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MODULATION OF PROLACTIN BINDING SITES IN VITRO BY MEMBRANE FLUIDIZERS

II. AGE-DEPENDENT EFFECTS ON RAT VENTRAL PROSTATIC MEMBRANES

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The objectives of this study were to determine (i) if the age-related changes in ^{125}I-labeled ovine prolactin specific binding of rat ventral prostate was correlated with changes in membrane lipid microviscosity and (ii) if membrane fluidizers produced age-dependent effects on prolactin binding of prostatic membranes. The degree of fluidization was monitored by a fluorescence polarization method using 1,6-diphenylhexatriene. Membrane preparations of ventral prostate glands obtained from immature (24–25 days old), young-adult (80–90 days old) and aged (550–610 days old) male rats were used for prolactin binding and membrane lipid microviscosity measurements. Relative to immature rats, prostatic prolactin binding decreased approximately 50% in young-adult rats and 75% in aged rats. Membrane lipid microviscosity, relative to immature rats, was increased 72% in young-adult rats and 140% in aged rats. Prostatic membranes obtained from immature animals exhibited no significant effects of in vitro alcohol treatment on prolactin binding, whereas, those obtained from aged animals exhibited maximal increase in prolactin binding. The value of the microviscosity parameter, after in vitro alcohol exposure, exhibited no significant changes in immature animals, whereas, this parameter was decreased approximately 15% in young-adults and approximately 30% in aged animals. These data suggest that in vitro fluidization of prostatic membrane exhibits an age-dependent modification of prolactin binding.

Introduction

In recent studies on murine hepatic and rat prostatic membranes the factors known to modulate prolactin binding in vitro and in vivo were also reported to alter membrane lipid microviscosity [1–4]. These and other studies have suggested that changes in membrane lipid microviscosity may alter prolactin and other peptide hormone receptor levels, possibly by modifying the physical nature of the surrounding lipid bilayer [5–7]. In a recent study we reported that aliphatic alcohols, known to fluidize certain membranes, increased in vitro the apparent fluidity and prolactin binding capacity of prostatic membranes of adult rats in a dose-dependent manner [8].

The studies reported herein were undertaken to (1) document the age-dependent changes in the lipid microviscosity and prolactin binding capacity of rat prostatic membranes, and (2) determine if in vitro exposure of prostatic membranes obtained from animals at various ages to aliphatic alcohols produced age-dependent changes in prolactin binding.

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Materials and Methods

Animals. Sprague-Dawley male and female rats (80–100 days old), obtained from Charles River Breeding Laboratories, were maintained and bred in our colony in a 12 h light-dark cycle with water and Purina rat chow available ad lib. Male offspring at 24–25, 80–90 and 550–610 days of age were used for the present study.

Reagents. Ovine prolactin (NIH-P-S-13, 35 IU/mg), obtained from the National Pituitary Agency, was dissolved in 0.01 M NH_4OH in an ice bath and then diluted in 25 mM Tris/0.9% NaCl at pH 7.4.

Tissue preparation. After decapitation, the ventral prostate glands were excised, frozen in liquid nitrogen, and pulverized. The tissues were homogenized for 1 min using a Brinkmann Polytron set at 6 in approximately 10 vol. of 0.3 M sucrose buffered to pH 7.6 with 25 mM Tris-HCl. The supernatant from a 10 min preliminary $125 \times g$ centrifugation was recentrifuged at $15\,000 \times g$ for 20 min to obtain a membrane pellet. This membrane pellet ($15\,000 \times g$) exhibited higher prolactin binding (per mg protein) than the whole homogenate, or $125 \times g$ or $100\,000 \times g$ pellets [4,9]. On the basis of marker enzyme analysis, this membrane fraction of ventral prostate gland was found to be rich in both Golgi and plasma membranes with some mitochondrial contamination (Dave, J.R. and Witorsch, R.J., unpublished data).

