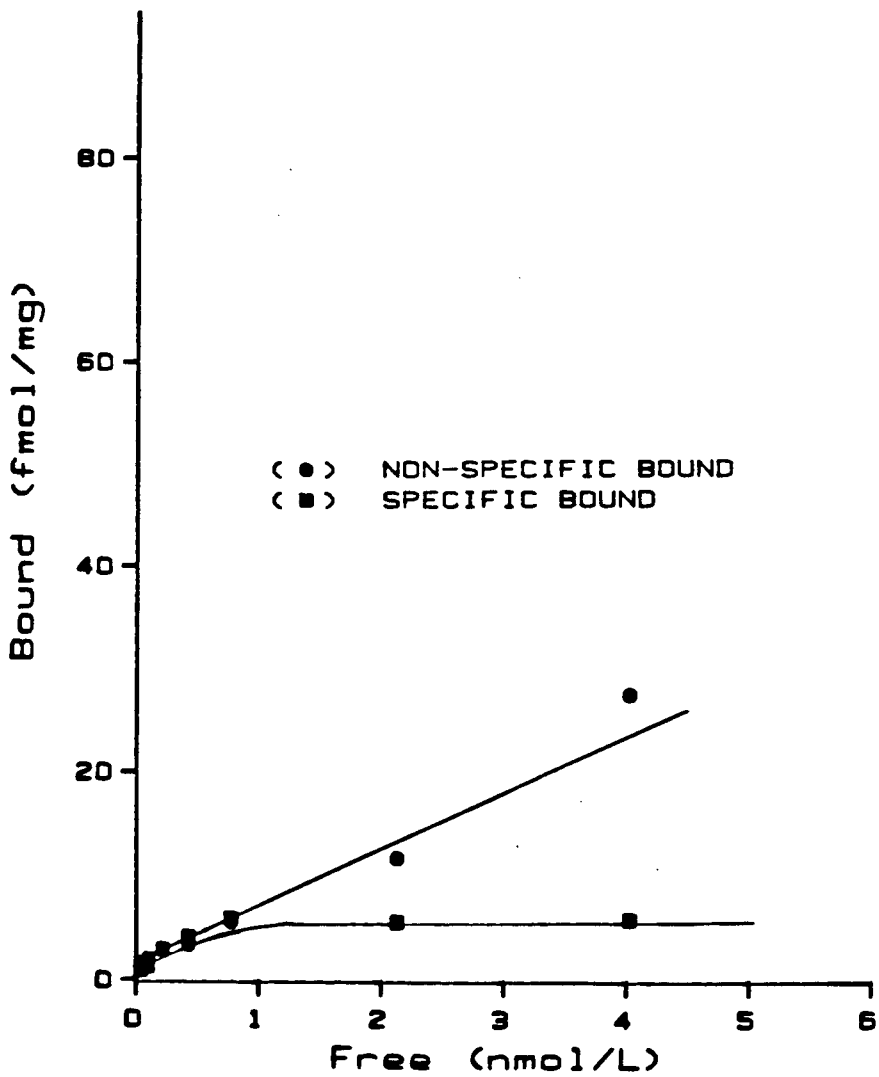

80
60
40
20
0
Bound (fmol/mg)
(●) NON-SPECIFIC BOUND
(■) SPECIFIC BOUND
0
1
2
3
4
5
6
Free (nmol/L)

Figure 4

SCATCHARD PLOT FOR BINDING STUDIES OF THE HEPATIC CYTOSOLIC HIGH AFFINITY-LOW CAPACITY ANDROGEN BINDING PROTEIN IN CONTROL, 4 AND 10 DAY STREPTOZOTOCIN DIABETIC ADULT MALE RATS

Data presented in a saturation plot in figure 3 was transformed and is presented in a Scatchard plot. Binding capacity and Kd values were determined by linear regression analysis of data.

FIGURE 4

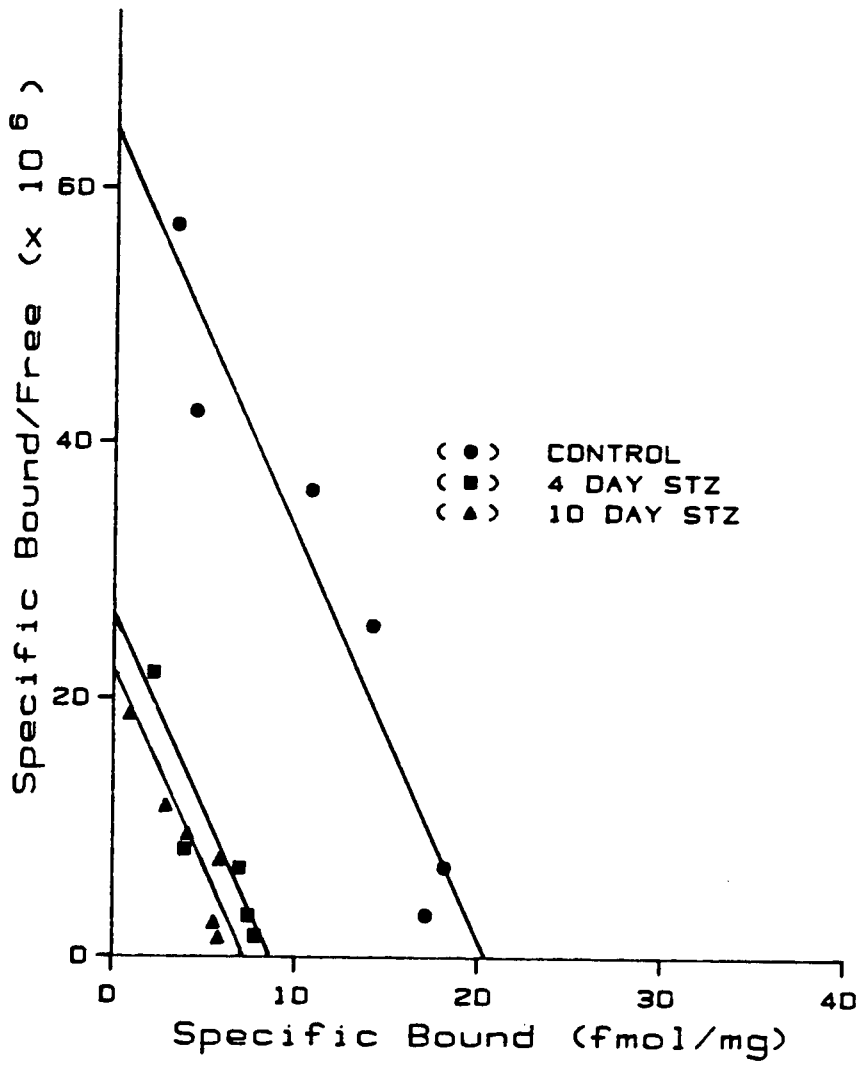

Specific Bound/Free (x 10 6)
60
40
20
0
0
10
20
30
40
Specific Bound (fmol/mg)
(●) CONTROL
(■) 4 DAY STZ
(▲) 10 DAY STZ

Table VIII

THE EFFECT OF 4 AND 10 DAY STZ DIABETES ON THE BINDING PARAMETERS OF THE HEPATIC CYTOSOLIC HIGH AFFINITY-LOW CAPACITY ANDROGEN BINDING PROTEIN

Standard assay conditions were used with hepatic cytosol from untreated control, citrate buffer injected, and 4 and 10 day STZ diabetic adult male rats (240-390 g).

Animal Model	Kd (nM)	Binding Capacity (fmol/mg)	N
Control	0.30 ± 0.02	20.76 ± 1.49	43
Citrate control	0.33 ± 0.04	22.51 ± 2.48	26
4 Day STZ	0.31 ± 0.05	9.71 ± 1.22 *	22
10 Day STZ	0.36 ± 0.04	8.31 ± 1.21 *	23

* Significantly different from control ($P < 0.05$) using ANOVA and Newman-Keul's range test

Table IX

THE EFFECT OF 4 AND 10 DAY STREPTOZOTOCIN DIABETES ON THE TEMPERATURE STABILITY OF THE HEPATIC CYTOSOLIC HIGH AFFINITY-LOW CAPACITY ANDROGEN BINDING PROTEIN OF ADULT MALE RATS

Standard assay conditions were used, except that the 50 % ammonium sulfate fractionated cytosol was preincubated at 24°C for 0 to 6 hours before the start of the 3 hour 4°C incubation.

1. Control *versus* 4 day STZ diabetic

Time (hours)	Kd (nM)		Binding Capacity (fmol/mg)	
	Control	STZ diabetic (4 day)	Control	STZ diabetic (4 day)
0	0.19	0.14	10.63	10.07
0.5	0.34	0.08	7.10	4.95
1.5	0.17	0.08	1.47	1.68
3.0	NS	NS	NS	NS
6.0	NS	NS	NS	NS

NS denotes no Scatchard plot

TABLE IX

2. Control versus 10 day STZ diabetic

Time (hours)	Kd (nM)		Binding capacity (fmol/mg)	
	Control	STZ diabetic	Control	STZ diabetic (10 day)
0	0.22	0.13	23.40	4.38
0.5	0.24	0.10	19.36	4.21
1.5	0.35	0.53	8.15	3.92
3.0	NS	NS	NS	NS
6.0	NS	NS	NS	NS

NS denotes no scatchard plot

2. Temperature stability of the high affinity-low capacity androgen binding protein

The lower binding capacity of the HALC ABP seen in STZ diabetic hepatic cytosol may simply be due to decreased stability of the HALC ABP prepared from the STZ diabetic animal. In order to test this possibility partially purified cytosol from control and diabetic rat liver was allowed to stand, at 24°C in a water bath for 0, 0.5, 1.5, 3 and 6 hours before the start of the incubation to determine the relative stability of the HALC ABP. this experiment was done using hepatic cytosol from four and ten day diabetic rats. Results are in tables IX, 1 and 2. The Kd showed no change in this experiment. The binding capacity decreased as time of preincubation at 24°C increased. There appears to be no difference in the rate of degradation between the HALC ABP prepared from control and 4 day STZ diabetic rat liver. There is no apparent change in the binding capacity of the HALC ABP prepared from the liver of the 10 day STZ diabetic rat, until at 3 hours it is no longer detectable.

3. Effect of the induction of streptozotocin diabetes on the specificity of the high affinity-low capacity androgen binding protein

In order to determine whether STZ diabetes had an effect

on specificity of the HALC ABP binding, inhibition studies with steroidal and non-steroidal competitors in control and STZ diabetics were undertaken. Data was plotted as percent total bound remaining versus free competitor added and as percent specific bound remaining versus free competitor added. IC_{50} values were determined from a logit (percent specific bound remaining) versus log (free) plot, and by using the least squares method of linear regression. Table X, 1 and 2, and figure 5, a and b summarize the results of these experiments. The order of affinity of competitors was R1881 = 5α-DHT > progesterone > testosterone enanthate = estradiol > androstenedione. 5β-DHT and diethylstilbesterol produced insignificant displacement of R1881 from specific binding sites. Results were generally poorer (large standard errors) in diabetics but showed a similar pattern of affinity.