Prolactin binding assay. Ovine prolactin was iodinated with ^{125}I (Amersham) by a modification of the lactoperoxidase method of Thorell and Johansson [4,10]. The specific activity of iodinated prolactin was 90 μCi/μg. The membrane pellet was resuspended in 10 mM $MgCl_2$/25 mM Tris at pH 7.6 to provide approximately 3 mg protein/ml [11]. Membranes were exposed to aliphatic alcohols in two ways, either by coincubation with ligand or preincubation prior to the exposure of ligand. 100 μl of membrane suspension was incubated overnight at room temperature with varying concentrations of ethanol, 1-propanol or 1-butanol and 75 000 to 85 000 cpm of ^{125}I-labeled ovine prolactin with and without unlabeled ovine prolactin in a final volume of 0.5 ml of buffer (10 mM $MgCl_2$/0.1% bovine serum albumin/25 mM Tris-HCl, pH 7.6). In other experiments 100 μl of membrane suspension was exposed to the varying concentrations of alcohols for 15 min at room temperature in 0.5 ml of buffer (25 mM Tris-HCl, pH 7.6) and washed free of alcohols (by spinning the tubes at 2500 rpm for 10 min and washing the pellets twice with 1.0 ml buffer) prior to the addition of ligand. Binding assay was terminated by adding 1.0 ml of chilled buffer. The tubes were centrifuged at 2500 rpm for 20 min. The pellets were washed with an additional 1 ml of buffer and counted in a Nuclear-Chicago gamma counter. Each sample was assayed in triplicate. The diminution of radioactivity by coincubation with 1 μg unlabeled ovine prolactin represented specifically bound hormone. Under these assay conditions we routinely obtained 25–30% total and 8–12% non-specific binding of added iodinated prolactin to the prostatic membranes.

Membrane microviscosity. The fluorescence polarization technique, with 1,6-diphenylhexatriene was used for derivation of the steady-state polarization constant, P, and microviscosity parameter [12,13]. Membrane suspensions were incubated at 24°C with an equal volume of dispersion of 2 μM 1,6-diphenylhexatriene in phosphate-buffered saline at pH 7.1 for 45 min. Alcohols were added for a further 15 min of incubation. The unincorporated 1,6-diphenylhexatriene was removed by two washes with phosphate-buffered saline. This treatment also removed the alcohols whose presence otherwise might contribute some probe partitioning effects on the steady-state polarization measurements. Steady-state polarization measurements were made at 25°C on a SLM subnanosecond fluorometer (4800 series). The ratio output ($I_{\parallel}/I_{\perp}$) and $I_{\perp}$ from the photomultiplier tubes were entered directly into a 9815 Hewlett-Packard calculator for programming. $I_{\perp}$ and $I_{\parallel}$ are the fluorescence intensities polarized perpendicular and parallel to the polarization of the excitation beam [13]. Appropriate measures were taken to avoid light scattering and signal-to-noise effects on the polarization measurements. The values of steady-state polarization were used to determine the microviscosity parameter defined as $((r_0/r) - 1)^{-1}$, where r is the value of the anisotropy calculated from the measured polarization constant, P, according to the equation $r = 2P/(3 - P)$ and r_0 is the limiting value of the anisotropy of 1,6-diphen-

ylhexatriene [13] which in the present study was taken as 0.362. Data presented in the text are expressed both as P values and as 'microviscosity parameter' $((r_0/r)-1)^{-1}$, for comparison with other systems.

Results

Table I shows an age-dependent inverse relationship in prostatic prolactin binding capacity and membrane lipid microviscosity. Relative to immature rats, prostatic prolactin binding decreased approximately 50% in young-adult and 75% in aged rats. Membrane lipid microviscosity, relative to immature rats, was increased 72% in young-adult rats and 140% in aged rats.