VII. Effect of hormone replacement on the hepatic cytosolic high affinity-low capacity androgen binding protein

1. Effects of once daily admininstration of protamine zinc insulin and twice daily administration of Toronto insulin

Ortiz-Cara et al (1984) showed restoration of the pattern of secretion and amount of thyrotropin stimulating

Table X

THE EFFECT OF 10 DAY STREPTOZOTOCIN DIABETES ON THE SPECIFICITY OF THE HEPATIC CYTOSOLIC HIGH AFFINITY-LOW CAPACITY ANDROGEN BINDING PROTEIN OF MALE RATS

1. Control rats

Adult (240-390 g) male Wistar rat hepatic cytosol (0.5-1.5 mg protein/mL) was prepared and incubations conducted as described in the Methods section. IC_{50} values were determined using a logit-log plot and the least squares method of linear regression. Experiments were conducted several times, therefore data is expressed as average ± SEM.

Competitors	IC_{50} (nM)	N
Androgens		
R1881	0.45 ± 0.16 a	10
5α-DHT	1.42 ± 0.40 a	6
Testosterone enanthate	56.60 ± 15.24 b,c	4
Androstenedione	83.14 ± 24.48 c	7
5β-DHT	*	3
Estrogens		
17β-Estradiol	56.7 ± 5.44 b,c	5
Diethylstilbesterol	*	6
Progestins		
Progesterone	2.40 ± 7.88 a,b	7

* although some displacement occurred, it was not consistent and did not increase with increasing concentrations of competitor
Similar letters or combinations of letters indicate no significant difference at $p < 0.05$ level using ANOVA and Newman Keuls range test

Table X

2. Ten day STZ diabetic rats

Adult (240-390 g) male Wistar rat hepatic cytosol (0.5-1.5 mg protein/mL) was prepared and incubations conducted as described in the Methods section. IC_{50} values were determined using a logit-log plot and the least squared method of linear regression. Experiments were conducted several times, therefore data is expressed as average ± SEM.

Competitor	IC_{50} nM	N
Androgens		
R1881	0.18 ± 0.17 a	5
5α-DHT	1.66 ± 1.28	3
Testosterone enanthate	167.25 ± 82.18 b	4
5β-DHT	*	6
Estrogens		
17β-Estradiol	111.00 ± 66.78	4
Progestins		
Progesterone	37.45 ± 13.91	7

* although some displacement occurred, it was not consistent and did not increase with increasing concentrations of competitor

Similar letters indicate no significant differences at the $P < 0.05$ level using to ANOVA and Newman-Keul's range test
No letters indicate values are not significantly different from a or b

Figure 5

SPECIFICITY STUDIES OF THE HIGH AFFINITY-LOW CAPACITY ANDROGEN BINDING PROTEIN IN HEPATIC CYTOSOL FROM CONTROL AND 10 DAY STREPTOZOTOCIN DIABETIC ADULT MALE RATS

Adult (240-390 g) male Wistar rat hepatic cytosol (0.5 to 1.5 mg/mL) was incubated with 1 nM of tritiated R1881 and 100 fold molar excess of triamcinolone acetonide in the presence of increasing concentration of several steroidal and non-steroidal competitors, for 3 hours at 4°C. Percentage of specific binding was calculated as the fraction of R1881 displaced relative to total R1881 bound multiplied by 100. This value was plotted as a function of the logarithm of the free concentration of the competitor present in the incubation mixture. Figure a shows typical tritiated-R1881 competitor curves for R1881, testosterone enanthate, 5β-DHT, androstenedione, 5α-DHT, estradiol, diethylstilbesterol, and progesterone in control adult male Wistar rats. Figure b shows typical tritiated R1881 competitor curves for R1881, 5α-DHT, testosterone enanthate, 5β-DHT, estradiol, and progesterone in 10 day STZ diabetic adult male Wistar rats.

FIGURE 5 a

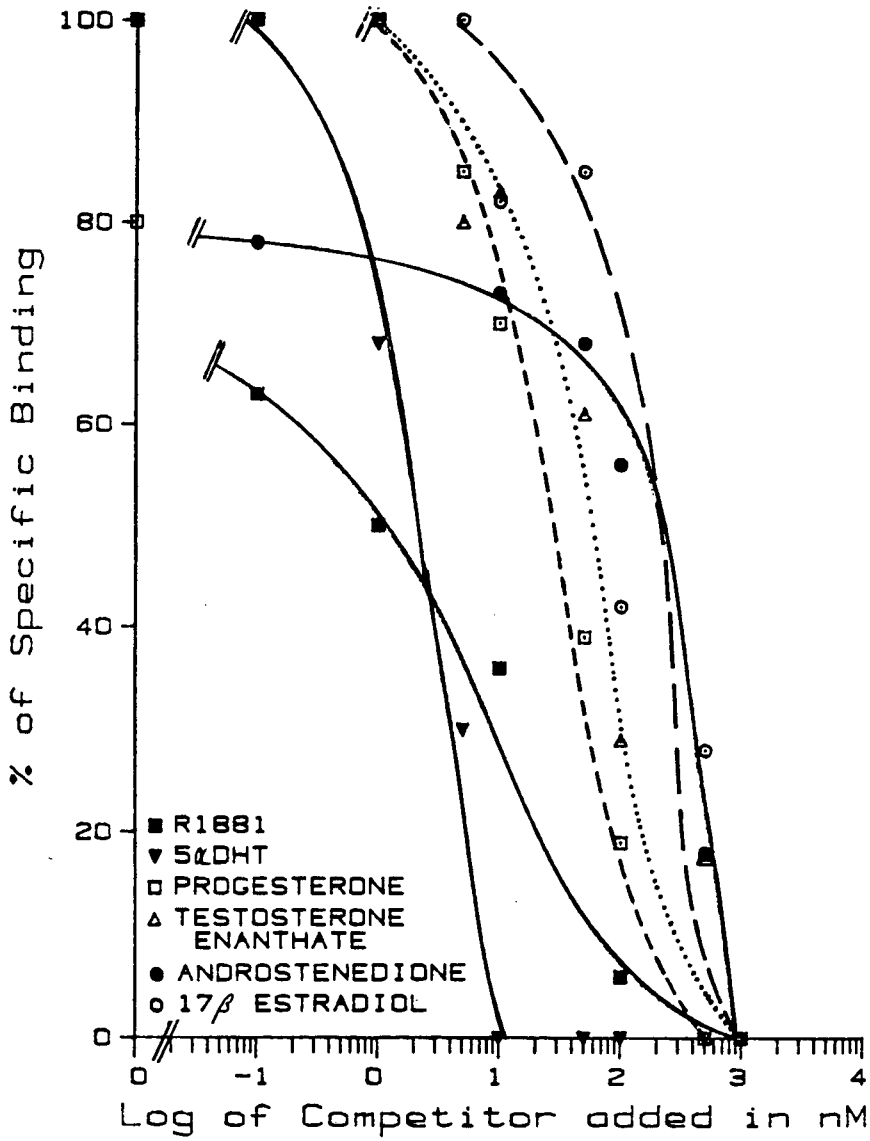

100
80
60
40
20
0
% of Specific Binding
R1881
5αDHT
PROGESTERONE
TESTOSTERONE ENANTHATE
ANDROSTENEDIONE
17β ESTRADIOL
0
-1
0
1
2
3
4
Log of Competitor added in nM

FIGURE 5 b

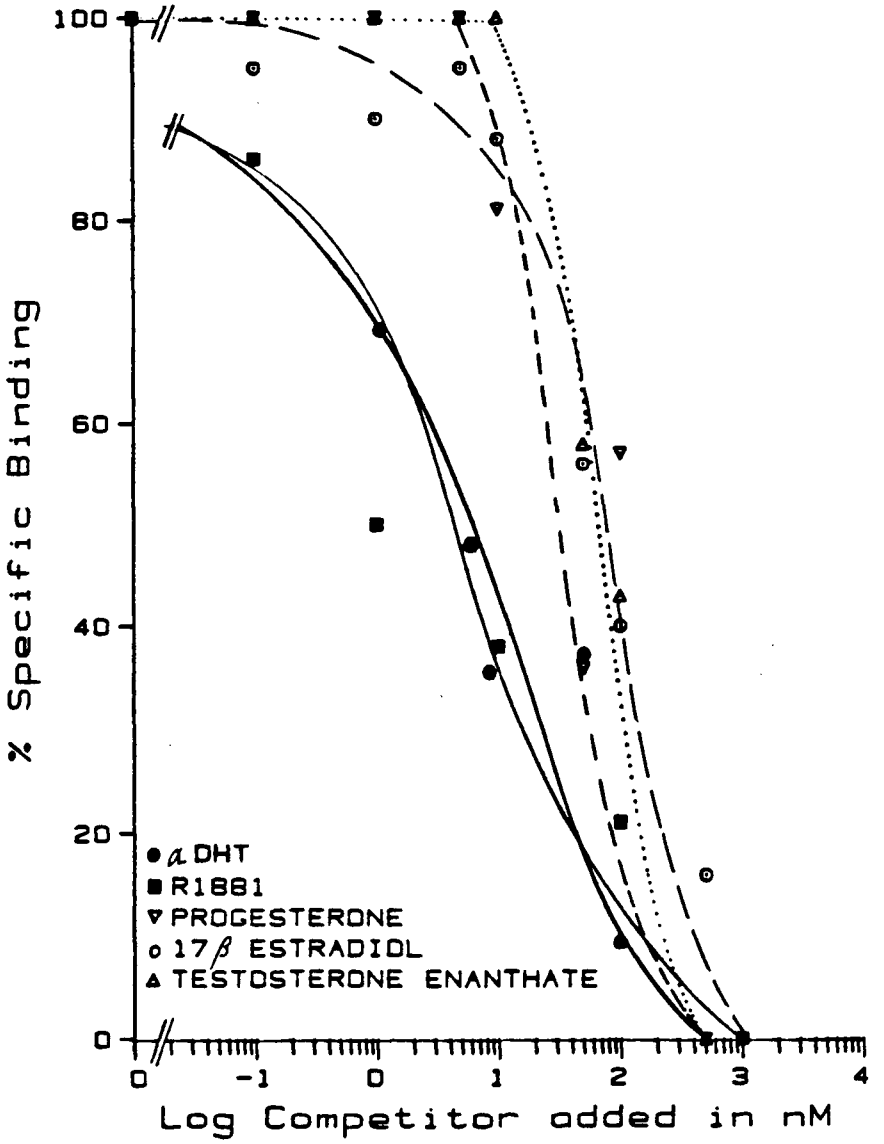

100
80
60
40
20
0
% Specific Binding
● α DHT
■ R1881
▼ PROGESTERONE
o 17β ESTRADIOL
▲ TESTOSTERONE ENANTHATE
0
-1
0
1
2
3
4
Log Competitor added in nM

Table XI

THE EFFECT OF TREATMENT WITH REGULAR (TORONTO) AND PROTAMINE ZINC INSULIN ON THE HEPATIC CYTOSOLIC HIGH AFFINITY-LOW CAPACITY ANDROGEN BINDING PROTEIN OF 4 DAY STREPTOZOTOCIN DIABETIC MALE RATS

Adult (240-390 g) male rats were treated s.c. with regular (Toronto) insulin (15 U/kg, twice a day at 0830 and 1700) or with PZI (10 U/kg, once a day). Treatment was started at the time of induction of diabetes with STZ. Standard assay conditions were used.