Figs. 1A–C show that overnight incubation of prostatic membrane preparations obtained from rats at various ages with varying concentrations of alcohols and ^{125}I-labeled ovine prolactin resulted in age-dependent changes in specific prolactin binding. These changes in specific prolactin binding produced by alcohols also appeared to be dependent on membrane lipid microviscosity. As shown in Fig. 1A, ethanol at concentrations ranging from 1% to 16.7% had no significant effects on specific prolactin binding to prostatic membranes obtained from immature rats. However, it produced a dose-dependent increase and decline in prolactin binding of prostatic membranes from older rats. In three different experiments prolactin binding increased approximately 60% in young-adult rats and 170% in aged rats. A maximal increase in prolactin binding was observed in both groups at 4.8% ethanol.

Propanol at concentrations ranging from 1% to 16.7% produced a progressive decrease in specific prolactin binding to prostatic membranes from immature animals and a dose-dependent increase and decline in the same in young-adult and aged rats (Fig. 1B). In three different experiments prolactin binding increased approximately 50% in young-adult rats and 140% in aged rats. A maximal increase in prolactin binding was observed in both groups at 3.8% propanol.

Butanol from 1% to 9.1% concentrations produced a progressive decline in prolactin binding in immature rats and a dose-dependent increase and decline in the same in older rats (Fig. 1C). In three different experiments prolactin binding increased approximately 40% in young-adult rats and 110% in aged rats. A maximal increase in prolactin binding was observed in both groups at 1.0% butanol.

Pre-exposure of prostatic membranes of young-adult rats at room temperature to 4.8% ethanol followed by a wash and subsequent addition of ^{125}I-labeled ovine prolactin resulted in a time-dependent increase in prolactin binding (data reported in Ref. 8). A maximal 61% increase in prolactin binding was observed after 15 min of ethanol pre-incubation which was consistent with that observed by overnight incubation of membranes with 4.8% ethanol and ^{125}I-labeled ovine prolactin. In other experiments 15 min of membrane preincubation (obtained from animals in the three age groups) with either 4.8% ethanol, 3.8% 1-propanol or 1% 1-butanol prior to addition of ligand increased prolactin binding to a value which was consistent in magnitude with that observed by overnight incubation of membranes with the respective alcohol and ^{125}I-labeled ovine prolactin (data not presented).

The physical effects of the alcohols on prostatic membranes were quantitated by fluorescence polarization measurements. Table II shows the effects of in vitro treatment of alcohols on ap-

TABLE I

DEMONSTRATION THAT SPECIFIC BINDING OF PROLACTIN TO PROSTATIC MEMBRANES DECREASED AND MEMBRANE LIPID MICROVISCOSITY INCREASED AS A FUNCTION OF AGE

Prostatic membranes from Sprague-Dawley rats were assayed for their prolactin-binding capacity using ^{125}I-labeled ovine prolactin and membrane lipid microviscosity using 1,6-diphenylhexatriene as described in the text. Each value represents the mean ± S.D. of three different experiments each assayed individually in triplicate. Values in parentheses are percent of values obtained for 24–25-day-old rat prostatic membranes.

Age (days)	^{125}I-prolactin specific binding (cpm/mg protein)	Polarization constant, P	Microviscosity parameter, $((r_0/r)-1)^{-1}$
24– 25	64839 ± 2386	0.108 ± 0.003	0.260 ± 0.006
80– 90	31953 ± 3153 (49)	0.159 ± 0.002 (147)	0.448 ± 0.004 (172)
550–610	16058 ± 2936 (25)	0.195 ± 0.001 (181)	0.624 ± 0.002 (240)