Animal Model	Kd (nM)	Binding Capacity (fmol/mg)	N
Citrate control	0.33 ± 0.04	22.51 ± 2.48	26
4 Day STZ	0.31 ± 0.05	9.71 ± 1.22 *	22
4 Day STZ + PZI	0.60 ± 0.19	11.56 ± 3.72 *	4
4 Day STZ + Toronto Insulin	0.28 ± 0.06	6.66 ± 1.30 *	8

* Indicates significant difference from control at $P < 0.05$ level using ANOVA and Newman-Keul's Range test

hormone, triiodothyronine, and thyroxine, when regular insulin was administered twice a day, in a dose of 15 U/kg, to male Wistar rats. We attempted to restore the HALC ABP using the same regimen. The results of this study are shown in table XI. Toronto insulin administered twice daily had no restorative effect on the HALC ABP. PZI (10 U/kg) administration once daily also was ineffective in restoring HALC ABP levels. Testape® results were negative for insulin treated diabetics.

2. Effects of testosterone enanthate and testosterone enanthate with protamine zinc insulin treatment

No change in Kd values in either testosterone enanthate (1 mg/kg) or testosterone (1 mg/kg) and PZI (10 U/kg) treated 4 day STZ diabetic rats was found (Table XII). The decreased binding capacity was not reversed in testosterone enanthate treated 4 day STZ diabetic rats. Treatment with testosterone enanthate and PZI resulted in a partial restoration of the binding capacity of the HALC ABP to an intermediate value, not statistically different from either control or STZ diabetics.

3. Effects of triiodothyronine treatment

In an attempt to restore control levels of the binding

Table XII

THE EFFECT OF TREATMENT OF 4 DAY STREPTOZOTOCIN DIABETIC MALE RATS WITH TESTOSTERONE ENANTHATE, OR TESTOSTERONE ENANTHATE WITH PROTAMINE ZINC INSULIN ON THE HEPATIC CYTOSOLIC HIGH AFFINITY-LOW CAPACITY ANDROGEN BINDING PROTEIN

Adult (240-390 g) male STZ diabetic rats were treated subcutaneously with testosterone enanthate (1 mg/kg subcutaneously once daily) or with testosterone enanthate and and protamine zinc insulin (10 U/kg, once daily). Treatment was started at induction of diabetes with STZ. Standard incubation conditions were used.

Animal Model	Kd (nM)	Binding Capacity (fmol/mg)	N
Citrate control	0.33 ± 0.04	22.51 ± 2.48 a	26
4 Day STZ	0.31 ± 0.05	9.71 ± 1.22 b	22
4 Day STZ + testosterone enanthate	0.41 ± 0.07	10.05 ± 1.79 b	8
4 Day STZ + testosterone enanthate and ovine growth hormone	0.45 ± 0.07	15.70 ± 2.91	11

Similar letters indicate values which are not significantly different at the $P < 0.05$ level using ANOVA and Newman-Keul's Range test

Table XIII

THE EFFECT OF TREATMENT WITH TRIIODOTHYRONINE ON THE HEPATIC CYTOSOLIC HIGH AFFINITY-LOW CAPACITY ANDROGEN BINDING PROTEIN OF 4 DAY STREPTOZOTOCIN STZ DIABETIC MALE RATS

Hepatic cytosol (0.5-1.5 mg protein/mL) from control and four day STZ and four day STZ diabetic rats treated with triiodothyronine adult male rats (240-390 g) was assayed according to standard assay conditions.

Animal Model	Kd (nM)	Binding Capacity (fmol/mg)		N
Citrate control	0.33 ± 0.04	22.51 ± 2.48		26
4 Day STZ	0.31 ± 0.05	9.71 ± 1.22	*	22
4 Day STZ + triiodothyronine	0.12 ± 0.01	9.49 ± 1.97	*	6

* Indicates signifigant differences at the $P < 0.05$ level using ANOVA and Newman-Keul's Range test

capacity of HALC ABP, triiodothyronine was administered to STZ diabetic rats. The dose of triiodothyronine used was 3 μg per 100 g daily. This dose has been shown to restore normal thyroid index in STZ diabetic male Wistar rats (Tahaliani, 1983). Results of the study may be seen in table XIII; the hormone treatment had no effect on Kd or binding capacity values.

4. Effects of growth hormone replacment

(a)Effect of continous infusion of ovine growth hormone by osmotic minipump

Mature male rats were implanted with minipumps, then STZ was administered to induce diabetes. The infusion rate of oGH was 0.02 U/hr over the 4 days of diabetes. Control and STZ rats were operated on, but no minipumps were implanted. No significant change in Kd values occured (table XIV). The binding capacity of the HALC ABP in oGH treated 4 day STZ diabetic rats was not restored to control values.

(b)Effect of multiple subcutaneous ovine growth hormone doses

Replication of the normal male secretion pattern of oGH was attempted by treating with 7 s.c. doses of oGH (in

Table XIV

THE EFFECT OF INFUSION OF OVINE GROWTH HORMONE BY MINIPUMP ON THE HEPATIC CYTOSOLIC HIGH AFFINITY-LOW CAPACITY ANDROGEN BINDING PROTEIN OF STREPTOZOTOCIN DIABETIC MALE RATS

Ovine growth hormone was administered in a dose of 0.02 U/hour starting at induction of diabetes with STZ in adult (240-390 g) male rats. Rats were killed after 4 days. Control and 4 day STZ diabetic control rats were operated on but no minipump was implanted. Standard assay conditions were used.

Animal Model	Kd (nM)	Binding Capacity (fmol/mg)	N
Citrate control	0.33 ± 0.04	22.51 ± 2.48 a	26
Internal control	0.50 ± 0.07	14.66 ± 2.60	3
4 Day STZ	0.55 ± 0.32	7.66 ± 1.45	3
4 Day STZ + ovine growth hormone	0.35 ± 0.11	7.16 ± 1.92 b	6

Similar letters indicate values which are not significantly different at the $P < 0.05$ level using ANOVA and Newman-Keul's Range test

Table XV

THE EFFECT OF MULTIPLE SUBCUTANEOUS DOSES OF OVINE GROWTH HORMONE ON THE HEPATIC CYTOSOLIC HIGH AFFINITY-LOW CAPACITY ANDROGEN BINDING PROTEIN OF FOUR DAY STREPTOZOTOCIN DIABETIC MALE RATS

Ovine growth hormone was administered s.c. 7 times a day (0240, 0615, 1015, 1415, 1815, 2100, and 2340) in a dose of 30 μg in saline, pH 8.5, per rat per dose starting at induction of diabetes with STZ in adult (240-390 g) male rats. Control and STZ diabetics were administered saline at pH 8.5 in the same way. Rats were killed 4 days after induction of diabetes. Standard assay conditions were used.

Animal Model	Kd (nM)	Binding Capacity (fmol/mg)	N
Citrate control	0.33 ± 0.04	22.51 ± 2.48 a	26
Internal Control	0.20 ± 0.01	14.75 ± 1.93	4
4 Day STZ	0.31 ± 0.11	9.25 ± 4.26	4
4 Day STZ + ovine growth hormone	0.33 ± 0.11	7.28 ± 2.88 b	7

Similar letters indicate values that are not significantly different from each other at the $P < 0.05$ level using ANOVA and Newman-Keul's Range test

saline, pH 8.5), daily. Control and 4 day STZ diabetic rats were given 3 daily doses of saline (pH 8.5). Results in table XV show no significant changes in Kd values, and no restoration of the the binding capacity of the 4 day STZ diabetic oGH treated group to control levels.

(c)Effect of multiple dosing of ovine growth hormone using a vena cava catheter

Intravenous catheters were implanted into the vena cava. Ovine GH was administered 7 times a day, in a dose of 30 µg/rat/dose in saline, pH 8.5 in order to more accurately duplicate normal oGH secretory patterns in the male rat. Control and 4 day STZ rats had vena cava catheters implanted through which saline, pH 8.5, was administered 7 times a day. Technical difficulty encountered in the use of the vena cava catheters reduce the number of animals to the point that statistical analysis was not possible. Still, from table XVI it is apparent that control and 4 day STZ binding capacity values are identical and considerably below normal control binding capacity values. In this experiment, vena cava catheters were implanted under halothane anaesthetic. Halothane has been suspected of causing hepatotoxicity (Brown and Sipes, 1977), so an experiment using ether, the anaesthetic commonly used in our laboratory, was done.

Table XVI

THE EFFECT OF MULTIPLE INTRAVENEOUS DOSES OF OVINE GROWTH HORMONE BY VENA CAVA CATHETER ON THE HEPATIC CYTOSOLIC HIGH AFFINITY-LOW CAPACITY ANDROGEN BINDING PROTEIN OF STREPTOZOTOCIN DIABETIC MALE RATS

Ovine growth hormone was administered in a dose of 30 μg in saline, pH 8.5, 7 times a day (0240, 0615, 1015, 1415, 1815, 2100, and 2340) by vena cava catheter starting at the induction of diabetes with STZ in adult male rats (240-390 g). Control and STZ diabetic controls also had vena cava catheters implanted and saline, pH 8.5 was administered 7 times a day. Rats were killed 4 days after induction of diabetes. Standard assay conditions were used.

Animal Model	Kd (nM)	Binding Capacity (fmol/mg)	N
Citrate control	0.33 ± 0.04	22.51 ± 2.48	26
Internal control	0.08 ± 0.02	8.91 ± 2.02	3
4 Day STZ	0.11 ± 0.03	8.94 ± 0.17	2
4 Day STZ + ovine growth hormone	0.15 ± 0.03	6.17 ± 1.16	3

Statistical tests were not done due to the low number of animals used

(d)Effects of ether *versus* halothane general anaesthesia in control rats

Vena cava catheters were implanted into control rats under ether or halothane anaesthetic. Animals were killed 4 days later; cannulas were not used. Although performed on a limited number of animals, results (table XVII) show that there is no advantage to either ether or halothane as a general anaesthetic for our purposes.

(e)Effects of multiple tail vein injections of ovine growth hormone

Pursuing restoration of the normal oGH secretory pattern, we next did experiments in which oGH (30 μg/dose in saline, pH 8.5) was administered 4 times a day using the tail vein. Again control and 4 day STZ controls received equal amounts of saline, pH 8.5. Results are given in table XVIII. No significant difference in Kd values, no restoration to control binding capacities occured in oGH treated 4 day STZ diabetic rats.

Table XVII

THE EFFECT OF HALOTHANE VERSUS ETHER ANAESTHESIA ON THE HEPATIC CYTOSOLIC HIGH AFFINITY-LOW CAPACITY ANDROGEN BINDING PROTEIN OF ADULT MALE RATS

Vena cava catheters were implanted into adult (240-390 g) male rats) under ether or halothane anaesthesia. Catheters were not used. Rats were killed four days after surgery. Standard assay conditions were used.

Animal Model	Kd (nM)	Binding Capacity (fmol/mg)
Control	0.30 ± 0.02	20.76 ± 1.49 (43)
Ether	0.26	13.81
Ether	0.19	12.06
Halothane	0.26	14.59
Halothane	0.48	34.00

( ) indicates N

Statistical analysis was not done due to the low number of animals studied.

Table XVIII

THE EFFECT OF MULTIPLE INTRAVENEOUS DOSES OF OVINE GROWTH HORMONE BY THE TAIL VEIN ON THE HEPATIC CYTOSOLIC HIGH AFFINITY-LOW CAPACITY ANDROGEN BINIDNG PROTEIN OF 4 DAY STREPTOZOTOCIN DIABETIC MALE RATS

Ovine growth hormone in a dose of 30 μg in saline, pH 8.5, was administered by the tail vein 4 times a day (0900, 1300, 1700, 2100 hours) starting with the induction of diabetes with STZ in adult (240-390 g) male rats. Control and STZ controls were injected with saline, pH 8.5 in the same way. Rats were killed 4 days after induction of diabetes. Standard assay conditions were used.