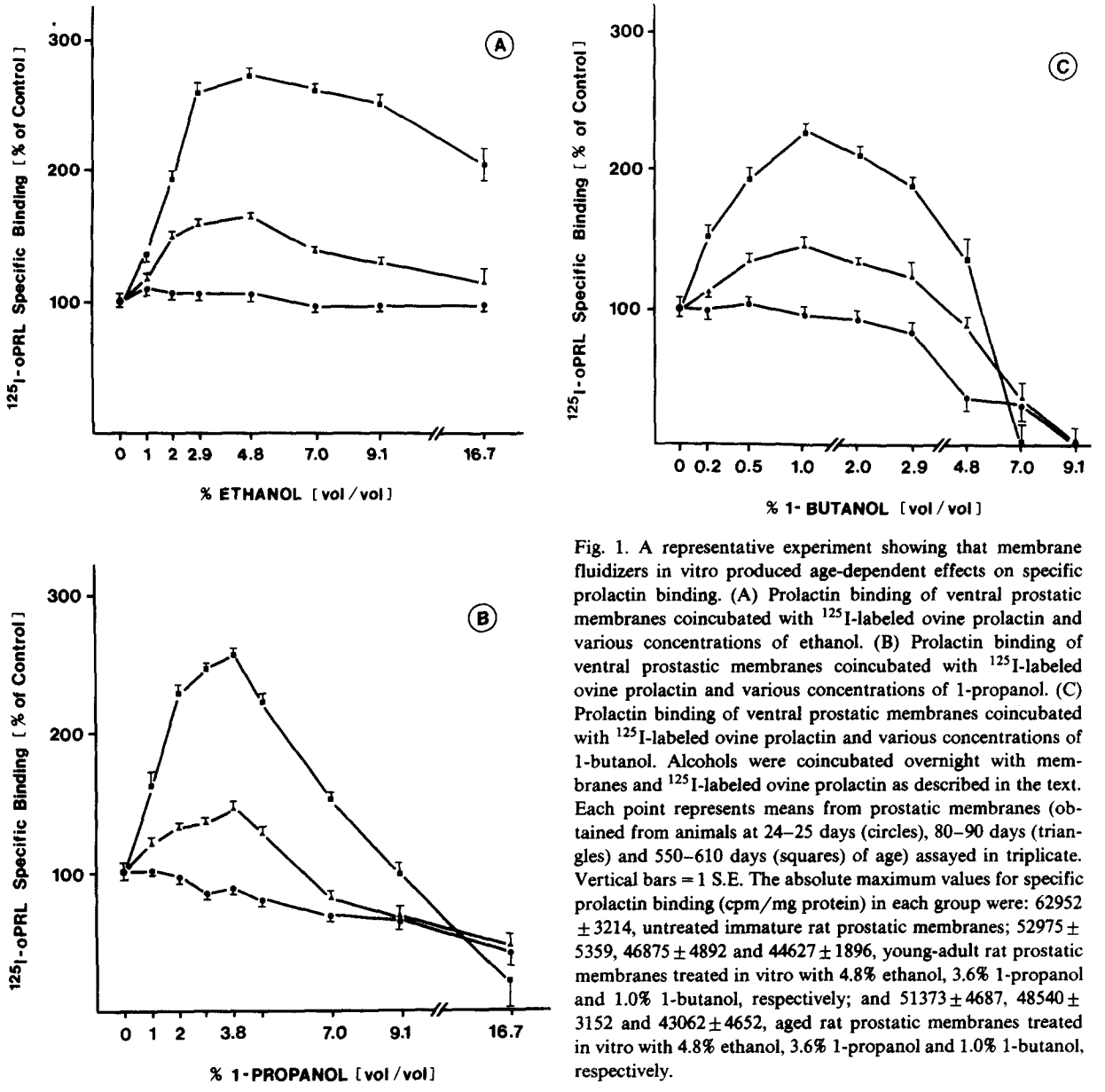

Fig. 1. A representative experiment showing that membrane fluidizers in vitro produced age-dependent effects on specific prolactin binding. (A) Prolactin binding of ventral prostatic membranes coincubated with ^{125}I-labeled ovine prolactin and various concentrations of ethanol. (B) Prolactin binding of ventral prostastic membranes coincubated with ^{125}I-labeled ovine prolactin and various concentrations of 1-propanol. (C) Prolactin binding of ventral prostatic membranes coincubated with ^{125}I-labeled ovine prolactin and various concentrations of 1-butanol. Alcohols were coincubated overnight with membranes and ^{125}I-labeled ovine prolactin as described in the text. Each point represents means from prostatic membranes (obtained from animals at 24–25 days (circles), 80–90 days (triangles) and 550–610 days (squares) of age) assayed in triplicate. Vertical bars = 1 S.E. The absolute maximum values for specific prolactin binding (cpm/mg protein) in each group were: 62952 ± 3214, untreated immature rat prostatic membranes; 52975 ± 5359, 46875 ± 4892 and 44627 ± 1896, young-adult rat prostatic membranes treated in vitro with 4.8% ethanol, 3.6% 1-propanol and 1.0% 1-butanol, respectively; and 51373 ± 4687, 48540 ± 3152 and 43062 ± 4652, aged rat prostatic membranes treated in vitro with 4.8% ethanol, 3.6% 1-propanol and 1.0% 1-butanol, respectively.

parent microviscosity (inversely related to membrane lipid fluidity) expressed both as the measured values of steady state polarization and calculated values of microviscosity parameter $((r_0/r)-1)^{-1}$. Exposure of alcohols decreased the value of prostatic membrane microviscosity parameter in young-adult and aged rats. However, no significant changes in the same were observed in immature rat prostatic membranes. The relative degree of fluidization by the alcohols was greater in magnitude in membranes obtained from aged rats than in young-adult rats. The microviscosity parameter values were 80%, 88% and 92% of control in young-adult rats and 64%, 70% and 72% of control in aged rats after the exposure to 4.8% ethanol, 3.8% 1-propanol and 1% 1-butanol, respectively. At higher alcohol concentrations the value of microviscosity parameter was consistently increased (data not presented).

TABLE II

EFFECTS OF IN VITRO EXPOSURE OF ALIPHATIC ALCOHOLS ON STEADY-STATE POLARIZATION AND MICROVISCOSITY PARAMETER OF PROSTATIC MEMBRANES OBTAINED FROM ANIMALS AT DIFFERENT AGES

Steady-state polarization, proportional to the apparent microviscosity, was measured at 25°C using 1,6-diphenylhexatriene. Microviscosity parameter was calculated according to Ref. 13, as described in the text. Each value represents the mean from two experiments assayed individually in triplicate ± S.D. Values in parentheses are percent of control values.

Age (days)	Treatment (in vitro)	Polarization constant, P	Microviscosity parameter, $((r_0/r)-1)^{-1}$
24– 25	none	0.112 ± 0.002	0.272 ± 0.004
	ethanol 4.8%	0.109 ± 0.002	0.263 ± 0.004 (96)
	propanol 3.8%	0.118 ± 0.003	0.292 ± 0.006 (107)
	butanol 1.0%	0.113 ± 0.001	0.276 ± 0.002 (101)
80– 90	none	0.154 ± 0.001	0.426 ± 0.002
	ethanol 4.8% [a]	0.132 ± 0.001	0.341 ± 0.002 (80)
	propanol 3.8% [a]	0.142 ± 0.001	0.376 ± 0.001 (88)
	butanol 1.0% [a]	0.145 ± 0.001	0.390 ± 0.001 (92)
550–610	none	0.192 ± 0.002	0.607 ± 0.004
	ethanol 4.8%	0.145 ± 0.002	0.390 ± 0.003 (64)
	propanol 3.8%	0.153 ± 0.001	0.422 ± 0.001 (70)
	butanol 1.0%	0.156 ± 0.001	0.435 ± 0.001 (72)

[a] Values taken from Ref. 8. The measurements of membrane microviscosity in both these studies (present and earlier [8]) were carried out simultaneously.