Animal Model	Kd (nM)	Binding Capacity (fmol/mg)	N
Citrate control	0.33 ± 0.04	22.51 ± 2.48	26
Internal control	0.25 ± 0.03	20.98 ± 3.20	8
4 Day STZ	0.33 ± 0.06	7.75 ± 0.75 *	6
4 Day STZ + ovine growth hormone	0.44 ± 0.18	5.41 ± 1.93 *	4

* Indicates significant difference from either control at the $P < 0.05$ level using ANOVA and Newman-Keul's Range test

(f)Effects of multiple tail vein injections of ovine growth hormone, daily injections of testosterone enanthate, subcutaneously, and daily injection of protamine zinc insulin, subcutaneously

Previous results using PZI and testosterone enanthate replacement showed partial restoration of HALC ABP levels. Therefore, to determine whether oGH, testosterone enanthate, and PZI were all required for normal HALC ABP binding capacity levels, a set of experiments in which daily subcutaneously PZI (10 U/kg) and testosterone enanthate (1mg/kg) dosing along with 4 daily tail vein doses of oGH (30 μg) was carried out. Control and 4 day STZ rats received saline, pH 8.5, 4 times a day and daily s.c. injections of corn oil, which was the vehicle used to deliver testosterone enanthate. Results (table XIX) show no significant change in Kd values, and no restoration to control binding capacities of 4 day STZ diabetic testosterone enanthate, PZI, and oGH treated animals.

Table XIX

THE EFFECT OF SUBCUTANEOUS ADMINISTRATION OF TESTOSTERONE ENANTHATE AND PROTAMINE ZINC INSULIN WITH MULTIPLE TAIL VEIN INJECTIONS OF OVINE GROWTH HORMONE ON THE HEPATIC CYTOSOLIC HIGH AFFINITY-LOW CAPACITY ANDROGEN BINDING PROTEIN OF 4 DAY STREPTOZOTOXIN DIABETIC MALE RATS

Adult (240-390 g) male rats were treated once daily with TE, 1 mg/kg s.c.; with PZI, 10 U/kg, s.c.; and with oGH four times a day (0900, 1300, 1700, 2100 hours) in a dose of 30 µg in saline, pH 8.5 injected into the tail vein, starting at the induction of diabetes with STZ. Control and STZ controls received equal amounts of corn oil s.c. once a day, and saline, pH 8.5, into the tail vein 4 times a day. Rats were killed 4 days after the induction of diabetes. Standard assay conditions were used.

Animal Model	Kd (nM)	Binding Capacity (fmol/mg)		N
Citrate control	0.33 ± 0.04	22.51 ± 2.48		26
Internal control	0.23 ± 0.03	24.92 ± 2.88		6
4 Day STZ	0.23 ± 0.04	7.82 ± 1.19	*	4
4 Day STZ treated with testosterone enanthate, protamine zinc insulin, and ovine growth hormone	0.15 ± 0.06	10.12 ± 1.02	*	4

* Indicates significant difference from control value at $P < 0.05$ level using ANOVA and Newman-Keul's Range test

DISCUSSION

I. Ammonium sulfate fractionation

Ammonium sulfate fractionation was used to partially purify the HALC ABP in this study (table I). We found that 0-50 % fractionation produced binding with a Kd characteristic of the HALC ABP seen in whole cytosol, and gave a 2 to 3 fold purification of the HALC ABP based on increase in binding capacity (table I, and III).

The amount of ammonium sulfate required to fractionate our hepatic HALC ABP is different from the amount of ammonium sulfate used by others to partially purify the hepatic ABP, and the prostatic AR. Early work done with ammonium sulfate fractionation of hepatic cytosol by Ota et al, 1981, resulted in the sedimentation of an ABP in the 30 % ammonium sulfate pellet of hepatic cytosol. Precipitation was performed with 40 and 50 % as well but dpm/mg protein was maximum at 30 %. The sedimentation coefficient of this protein was not determined. Although specificity studies of an ABP of whole cytosol had been done by this group, no studies on the ammonium sulfate precipitated ABP were reported in this or subsequent papers. This may be important because we found an atypical Kd for the ABP in the 0-33 % fractionation. This may indicate the presence of more than 1 or a family of AR, as Lea et al,

1979, suggested exist in the prostate. Rumbaugh et al, 1984, used ammonium sulfate precipitation to decrease non-specific binding because it was impossible to obtain a Scatchard plot using [^{3}H] 5α-DHT in whole cytosol. Therefore, comparison of change due to the precipitation procedure is impossible. Decker and Levinson, 1985, used ammonium sulfate purification, and they, too, found an 8S androgen specific binding protein in the 0-33 % fraction, and no specific binding in the 33-60 % fraction. No characterization of the ABP found in the 0-33 % fraction was done.

Ammonium sulfate purification of prostatic cytosol also results in separation of 2 AR's with different steroid specificities. Fang and Liao, 1971, and Verhoeven et al, 1975, found an AR of higher affinity precipitated in 35 % ammonium sulfate, and a second class of AR precipitated in the 50-60 % ammonium sulfate fraction. Mainwaring and Peterken, 1971, found 2 AR's in fractionated prostatic cytosol, the 8 S form precipated at 33 % ammonium sulfate, and the 3.5 S form did not co-precipitate. Because of the differences in amount of ammonium sulfate required to fractionate the hepatic versus prostatic AR, a sucrose density gradient study to determine sedimentation coefficient of our HALC ABP would be benificial in testing our belief that the fractionated HALC ABP is the same as the HALC ABP from whole cytosol.

Freeze storage (-80°C) of the pellet prepared using 50 % ammonium sulfate (figure 2) proved successful for time periods of up to 60 days, and apparently did not change the AR. Similar pellets prepared from prostate cytosol and stored frozen for over 1 month had a changed initial velocity of interaction with 5α-DHT according to Feit and Muldoon (1983). A more rigorous study to examine changes in the HALC ABP with time is warranted in light of the results of Feit and Muldoon.

II. Inclusion of triamcinolone acetonide in the hepatic cytosolic high affinity-low capacity androgen binding protein assay

Triamcinolone acetonide is used to block binding of R1881 to a second, lower affinity-higher capactiy binding protein present in the hepatic cytosol (Eagon et al, 1983, Eisenfeld et al, 1983, Kyakumoto et al, 1984, and Sunahara et al, 1985).

The reason for the suggestion that the lower affinity ABP to which R1881 binds in hepatic cytosol is the glucocorticoid receptor is as follows. R1881 binds to the cytosolic progestin receptor in the prostate of rat and humans (Zava et al, 1979, and Menon et al, 1978), and some believe R1881 binds to a progestin receptor in rat liver cytosol (Levinson and Decker, 1985). However, Yamada

and Miyaji, 1982, and Kyakumoto et al, 1984, found that a cytosolic progestin receptor is present in minimal amounts, if any, in male liver cytosol. This appears to be the case in human liver cytosol, too (Aten et al, 1983). The glucocorticoid receptor is present in the 8S peak of glycerol density gradient centrifugation analysis of male rat hepatic cytosol (Kyakumoto et al, 1984), and it is most likely the lower affinity-higher capacity binding protein to which R1881 binds in the absence of triamcinolone acetonide (Kyakumoto et al, 1984, and Sunahara et al, 1985).

Partial purification with ammonium sulfate was successful in separating much of the non-specific binding from the HALC ABP. It was possible that partial purification also separated the low affinity (glucocorticoid receptor) ABP of the hepatic cytosol from the high affinity (HALC) ABP. Experiments were done in which triamcinolone acetonide was not included in the incubation mixture (figures 1, a and b). Two or more binding sites were evident in saturation binding curves and Scatchard plots. Therefore inclusion of triamcinolone acetonide, 100 fold molar excess, was continued.

III. Artifactual results from blood contamination

A report which showed serum non-specific binding

contaminants led to inaccuracies in liver polycyclic hydrocarbon binding assays (Poellinger et al, 1983) suggested the possibility of another confounding binding protein being present in the partially purified hepatic cytosol. This report also suggested that adequate perfusate volume could minimize or eliminate these contaminants. We found (Table III) no differences in Kd or in binding capacity in binding studies done with whole and fractionated cytosol from livers perfused with 10 or 100 mL TEDM buffer One 50 % ammonium sulfate fractionated cytsolic sample from a liver perfused with 100 mL TEDM buffer had a binding capacity much lower than expected, however, this sample also had a markedly different Kd value. The reason for this difference may have been physical trauma resulting from perfusion of the liver with 100 mL of buffer.

IV. Kd and binding capacity values of the hepatic high affinity-low capacity androgen binding protein

Scatchard analysis of the HALC ABP of mature male Wistar rats (tables IV, V, and VIII) resulted in a Kd of 0.30 $\pm$ 0.02 nM and a binding capacity of 20.8 $\pm$ 1.5 fmol/mg protein (N = 43). Others have reported the following vaules, Kd (in nM): 25 (Kyakumoto et al, 1984), 2.3 (Levinson and Decker, 1985), 0.3 (Sunahara et al, 1985), 1.5 (Eagon et al, 1984), and 0.10 (Turocy et al,

1985). Binding capacity values (in fmol/mg protein) are: 26.3 (Kyakumoto et al, 1984), 18.8 (Levinson and Decker, 1985), 5.9 (Sunahara et al, 1985), 10-15 (Eagon et al, 1983 a and b, and 1984), 15 (Turocy et al, 1985). All values resulted from the use of R1881 in binding studies. Banister et al, 1985b, reported values using mibolerone, another synthetic androgen. They found a Kd of 0.86 ± 0.4 nM, and a binding capacity of 8.36 ± 2.77 fmol/mg. It is unlikely that differences seen are caused by strain differences, since all except Levinson and Decker used Wistar rats. However, differences in pH, in whole versus ammonium sulfate partially purified cytosol, in Scatchard analysis versus sucrose density analysis, or in tissue:buffer homogenization volume ratios may contribute to differences seen in Kd and binding capacity values. It should be noted that all the reported values are remarkably close, considering the differences in techniques used.

V. Specificity of the hepatic high affinity-low capacity androgen binding protein

Further characterization of the hepatic HALC ABP by determination of specificity of binding has been done by several groups. Competitor studies done by Levinson and Decker (1985), were performed in the absence of triamcinolone acetonide. This group used sucrose density

gradients to analyze results of binding studies. However, the glucocorticoid receptor to which R1881 also binds in the absence of triamcinolone acetonide, is found in the 8S fraction, as is the AR (Kyakumoto et al, 1984). As a result, Levinson and Decker's competitor studies involved the displacement of R1881 from both glucocorticoid receptor and AR. Eagon et al, (1983 a and b), reported that only R1881 and 5α-DHT compete for the AR site; however a complete report of these data is not yet available. Kyakumoto et al, 1984, and Sunahara et al, 1985, did competitor studies with and without triamcinolone. In incubations containing triamcinolone acetonide, Kyakumoto et al, 1984, found the order of ability to displace 10 nM [^{3}H]R1881 from the receptor with 100 fold excess competitor was: R1881 > testosterone = androstenedione > 5α-DHT. Estradiol, cortisol, triamcinolone acetonide and progesterone had no ability to displace [^{3}H]R1881. Sunahara et al, 1985, found generally similar results. The order of ability to displace 1 nM [^{3}H]R1881 from the receptor with 100 fold excess of competitor was as follows: 5α-DHT > testosterone > R1881 > androstendione > estradiol. Diethylstilbesterol, progesterone, cortisol, and triamcinolone acetonide had no displacing ability. Although the order of displacing ability differs, R1881, testosterone and androstenedione were closely clustered in both studies. Kyakumoto and Sunahara found some differences in the ability of 5α-DHT to

displace [^{3}H]R1881. In the present study the order of displacement of 1 nM [^{3}H]R1881 with 100 fold excess competitor is: R1881 = 5α-DHT > progesterone > testosterone enanthate = androstendione = estradiol, and no displacement was seen with diethylstilbesterol. In these three studies there was agreement in the high specificity of the AR for R1881 and 5α-DHT; however in our studies the HALC ABP had greater affinity for estradiol and progesterone and less for androstenedione. The reason for this discrepancy is unclear. The most obvious conclusion was that co-purification of the glucocorticoid receptor occured. The amount of triamcinolone acetonide included in the incubation may have been inadequate to mask all of the glucocorticoid receptor. We saw no evidence for this in Scatchard plots (figure 1b), where non-linearity of the plot is indicative of more than 1 binding site. Increasing amounts of triamcinolone acetonide did not significantly reduce the binding capacity of the HALC ABP in male hepatic 50 % ammonium sulfate fractionated cytosol (table IV). Lea et al, 1979, has suggested a family of AR in the prostate; perhaps we selectively precipitated a subtype of hepatic AR which had higher progestin affinity.

VI. Sex and age differences in the hepatic high affinity-low capacity androgen binding protein

Sex and age differences in the HALC ABP were investigated using 50 % ammonium sulfate fractionated cytosol. Previous experiments to determine sex specificity have produced inconsistent reports between laboratories. Partial purification with 50 % ammonium sulfate enables quantitation of smaller amounts of HALC ABP and should more accurately answer the question of the presence of small amounts of HALC ABP. In our study immature male and female rats had equal amounts of HALC ABP which were similar to those found in mature females, and which was 1/4 to 1/2 the amount found in mature males. Others have reported no HALC ABP in immature rats (Eagon et al, 1984), low amounts in immature males, and none in immature females (Sunahara et al, 1985). The androgen binding site studied by Rumbaugh and Lucier, 1982, and Rumbaugh et al, 1984, was absent in immature rats. However we question whether this 4S protein is the same as the high affinity binding protein studied by others, because of the 4S sedimentation coefficient, unknown specificity and the ability to measure this binding protein without the stabilizing effect of molybdate.

In our study, mature female Wistar rats appear to have 1/3 the amount of hepatic HALC ABP that is found in mature male rats (table IV). Others report hepatic AR present in

equal amounts in mature male and female rats (Eagon et al, 1983a and b, and Turocy et al, 1985), or absent in mature females (Levinson and Decker, 1985, and Sunahara et al, 1985).

Increasing amounts of triamcinolone acetonide (table IV) resulted in an apparent reduction in the amount of HALC ABP in females but not in males. The change in Kd of the HALC ABP in male with 100 versus 500 fold excess triamcinolone acetonide may indicate competition of triamcinolone for the HALC ABP, although this has not been demonstrated in competitor studies done with triamcinolone in whole cytosol. Because triamcinolone acetonide and R1881 are known to bind to progestin receptors in the rat prostate (Zava et al, 1979), they may have been binding to an hepatic progestin receptor not present in males (Kyakumoto et al, 1984). The presence of a progestin receptor in female rat liver cytosol has not been investigated. It is unlikely that the triamcinolone acetonide reducible binding is due to an increased amount of glucocorticoid receptor, which is also seen in males (Kyakumoto et al, 1984). The presence of a progestin receptor in female rat liver cytosol has not been investigated. It is unlikely that the triamcinolone acetonide reducible binding is due to an increased amount of glucocorticoid receptor, which is also seen in males, because the Kd of glucocorticoid receptor is clearly much less than the ABP (figure 1, a and b) and the

measured Kd of the hepatic HALC ABP of females (tables IV and V) is closer to the Kd of the HALC ABP of males than the Kd of the glucocorticoid receptor. The HALC ABP of female rats requires further investigation.

Results of castration of mature male rats are in table VI. There is no change in the Kd of binding capacity after one week of castration. Sunahara et al, 1985, showed that the Kd and binding capacity of the HALC ABP did not change at 18 hours, 4 or 10 days after castration. Eagon et al, 1984, found a temporary enhancement of the cytosolic AR at 48 hours after castration, then a decrease to undetectable levels 30 days after castration. Results appear to be consistent between groups, however verification of the increase at 48 hours, and the absence of the HALC ABP 30 days after castration is necessary. The binding capacity of neonatally castrated male rats was low or undetectable. Neonatally castrated testosterone-treated rats also had low binding capacities, but unlike neonatally castrated rats, produced reliable Scatchard plots. Several possibilities for these results exist. Stress during the neonatal period may have irreversible effects on the ability to produce normal levels of HALC ABP at maturity. Imprinting, where normal adult levels of enzyme activity or binding protein content are dependent on in utero or neonatal surges of a hormone, may occur with the HALC ABP. This does occurs with other hepatic steroid binding proteins (Sloop et al,

1983). To test this possibility, administration of testosterone to mature neonatally castrated and neonatally castrated, prepubertally, androgen-imprinted rats would be necessary.

VII. Effects of diabetes on the hepatic high affinity-low capacity androgen binding protein and the prostatic androgen receptor

Tesone et al, 1980, found a decreased number of available (measured by incubation of cytosol with [^{3}]R1881 under conditions of negligible exchange) and total (measured by incubation of cytosol with [^{3}H]R1881 under exchange conditions) AR in the prostate of 14 day STZ diabetic Wistar rats (control 94.2 $\pm$ 11.3, STZ diabetic 12.6 $\pm$ 3.0 fmol/mg protein), which could be partially restored by treatment with insulin (PZI, 2 IU/rat, 18.3 $\pm$ 2.4 fmol/mg protein) or treatment with testosterone (500 µg/rat s.c. daily, 41.1 $\pm$ 3.1 fmol/mg protein). There was no change in affinity constant (control 0.13 $\pm$ 0.01 X 10^9 M, diabetics 0.17 $\pm$ 0.02 X 10^9 M, diabetics treated with PZI 0.10 $\pm$ 0.02 X 10^9 M, or diabetics treated with testosterone 0.12 $\pm$ 0.01 X 10^9 M).

We found a decrease in binding capacity of hepatic cytosolic HALC ABP in 4 and 10 day STZ diabetic rats (control 22.51 $\pm$ 2.48, 4 day diabetic 9.71 $\pm$ 1.22, 10

day diabetic 8.31 ± 1.21 fmol/mg protein), and no change in Kd (control 0.33 ± 0.04, 4 day diabetic 0.31 ± 0.05, 10 day diabetic 0.36 ± 0.04 nM). The decreased binding capacity was not due to decreased temperature stability of the HALC ABP in STZ diabetic rats (table IX). No changes in specificity of the HALC ABP were apparent (Table X). The reduction in binding capacity in 4 day STZ diabetic rats was not reversible by treatment with PZI (10 U/kg daily, 11.56 ± 3.72 fmol/mg protein); Toronto insulin (15 U/kg twice a day, 6.66 ± 1.30 fmol/mg protein); testosterone enanthate (1 mg/kg daily, 10.05 ± 1.79 fmol/mg protein); triiodothyronine (30 µg/kg daily, 9.49 ± 1.94 fmol/mg protein); or oGH administered by minipump (0.02 U/hour for 4 days, 7.16 ± 1.92 fmol/mg protein), by multiple s.c. doses (30 µg/dose, 7 doses daily, 7.28 ± 2.88 fmol/mg protein), by multiple intravenous doses by vena cava catheter (30 µg/dose, 7 doses daily, 6.71 ± 1.16 fmol/mg protein), or by tail vein injection (30 µg/dose, 4 doses daily, 5.41 ± 1.93 fmol/mg protein).

Administration of PZI (10 U/kg daily) with testosterone enanthate (1 mg/kg s.c. daily) resulted in a partial restoration in the binding capacity of the hepatic HALC ABP to 15.7 ± 2.91 fmol/mg protein. It should be noted that results from the initial PZI with testosterone enanthate experiment produced results not different from control; however when repeated, results were not different from 4 day

STZ diabetic values. Treatment with PZI (10 U/kg daily) and testosterone enanthate (1 mg/kg s.c. daily), and with oGH (30 μg/dose, 4 doses daily by tail vein) did not reverse the decrease seen with 4 day STZ diabetic rats (10.12 ± 1.02 fmol/mg protein). Therefore, we conclude that restoration to control levels did not occur with PZI and testosterone enanthate alone. BioBreeding strain diabetic and non-diabetic male rats had HALC ABP binding capacity significantly less than control Wistar males (Wistar control 22.51 ± 2.48, BioBreeding strain non-diabetic 11.57 ± 2.23, BioBreeding strain diabetic 4.58 ± 0.73 fmol/mg protein). The binding capacity of the BioBreeding strain diabetic was not statistically significantly decreased from the BioBreeding strain non-diabetic (ANOVA and Newman-Keul's range test). An increase in number of animals studied would be required to properly test the significance of the apparent decrease in diabetics. There was no change in the Kd value of the HALC ABP between Wistar control, BioBreeding strain non-diabetic, and BioBreeding diabetic rats (0.33 ± 0.04, 0.36 ± 0.04, and 0.49 ± 0.32 fmol/mg protein, respectively).

VIII. Non-specific effects of streptozotocin

Because of the inability to restore HALC ABP binding

capacity levels with a dose of insulin which eliminates glucosuria in diabetic male Wistars, the suggestion that the decrease in binding capacity of the HALC ABP was a direct toxic effect of STZ was made. Although direct toxicity of STZ including degranulation of rough endoplasmic reticulum and swelling of the mitochondria has been studied in mice (Laguens et al, 1980), evidence for direct hepatic damage in STZ diabetic Wistar rats is limited to "focal necrosis" (Junod et al , 1967). Histologically, "essentially normal" livers were seen by Warren et al, 1983.

Synthesis of hepatic and secreted protein in the liver of STZ diabetic rats is decreased (McNurlan and Garlick, 1979). It is possible the decrease in the HALC ABP results from this general reduction in protein synthesis. Breakdown of protein is also increased 1 day after insulin treatment is stopped, and breakdown slows to rates below control rates 4 days after insulin is stopped (Albertse et al, 1980). Insulin-treated diabetic rats do not show the decreased rate of protein synthesis, nor the increased protein breakdown (Albertse et al, 1980). This is in contrast to what is seen with the hepatic HALC ABP since its decrease is not prevented by insulin treatment (table XI), These facts decrease the possibility that the decrease seen in the binding capacity of the HALC ABP is a non-specific effect of STZ. Also, the hepatic estrogen receptor of the male Wistar rat is not reduced in binding capacity by STZ administration

(W.A. Haniuk, 1985) which one would expect if the decrease in binding capacity of the HALC ABP was a non-specific effect of STZ.