Discussion

The observation we made in the present study that specific prolactin binding of prostatic membranes decreases progressively from weaning to senescence is consistent with earlier reports [14,15]. The cause of this post-pubertal decline in prostatic prolactin binding remains, at this time, speculative. However, in a recent study we observed that pretreatment of prostatic membranes from intact young-adult rats with dextran-coated charcoal for 30 min at room temperature prior to radioreceptor assay, resulted in 30–67% increase in ^{125}I-labeled ovine prolactin binding capacity [14]. Similar treatment failed to increase prolactin binding in immature or young-adult rats castrated 3 days previously [14]. Furthermore, in vitro addition of dihydrotestosterone, and not testosterone or estradiol, to immature rat prostatic membranes and to dextran-coated charcoal pretreated membranes of adult rats resulted in a dose-dependent decline in prolactin binding. The post-pubertal decline in prostatic prolactin binding observed in the present and earlier studies and effects of dextran-coated charcoal in intact adult prostate suggest that substances of testicular origin may inhibit, in part, prolactin binding of prostatic membranes.

The estimates of lipid microviscosity employed in this study represent averaged measures of changes throughout the membranes. These changes in specific or localized domains within the membranes may be more extensive than the averaged estimates. The demonstration of a high microviscosity in the prostatic membranes of aged animals and a low microviscosity in immature animals compared to that in the young-adults in this study is in agreement with the earlier findings in mouse liver [16]. Other investigators have reported that the activity of 3-hydroxybutyrate dehydrogenase in the mitochondrial fraction of the rat brain is inhibited after weaning due to increased membrane lipid bilayer rigidity [17]. Recently, changes in enzyme kinetics and Arrhenius plots of both adenylate cyclase and 5′-nucleotidase activity in rat liver plasma membranes during weaning were attributed to the altered lipid environment [18]. An increase in lymphocyte membrane microviscosity in aged mice has been re-

ported by Rivnay et al. [19] who have attributed these changes in microviscosity to elevations of serum cholesterol [19]. Support for this thesis also comes from another study in which liver cholesterol concentrations were reported to be 2-fold higher and phospholipid concentration 30% lower in 20-month-old animals when compared to month-old animals [20]. Thus, the increase in the membrane lipid microviscosity in the aged animals in the present study may be attributed to the altered serum and prostatic membrane lipid composition. While changes in the lipid microviscosity of membranes can modify biochemical events on the cell surface, the mechanism by which such events occur and their physiological significance remain, at the present time, unclear.

The non-specific fluidization effects of aliphatic alcohols on a variety of membrane systems have been well documented [7,21,22]. The age-dependent effects of alcohols on prolactin binding reported in this study suggest a direct relationship between prostatic prolactin receptors and membrane lipid fluidity. These and earlier observations in rat prostate and in other prolactin target organs support the hypothesis than an alteration in the fluidity of the membrane lipid bilayer affects the accessibility of the receptor for its hormone. Membrane proteins, including receptor proteins are now considered to float within the matrix of the lipid bilayer. Changes in the receptor protein functionality are presumably due to changes in the mobility or orientation of these proteins within the bilayer. Increased lipid fluidity would tend to increase the mobility of receptor protein and, thus, increase the detectablity of cryptic prolactin receptors. Such effects of increased membrane lipid fluidity leading to exposure of a larger proportion of the cryptic prolactin and other peptide hormone receptors have been reported by other investigators [23,24].

The data presented herein suggest that alcohols produced age-dependent and membrane lipid microviscosity-dependent changes in prostatic prolactin binding according to their capacity to induce membrane fluidization. Prostatic membranes from immature animals did not respond to this in vitro fluidization. This may be related to the differences in the lipid composition of these membranes. It also appears that the longer the carbon chain length of the alcohol, the lower the concentration required to produce a maximal increase in prolactin binding, whereas the magnitude of the peak response is inversely related to length of the alcohol. These differences in the magnitude of the peak response and in the concentration of alcohols required to produce a maximal increase in prolactin binding in the present study and earlier [8] studies may be related to the differences in stability of prolactin receptors to various alcohols. These data are also in agreement with findings that in vitro exposure of membrane fluidizers enhanced chemoattractant binding to human [25,26] and rabbit [27] polymorphonuclear leukocytes, gonadotropin binding to primate luteal membranes [23] and α-adrenergic binding in brain [7].