IX. Use of ovine growth hormone to restore hepatic high affinity-low capacity androgen binding protein binding capacity

Throughout this study GH replacment was undertaken using ovine rather than rat GH, due to limited availability of rat GH. There is evidence in the literature for a high degree of similarity between ovine and rat GH. Sequence studies show a high degree of similarity (Paladini et al, 1983). Cross reactivity of monkey anti-serum to rat GH with oGH in double antibody radioimmuno assay occurs (Hayashida, 1969). Ovine GH will not, however, mimic rat GH in induction of GH or prolactin receptors in female Wistar-Furth rats, nor increase the serum somatomedin C levels in hypophysectomized female rats (Baxter et al, 1984).

X. Effect of stress on the hepatic high affinity-low capacity androgen binding protein

During the course of this study, it has become apparent that stress, in particular surgical stress (see especially table XVII), has a great effect on the measurable amounts of

hepatic HALC ABP. One possible solution to this problem is to allow much longer recovery periods after surgery.

XI. Subcellular location of the high affinity-low capacity androgen binding protein

Recently the classical theory of sex steroid receptor function has been questioned. Evidence from autoradiography, subcellular fractionation, immunochemistry, and cellular enucleation (King and Greene, 1984, Welshons et al, 1984, Sheridan et al, 1979, Sheridan, 1975) supports the occurence of unoccupied receptors either exclusively in the nucleus or in equilibrium between the cytosol and the nucleus. Data are not yet conclusive. A recent discussion of this subject by Walters (1985) points out that possibilities for error exist in each technique used, however several techniques support the hypothesis of nuclear unbound steroid receptors strengthening the argument. Most studies on unbound steroid receptor location have been done with estrogen and progestin receptors. Evidence for the existence of a nuclear unbound AR consists of studies where AR were found in the nucleus but not the cytoplasm in autoradiography of the rat brain (Sheridan, 1983). More study is required to conclusively answer this question, however we must consider its relevance to the intrepretation of our data. The receptor leached from the nucleus may be

leached randomly or result from a decreased affinity for nuclear binding sites. If the nuclear unbound receptor leaches because of decreased affinity for nuclear binding sites, we may be studying a sub-population of ABP. If we are studying a subpopulation the orginal question of a change in binding characteristics and stability or a real decrease in numbers of HALC ABP in diabetes will have to be investigated in different ways, and the question of physiological relevence of the subpopulation as well as the general population will have to be answered.

Although much has been said about the nuclear versus cytosolic receptor locations of an hepatic AR; a specific androgen membrane transport protein on the plasmalemma of canine and human prostate cells (Giorgi, 1976), an androgen receptor on the nuclear envelope of rat hepatic and prostatic cells (Lefebvre and Morante, 1982, and Lefebvre, 1985), and androgen binding sites in microsomes have been demonstrated and may prove to be very important in the regulation of androgen effects on the liver. It is possible that regulation of androgen effects on the liver occurs as a result of interaction with all, one, or none of these hepatic androgen "receptors".

XII. Possible functions of the hepatic high affinity-low capacity androgen binding protein

The function of the hepatic HALC ABP is not immediately obvious. Androgen administration changes the level of some plasma proteins, probably by the modification of their synthesis in the liver (Anderson and Kappus, 1982). Androgen administration also increases the activity of some drug metabolizing enzymes in the rat liver (Kato et al, 1974). Steroids are believed to take effect by interacting with cellular receptors.

In examining the aspect of androgen effects on drug metabolizing enzymes more closely we see that acute STZ and genetic (BioBreeding strain) diabetes in rats causes a decrease in the sex differences in drug metabolism in rats within 4 days (Warren et al, 1983). These changes are reversed if diabetics are treated with insulin.

We have investigated the HALC ABP in STZ diabetic male Wistar rats. Although we found a decrease in hepatic HALC ABP corresponding to feminization of enzyme metabolism seen in male rats 4 days after induction of diabetes, we were unable to prevent the decrease by maintaining the diabetic animals on insulin after STZ injection. Therefore it seems unlikely that the hepatic HALC ABP is a regulator of sex differences in enzyme metabolism seen in rats.

FUTURE EXPERIMENTS

I. Sucrose density analysis of whole and partially purified cytosol

Sucrose density gradient studies in whole and 50 % ammonium sulfate fractionated cytosol have shown repeatedly that the 8S form of the ABP is the AR. It would be informative to analyze the hepatic HALC ABP that results from our 0-50 % fractionation using this technique.

II. Effects of prolonged frozen storage of the hepatic HALC androgen binding protein

Studies on the prostatic AR have shown that after 1 month of frozen storage, changes in the initial velocity of interaction with 5α-DHT occur (Feit and Muldoon, 1983). Although no changes were apparent from Scatchard analysis of the hepatic HALC ABP after up to 60 days of -80°C storage, a more rigorous study is warranted to determine possible progressive degradation of the protein with duration of storage.

III. The hepatic high affinity-low capacity androgen binding protein of mature female Wistar rats

Further investigation of the identity and characteristics of the hepatic cytosolic R1881 binding protein in the adult female Wistar should be done. In particular the presence of a progestin receptor in hepatic cytosol should be investigated.

IV. Verification of the specificity of diabetes causing a decrease in number of hepatic high affinity-low capacity androgen binding sites in male rats

It would be interesting to further characterize the reduction in binding capacity seen in the ABP of diabetics. In order to verify the specificity of the reduction in the number of HALC ABP binding sites in STZ diabetes it may be desirable to use 3-O-methyl-D-glucose. 3-O-methyl-D-glucose prevents the induction of STZ diabetes presumably by preventing STZ uptake into β pancreatic cells. This has been suggested to allow classification of changes seen in STZ diabetic animals as "non-specific effects of STZ" and effects of diabetes. This method depends on the specificity of 3-O-methyl-D-glucose for STZ uptake into the pancreas (Ganda et al, 1976). If 3-O-methyl-D-glucose is not specific for pancreatic cells, but blocks entry of STZ into

all cells of the body, no direct toxicity of STZ will be seen either. The occurance of decreased binding capacity of the hepatic HALC ABP in diabetic animals which are diabetic, but not STZ diabetic, such as BioBreeding strain diabetics, would support diabetes rather than STZ as the cause of effects on the HALC ABP. Preliminary studies using BioBreeding strain diabetic and non-diabetic rats suggest this is the case. Verification of these results by using a larger number of animals would be worthwhile.

V. Regulation of the hepatic high affinity-low capacity androgen binding protein by ovine growth hormone

Results from experiments with oGH administration show that the absence of GH or absence of peaks of GH is not the determinant in the reduction seen in the HALC ABP. Because of the results of Baxter et al, 1984, verification of our results in which oGH was adminstered using rat GH would be worthwhile. Antibodies to GH may be effective in restoring normal trough blood levels of GH which may be critical in maintaining normal ABP capacities, depending upon the half life of the antibodies used. If somatostatin is responsible for reduced pulses of GH (Tannenbaum, 1981) then somatostatin antibodies could be used to restore peaks without altering trough levels of GH.

VI. Determination of the effect of stress on the hepatic high affinity-low capacity androgen binding protein

Experiments involving the direct administration of cortisol may provide some information on the effects of stress on the HALC ABP binding capacity. Experiments in which gentled animals are used may reduce some of the difficulties encountered. Also increasing the length of time after surgery before the use of animals in experiments may be worthwhile.

VII. Purification of the hepatic high affinity-low capacity androgen binding protein

It would be preferable to work with a purer system to avoid problems associated with small amounts of HALC ABP and competition of endogenous with exogenous ligands. Purification of an androgen receptor from the seminal vesicle of the steer has been done (Chang et al, 1982). Also, the development of a cytosolic or nuclear exchange assay so that all androgen receptor sites including those occupied by endogenous androgen could be measured, would be desirable.

VIII. Identification and location of the physiologically relevant androgen receptor

The function of the hepactic cytosolic AR is presently unknown. It is possible that the effects of androgen on hepatic protein synthesis are regulated by another (mitochondrial, plasmalema, or nuclear envelope) ABP. This should be investigated. The location of the unbound hepatic AR whether nuclear, cytoplasmic or both will probably be critical in understanding the mechanism of androgen action. As we have seen differential centrifugation, enucleation, and antibodies have been used in an attempt to answer this question. We have no novel approach to suggest, but the information is important to determine.

SUMMARY

1. Partial purification of rat hepatic cytosol using 50 % ammonium sulfate was found to be beneficial because it resulted in a 2 to 3 fold purification of the HALC ABP (table I, whole cytosol 5.86 ± 0.67, 50 % ammonium sulfate fractionated cytosol 10.37 ± 2.34; table III, 50 % ammonium sulfate fractionated cytosol 20.76 ± 1.49 fmol/mg protein) and no significant (ANOVA and Newman-Keul's range test) change in the Kd (whole cytosol 0.32 ± 0.04, 50 % ammonium sulfate fractionated cytosol 0.30 ± 0.02 nM, table I) occured. Ammonium sulfate fractionation also reduced the androgen metabolizing enzymes, which allowed increased accuracy of determination of ligand concentration, especially important in competitor studies.

2. The 50 % ammonium sulfate pellet may be frozen and stored at -80°C, preserving the HALC ABP without any apparent changes in the HALC ABP assay for at least 60 days (figure 2).

3. The HALC ABP was found to be present in the immature (table V, Kd 0.40, 0.07 nM; binding capacity 9.20, 9.57 fmol/mg) and mature female (Kd 0.15 ± 0.03 nM, binding capacity 7.04 ± 0.94 fmol/mg) and immature male (Kd 0.10, 0.15 nM, binding capacity 9.45, 5.40 fmol/mg). Amounts

were less than those seen in the mature male (Kd 0.30 ± 0.02, binding capacity 20.76 ± 1.49 fmol/mg).

4. The diabetic state induced by STZ caused a reduction in the binding capacity (table VIII, control 22.51 ± 2.48, 4 day STZ diabetic 9.71 ± 1.22 fmol/mg), but no change in the Kd (control 0.33 ± 0.04, 4 day STZ diabetic 0.31 ± 0.05 nM) of the hepatic cytosolic HALC ABP.

5. The diabetic state induced by STZ did not change the temperature sensitivity and did not appear to change the specificity of binding of the hepatic HALC ABP (tables IX, and X).

6. The lowered binding capacity of the STZ diabetic rat liver HALC ABP could not be reversed with insulin (table XI, PZI 10 U/kg s.c., daily; or Toronto insulin 15 U/kg s.c., twice daily), testosterone enanthate (table XII, 1 mg/kg s.c., daily), triiodothyronine (table XIII, 30 μg/kg s.c., daily), or oGH (0.02 U/hour for four days by minipump; or 30 μg/dose s.c., 7 doses daily; or 30 μg/dose by vena cava catheter, 7 doses daily; or 30 μg/dose by tail vein injection, 4 doses daily, tables XIV, XV, XVI, and XVIII). Combined treatment of 4 day STZ diabetic rats was attempted using both PZI (10 U/kg s.c, daily) and testosterone enanthate (1 mg/kg s.c., daily, table XII); and with PZI (10 U/kg s.c., daily), testosterone enanthate (1 mg/kg s.c.,

daily), and oGH (30 μg/dose, 4 doses a day administered by the tail vein, table XIX). Neither regimen was successful in increasing the HALC ABP capacity to control levels.

BIBLIOGRAPHY

Adachi, K., and Kano, M. (1972). The role of receptor proteins in controlling androgen action in the sebaceous glands of hamsters. Steroids 19: 567-574.

Albertse, E.C., Garlick, P.K., and Pain, V.M. (1980). The effect of streptozotocin diabetes on liver protein metabolism. Proc. Nutr. Soc. 39: 50 A.

Anderson, K.E., and Kappus, A. (1982). Hormones and liver function. In: Shiff, L., and Schiff, E.R. (eds) Diseases of the Liver, 5th edition. Lippincott, Toronto, p. 167.

Angervall, L., Hesselsjo, R., Nilsson, S., and Tisell, L.E. (1967). Action of testosterone on ventral prostate, dorsolateral prostate coagulating glands and seminal vesicles of castrated alloxan-diabetic rats. Diabetologia 3: 395-401.

Aten, R.F., Eisenfeld, A.J., and Beattie, C.W. (1983). Androgen and estrogen receptors in human liver. The Endocrine Society. 65: 245. (abstract number 660).

Bannister, P., Sheridan, P., and Lowowsky, M.S. (1985a). Use of a new radioactive ligand, 7α,17α-dimethyl [17α-methyl ^{3}H]19-nortestosterone for the estimation of androgen receptors in rat liver cytosol. J. Steroid Biochem. 23: 121-123.

Bannister, P., Sheridan, P., and Losowsky, M.S. (1985b). Identification and characterization of the human hepatic androgen receptor. Clin. Endocrinol. 23: 495-502.

Baxter, R.C, Bryson, J.M., and Turtle, J.R. (1981). Changes in rat liver prolactin binding sites in diabetes are sex dependent. Metabolism 30: 211-216.

Baxter, R.C., Zaltsman, Z., and Turtle, J.R. (1984). Rat growth hormone (GH) but not prolactin (PRL) induces both GH and PRL receptors in female rat liver. Endocrinology 114: 1893-1901.

Beato, M. and Feigelson, P. (1972). Glucocorticoid-binding proteins of rat liver cytosol. I. Separation and identification of the binding proteins. J. Biol. Chem. 247: 7890-7896.

Bergink, E.W., Geelen, J.A.A., and Turpijn, E.W. (1985). Metabolism and receptor binding of nandrolone and testosterone under *in vitro* and *in vivo* conditions. Acta Endocrinol. 110 suppl. 271: 31-36.

Bonne, C. and Raynaud, J-P. (1975). Methyltrienolone, a specific ligand for cellular androgen receptors. Steroids 26: 227-232.

Bonne, C. and Raynaud, J-P. (1976). Assay of androgen binding sites by exchange with methyltrienolone (R1881). Steroids 27: 497-507.

Bradford, M.M. (1976). A rapid and sensitive method for the Quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analy. Biochem. 72: 248-254.

Brown, B.R., and Sipes, I.G. (1977). Biotransformation and hepatotoxicity of halothane. Biochem. Pharmacol.26: 2091-2094.

Bryan, R.M., Mercer, R.J., Bennett, R.C., Rennie, G.C., Lie, T.H., and Morgan, F.J. (1984). Androgen receptors in breast cancer. Cancer 54: 2436-2440.

Chang, C.H., Rowley, D.R., Lobl, T.J., and Tindall, D.J. (1982). Purification and Characterization of Androgen Receptor from Steer Seminal Vesicle. Biochem. 21: 4102-4109.

Clark, J.H. and Peck Jr., E.J. (1980). Steroid hormone receptors. Basic principles and measurement. In: Laboratory Methods Manual for Hormone Action and Molecular Endocrinology, 4th edition. (Ed. by W.T. Schrader and B.J. O'Malley). Dept. Cell Biol., Baylor College of Medicine, Texas Medical Center, Houston, TX.

Dahmer, M.K., Housley, P.R., and Pratt, W.B. (1984). Effects of molybdate and endogenous inhibitors on steroid-receptor inactivation, transformation and translocation. Ann. Rev. Physiol. 46: 67-81.

Decker, D. and Levinson, D.J. (1983). Characterization of a novel 8S androgen receptor in the male rodent liver that is absent in the testicular feminizing syndrome (tfm/y). The Endocrine Society. 65: 137 (abstract number 227).

Deslypere, J.P., Sayed, A., Verdonck, L., and Vermeulen, A. (1980). Androgen concentrations in sexual and non-sexual skin as well as in striated muscle in man. J. Ster. Bioch. 13: 1455-58.

Dube, J.Y., Tremblay, R.R., Lesage, R., and Verret, G. (1975). *In vivo* uptake and metabolism of testosterone by the head appendages of the cock. Mol. and Cell. Endocrin. 2: 213-220.

Eagon, P.K., Willett, J.E. and Rogerson, B.J. (1983a). Androgen receptor in male rat liver. Fed. Proc.42(7): 1878. (abstract number 707).

Eagon, P.K., Willet J.E., Seguiti, S.M., Rogerson B.J. (1983b). Androgen receptor in male rat liver cytosol: separation from a male specific estrogen binder. 65th Annual Meeting of The Endocrine Society, San Antonio TX, p.243 (abstract number 650).

Eagon, P.K., Willet, J.E., Seguiti, S.M., Rogerson B.J. (1984). Androgen receptor in male rat liver. 7th International Congress of Endocrinology, Quebec City, Canada, 1984, p.649 (abstract number 777).

Eisenfeld, A.J., Sheets, C.S. and Aten, R.F. (1983). Androgen receptor in the mammalian liver. The Endocrine Society. 65: 137 (abstract number 226).

Eppenberger, U., and Hsia, S.L. (1972). Binding of steroid hormone by the 105,000 x g supernatant fraction from homogenates of rat skin and variations during the hair cycle. J. Biol. Chem. 247: 5463-5469.

Fang, S., and Liao, S. (1971). Androgen Receptors: Steroid- and tissue-specific retention of a 17β-hydroxy-5α-androstan-3-one-protein complex by the cell nuclei of ventral prostate. J. Biol. Chem. 246:16-24.

Feit, E.I., and Muldoon, T.G. (1983). Differences in androgen-binding properties of the two molecular forms of androgen receptor in rat ventral prostate cytosol. Endocrinology 112: 592-600.

Foglia, V.G., Rosner, J.M., de Peralta Ramos, M.C., and Lema, B.E. (1969). Sexual disturbances in the male diabetic rat. Hormone and Metab. Res. 1: 72-77.

Ganda, O.P., Rossini, A.A., and Like, A.A. (1976). Studies on streptozotocin diabetes. Diabetes 25: 595-603.

Giannopoulos, G. (1973). Binding of testosterone to uterine components of the immature rat. J. Biol. Chem. 248: 1004-1010.

Giorgi, E.P. (1976). Studies on androgen transport into canine prostate *in vitro*. J. Endocrinol. 68: 109-119.

Gustafsson, J.A., Pousette, A., Stenberg, A., and Wrange, O. (1975). High-affinity binding of 4-androstene-3,17-dione in rat liver. Biochem. 14: 3942-3948.

Gustafsson, J.A., and Stenberg, A. (1974). Influence of adrenal steroids on liver enzymes of neonatally castrated rats. J. Endocrinol. 63: 103-116.

Haniuk, W.A. (1985). The effects of streptozotocin induced diabetes on the binding kinetics of the hepatic cytosolic estrogen receptor in the adult male wistar rat. Directed studies thesis. University of British Columbia, Vancouver, B.C.

Hawkins, E.F., Lieskovsky, G., and Markland F.S. Jr. (1981). Molybdate and the measurements of androgen receptors in prostate cancer. J. Clin. Endocrin. Metab. 53: 456-458.

Hayashida, T. (1969). Relatedness of pituitary growth hormone from various vertebrate classes. Nature (London) 222: 294-295.

Howland, B.E., and Zebrowski, E.J. (1976). Some effects of experimentally induced diabetes on pituitary-testicular relationships in rats. Horm. Metab. Res. 8: 465-469.

Jackson, F.L., and Hutson, J.C. (1984). Altered reponses to androgen in diabetic male rats. Diabetes 33: 819-824.

Jansson, J.O., Ekberg, S., Isaksson, O.G.P., and Eden, S. (1984). Influence of gonadal steroids on age- and sex-related secretory patterns of growth hormone in the rat. Endocrinology. 114: 1287-1294.

Jennings, A.S. (1984). Regulation of hepatic triiodothyronine production in the streptozotocin-induced diabetic rat. Endocrinol. Metab. 10: E526-E533.

King, W.J., and Greene, G.L. (1984). Monoclonal antibodies localize oestrogen receptor in the nuclei of target cells. 307: 745-747.

Junod, A., Lambert, A.E., Orci, L., Pictet, R., Gonet, A.E., and Renold, A.E. (1967). Studies of the diabetogenic action of streptozotocin. Proc. Soc. Exp. Biol. Med. 126: 201-205.

Kato, J. (1975). The role of hypothalamic and hypophyseal 5αdihydrotestosterone, estradiol and progesterone receptors in the mechanism of feedback action. J. Ster. Bioch. 6: 979-987.

Kato, R. (1974). Sex-related differences in drug metabolism. Drug Metab. Rev. 3: 1-32.

Keenan, B.S., Meyer, W.J., Hadjian, A.J., and Migeon C.J. (1975). Androgen receptor in human skin fibroblasts: Characterization of a specific 17β-hydroxy-5α-androstan-3-one-protein complex in cell sonicates and nuclei. Steroids 25: 535-552.

Kirdani, R.Y., Emrich, L.J., Pontes, E.J., Priore, R.L., and Murphy, G.P. (1985). A comparison of estrogen and androgen receptor levels in human prostatic tissue from patients with non-metastatic and metastatic carcinoma and benign prostatic hyperplasia. J. Ster. Biochem. 22: 569-575.

Kreig, M., and Voight, K.D. (1977). Biochemical substrate of androgen actions at a cellular level in prostate, bulbocavernosus/levator ani and in skeletal muscle. Acta Endocrin. 85 suppl. 214: 43-89.

Krieg, M., Smith, K., and Bartsch, W. (1978). Demonstration of a specific androgen receptor in rat heart muscle: Relationship between binding, metabolism, and tissue levels of androgens. Endocrinology 103: 1686-1694.

Kyakumoto S., Sato, N., Nemoto, T., Ohara-Nemoto, Y., and Ota, M. (1984). Binding of [^{3}H]methyltrienolone to androgen receptor in rat liver. Biochim. et Biophys. Acta 800: 214-219.

Laguens, R.P., Candela, S., Hernandez, R.E., and Gagliardino, J.J. (1980). Streptozotocin induced liver damage in mice. Horm. Metab. Res. 12: 197-201.

Lea, O.A., Wilson, E.M., and French. F.S. (1979). Characterization of different forms of the androgen receptor. Endocrinology 105: 1350-1360.

Lefebvre, Y.A., and Morante, S. (1982) Binding of dihydrotestosterone to a nuclear-envelope fraction from the male rat liver. Biochem. J. 202: 225-230.

Lefebvre, Y.A., Golsteyn, E.J., and Michiel, T.L. (1985). Androgen interactions with intact nuclear envelopes from the rat ventral prostate. J. Ster. Bioch. 23: 107-113.

Levinson, D.J., and Decker, D.E. (1985). Characterization of a [^{3}H]methyltrienolone (R1881) binding protein in rat liver cytosol. J. Steroid Biochem. 22: 211-219.

Mainwaring, W.I.P., and Peterken, B.M. (1971). A reconstituted cell-free system for the specific transfer of steroid-receptor complexes into nuclear chromatin isolated from rat ventral prostate gland. Biochem. J. 125: 285-295.