In conclusion, the present study documents age-dependent changes in prostatic membrane lipid microviscosity and prolactin binding in rat from weaning to senescence. The age-dependent effects produced by in vitro fluidization of prostatic membranes further suggest that the changes in prolactin binding from weaning to senescence appears to be mainly due to a change in microviscosity-dependent availability of prolactin receptor rather than apparent receptor number. However, whether the effects produced by 'testicular substances' on prostatic prolactin binding during maturation are mediated via membrane lipid microviscosity changes, remains to be determined.

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References

1 Dave, J.R. and Knazek, R.A. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 6597–6600
2 Dave, J.R., Knazek, R.A. and Liu, S.C. (1981) Biochem. Biophys. Res. Commun. 100, 45–51
3 Dave, J.R., Brown, N.V. and Knazek, R.A. (1982) Biochem. Biophys. Res. Commun. 108, 193–199
4 Dave, J.R. and Witorsch, R.J. (1983) Prostate 4, 119–128

5 Dave, J.R., Knazek, R.A. and Liu, S.C: (1981) Biochem. Biophys. Res. Commun. 103, 727–738
6 Huhtaniemi, I.T., Katikineni, M., Chan, V. and Catt, K.J. (1981) Endocrinology 108, 58–65
7 Ciofalo, F.R. (1978) Proc. West. Pharmacol. Soc. 21, 267
8 Dave, J.R. and Witorsch. R.J. (1983) Biochem. Biophys. Res. Commun. 113, 220–228
9 Keenan, E.J., Ramsey, E.E. and Kemp, E.D. (1981) in The Prostatic Cell: Structure and Function (Murphy, G.P., Sandberg, A.A. and Karr, J.P., eds.), Part B, pp. 9–18, Alan R. Liss, New York
10 Thorell, J.I. and Johansson, B.G. (1971) Biochim. Biophys. Acta 251, 363–369
11 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
12 Chen, R.E., Edelhoch, H. and Steiner, R.J. (1969) in Physical Principles and Techniques of Protein Chemistry (Leach, S.J., ed.), Part A, pp. 171–240, Academic Press, New York
13 Shinitzky, M. and Barenholz, Y. (1978) Biochim. Biophys. Acta 515, 367–394
14 Dave, J.R. and Witorsch, R.J. (1982) Endocrinology 111, 2144–2146
15 Aragona, C. and Friesen, H.G. (1975) Endocrinology 97, 677–684
16 Dave, J.R. and Knazek, R.A. (1983) Mech. Aging Dev. 23, 235–243
17 Houslay, M.D., Palmer, R.W. and Duncan, J.S. (1978) J. Pharm. Pharmac. 30, 711–713
18 Dipple, I. and Houslay, M.D. (1982) Biochem. Biophys. Res. Commun. 105, 259–263
19 Rivnay, B., Globerson, A. and Shinitzky, M. (1979) Mech. Aging Dev. 10, 71–79
20 Carlson, L.A., Froberg, S.O. and Nye, E.R. (1968) Gerontologia 14, 65–79
21 Chin, J.H., Goldstein, D.B. and Parsons, L.M. (1979) Alcoholism: Clin. Exptl. Res. 3,47–49
22 Sauerheber, R.D., Esgate, J.A. and Kuhn, C.E. (1982) Biochim. Biophys. Acta 691, 115–124
23 Cameron, J.L. and Stouffer, R.L. (1982) Endocrinology 110, 1451–1453
24 Bhattacharya, A. and Vonderhaar, B.K. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 4489–4492
25 Yuli, I., Tomonaga, A. and Snyderma, R. (1982) Proc. Natl. Acad. Sci. U.S.A. 79, 5906–5910
26 Liar, C.S. and Preer, R.J. (1980) Biochem. Biophys. Res. Commun. 93, 566–571
27 Fletcher, M.P., Seligmann, B.E. and Gullin, J.I. (1982) J. Immunol. 128, 941–948