Mainwaring, W.I.P., and Irving, R., (1973). The use of deoxyribonucleic acid-cellulose chromatography and isoelectric focusing for the characterization and partial purification of steroid-receptor complexes. Biochem. J. 134: 113-127.

Mainwaring, W.I.P., and Milroy, E.J.G. (1973). Characterization of the specific androgen receptors in the human prostate gland. J. Endocrin. 57: 371-384.

McGill, H.C. Jr., Anselmo, V.C., Buchanan, J.M, and Sheridan P.J. (1980). The heart is a target organ for androgen. Science 207: 775-777.

McGill, H.C. Jr., and Sheridan, P.J.(1981). Nuclear uptake of sex steroid hormones in the cardiovascular system of the baboon. Circ. Res. 48: 238-244.

McNurlan, M.A., and Garlick, P.J. (1979). Rates of protein synthesis in rat liver and small intestine in protein deprivation and diabetes. Proc. Nutr. Soc. 38: 133A.

Mennon, M., Tananis, C.E., Hicks, L.L., Hawkins, E.F., McLoughlin, M.G., and Walsh, P.C. (1978). Characterization of the binding of a potent synthetic androgen, methyltrienolone, to human tissue. J. Clin. Inves. 61: 150-162.

Milan, B., and Roy, A.K. (1973). Androgen "receptor" in rat liver: Cytosol "receptor" deficiency in pseudohermaphroditic male rats. Nature New Biology 242: 248-250.

Mitsuma, T., and Nogimori, T. (1982) Effects of streptozotocin-induced diabetes mellitus on hypothalamic-pituitary-thyroid axis in rats. Endocrinol. Japon. 29: 695-700.

Mode, A., Gustafsson, J.-A., Jansson, J.O., Eden, S., and Isaksson, O. (1982). Association between plasma level of growth hormone and sex differentiation of hepatic steroid metabolism in the rat. Endocrinology 111: 1692-1697.

Murray, F.T., Orth, J., Gunsalas, G., Weisz, J, Li, J.B., Jefferson, L.S., Musto, N.A., and Bardin, C.W. (1981). The pituitary-testicular axis in the STZ diabetic male rat: Evidence for gonadotropin in sertoli cell and leydig cell dysfunction. Int. J. Androl. 4: 265-280.

Norris, J.S., and Smith, R.G. (1983). Androgen Receptors. In: Principles of recepterology. M.K. Agarwal (ed). deGruyter, New York.

Ortiz-Caro, J., Gonzalez, C., and Jolin, T. (1984). Diurnal variations of plasma growth hormone, thyrotropin, thyroxine, and triiodothyronine in streptozotocin-diabetic and food-restricted rats. Endocrinology 115: 2227-2232.

Ota, M., Sato, N., Takahashi, S., and Ono, S. (1981). Partial Purification of Cytoplasmic Androgen Receptor in Rat Liver. Endocrinol. Japon. 28: 829-832.

Ota, M, Kyakumoto, S., and Nemoto, T. (1985). Demonstration and characterization of cytosol androgen receptor in rat exorbitial lacrimal gland. Bioch. International. 10: 129-135.

Pajunen, A.E.I., Isomaa, V.V., Jannes, O.A., and Bardin, C.W. (1982). Androgenic regulation of ornithine decarboxylase activity in mouse kidney and its relationship to changes in cytosol and nuclear androgen receptor concentrations. J. Biol. Chem. 257: 8190-8198.

Palandi, A.C., Pena, C., and Poskus, E. (1983). Molecular biology of growth hormone. CRC Crit. Rev. in Bioch. 15: 25-56.

Paz, G., Homonnai, Z.T., Harrell, A., and Kraicer, P.F. (1978). Improvement of the fertility of streptozotocin diabetic male rats following treatment with insulin and human chorionic gonatropin. Israel J. Med. Sci. 14: 1073-1078.

Pittman, C.S., Lindsay, R.H., Senga, O., Chambers, J.B.Jr., and Hill, J.B.Jr. (1981). The effects of diabetes mellitus on the 3,5,3'triiodothyronine production. Life Sci. 28: 1677-1682.

Poellinger, L., Lund, J., Gillner, M., Hansson, L.A., and Gustafsson, J.A. (1983). Physicochemical characterization of specific and non-specific polyaromatic hydrocarbon binders in rat and mouse liver cytosol. J. Biol. Chem. 258: 13535-13542.

Poortman, J., Prenen, J.A.C., Schwarz, F., and Thijssen, J.H.H. (1975). Interaction of Δ^5-androstene-3β,17β-diol with estradiol and dihydrotestosterone receptors in human myometrial and mammary cancer tissue. J. Clin. Endocrinol. Metab. 40: 373-379.

Rosen, V., Jung, I., Baulieu, E.E., and Robel, P. (1975). Androgen binding proteins in human benign prostatic hypertrophy. J. Clin. Endocrinol. Metab. 41: 761-770.

Roy, A.K., Milin, B.S., and McMinn, D.M. (1974). Androgen receptor in rat liver: Hormonal and developmental regulation of the cytoplasmic receptor and it's correlation with androgen-dependent synthesis of α_{2u}-globulin. Biochem. Biophys. Acta 354: 213-232.

Rumbaugh, R.C. and Lucier, G.W. (1982). Developmental correlation between rat hepatic cytosolic androgen binding protein and androgen inducible microsomal monooxygenase activity. 64th Annual Meeting of the Endocrine Society, San Francisco, CA. (abstract number 599).

Rumbaugh, R.C., McCoy, Z., and Lucier, G.W. (1984). Correlation of hepatic cytosolic androgen binding proteins with androgen induction of hepatic microsomal ethylmorphine N-dementylase in the rat. J. Steroid Bioch. 21: 243-252.

Sanborn, B.M., Wagle, J.R., and Steinberger, A. (1984). Control of androgen cytosol receptor concentrations in sertoli cells: Effect of androgens. Endocrinology 114: 2388-2393.

Scatchard, G., (1949). The attraction of proteins for small molecules and ions. Ann. N.Y. Acad. Sci. 51: 660-672.

Sheets, C.S., Aten, R.F., Kates, R.S., Preston, P.E., and Eisenfeld, A.J. (1985). Androgen receptor in the rabbit liver and apparent translocation to the nucleus in vivo. Endocrinology 116: 677-685.

Skeet, P., Chochrane, R.A., and Joels, L. (1984). The role o androgens in the effect of diabetes mellitus on hepatic drug metabolism in the male rat. Acta Endocrinol. 107: 506-512.

Sheridan, P.J. (1975). Is there an alternative to the cytoplasmic receptor model for the mechanism of action of steroids? Life Sci. 17: 497-502.

Sheridan, P.J. (1983). Androgen receptros in the brain: What are we measuring? Endocrine Rev. 4: 171-177.

Sheridan, P.J., Buchanan, J.M., Anselmo, V.C., and Martin, P.M. (1979). Equilibrium: The intracellular distribution of steroid receptors. Nature 282: 579-582.

Sloop, T.C., Clark, J.C., Rumbaugh, R.C., and Lucier, G.W. (1983). Imprinting of hepatic estrogen-binding proteins by neonatal androgens. Endocrinology. 112: 1639-46.

Snochowski, M., Pousette, A., Ekman, P., Bression, D., Andersson, L., Hogberg, B., and Gustafsson, J.A. (1977). Characterization and measurement of the androgen receptor in human benign prostatic hyperplasia and prostatic carcinoma. J. Clin. Endocrinol. Metab. 45: 920-930.

Sunahara, G.I. (1984). Characterization studies of the wistar rat hepatic cytosolic methyltrienolone (R1881) binding protein. Ph.D. thesis. University of British Columbia, Vancouver, B.C.

Sunahara, G.I., Finlayson, M.J.P., Warren, B.L., and Bellward, G.D. (1985). Characterization studies of a rat hepatic cytosolic androgen-binding protein. Can. J. Physiol. Pharmacol. 63: 952-957.

Tahiliani, A.G. (1983). Effects of insulin or thyroid treatment on diabetes-induced myocardial abnormalities. Ph.D. Thesis. University of British Columbia, Vancouver, B.C.

Tannebaum, G.S., Colle, E., Gurd. W., Wanamarker, L. (1981). Dynamic time course studies of the spontaneously diabetic BB Wistar rat. I. Longitudinal profiles of plasma growth hormone, insulin, and glusose. Endocrinology 109: 1872-1879.

Tannebaum, G.S. (1981). Growth Hormone Secretory Dynamics in Streptozotocin Diabetes. Evidence of a Role for Endogeneous Circulation Somatostatin. Endocrinology 108: 76-82.

Tesone, M. Biella de Souza Valle, L., Foglia, V.G., and Charreau, E.H. (1976). Endocrine function of the testis in streptozotocin diabetic rats. Acta Physiol. Lat. 26: 387-394.

Tesone, M., Oliveira-Filho, R.M., Biella de Souza Valle, L., Calvo, J.C., Baranao, J.L.S., Foglia, V.G., and Charreau, E.H. (1980). Androgen receptors in the diabetic rat. Diabetologia 18: 385-390.

Thieulant, M.L., Mercier, L., Samperez, S., and Jouan, P. (1975). Dihydrotestosterone-protein binding in the cytosol of rat anterior hypophysis in vitro: Evidence for a specific receptor. J. Ster. Bioch. 6: 1257-1260.

Traish, A.M., Muller, R.E., and Wotiz, H.H. (1981). A new procedure for the quantitation of nuclear and cytoplasmic androgen receptors. J. Biol. Chem. 256: 12028-12033.

Turocy, J.F., Chiang, A.N., Seely, D.H., and Eagon, P.K. (1985). Effects of H_2-antagonists on androgen imprinting of male hepatic functions. Endocrinology 117: 1953-1961.

Verhoeven, G., Heyns, W., and DeMoor, P. (1975). Ammonium sulfate precipitation as a tool for the study of androgen receptor proteins in rat prostate and mouse kidney. Steroids. 26: 149-167.

Walters, M.R. (1985). Steroid hormone receptors and the nucleus. Endocrin. Revs. 6: 512-543.

Warren, B.L., Pak, R., Finlayson, M., Gontovnick, L., Sunahara, G., and Bellward, G.D. (1983). Differential effects of diabetes on microsomal metabolism of various substrates: Comparison of streptozotocin and spontaneously diabetic wistar rats. Biochem. Pharmac. 32: 327-335.

Welshons, W.V., Lieberman, M.E., and Gorski, J. (1984). Nuclear localization of unoccupied oestrogen receptors. Nature 307: 747-749.

Wilson, E.M., and French, F.S. (1976). Binding propeties of androgen receptors: Evidence for identical receptors in rat testis, epididymis, and prostate. J. Biol. Chem. 251: 5620-5629.

Wilson, E.M., and French, F.S. (1979). Effects of proteases and protease inhibitors on the 4.5 S and 8 S androgen receptor. J. Biol. Chem. 254: 6310-6319.

Yamada, M., and Miyaji, H. (1982). Binding of sex hormones by male rat liver microsomes. J. Steroid Biochem. 16: 437-446.

Zava, D.T., Landrum, B., Horwitz, K.B., and McGuire, W.L. (1979). Androgen receptor assay with tritiated-methyltrienolone (R1881) in the presence of progesterone receptors. Endocrinology 104: 1007-1012.