J. steroid Biochem. Vol. 23, No. 3, pp. 333–338, 1985
Printed in Great Britain.

0022-4731/85 $3.00 + 0.00

A STUDY OF [^{3}H]ALDOSTERONE BINDING BY NUCLEAR AND CYTOPLASMIC RECEPTORS OF THE RAT KIDNEY WITH DIFFERENT CONTENT OF ALDOSTERONE IN THE ORGANISM

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(*Received* 1 *August* 1984)

Summary—The distribution of specific mineralocorticoid receptors in rat kidney cells was found to depend on aldosterone concentration. With increasing aldosterone concentrations the number of specific receptors for aldosterone in the cytoplasm decreased and their quantity in the nuclei increased. This was evidently due to their enhanced transport from the cytoplasm to the nuclei.

It was found that aldosterone (its complex with the receptor) attached to non-histone proteins of chromatin and that the structural integrity of DNA is needed to provide the binding of this complex to the cell nuclei.

The quantity of aldosterone acceptor sites in the kidney cell nuclei remained constant when aldosterone concentrations in the organism changed, and, hence, the functional states of the target organ changed too.

INTRODUCTION

It is known that many hormones affect the organism by initiating or enhancing the transcription of certain genes [1, 2, 3]. The first step of the mechanism for the action of a steroid hormone in target cells is the formation of a hormone complexed with specific receptor proteins of the cytoplasm. This complex is then transported to the cell nuclei, where, interacting with chromatin, it triggers enchanced mRNA transcription and, consequently, the induction of enzymes and other proteins responsible for the physiological effect of the hormone [4, 5]. Alterations in the quantity and distribution of specific receptors in the target cells may, probably, affect the sensitivity of these cells to the corresponding hormones.

Aldosterone, the main mineralocorticoid hormone in the organism, regulates ion transport and stimulates sodium ion reabsorption in the renal tubule.

In the cytoplasm and renal cell nuclei of adrenalectomized rats, protein receptors were found, which possess high affinity for aldosterone [6, 7]. As to estradiol, increase in its concentration is followed by a decrease in hormone–receptor complexes in the cytoplasm and an increase in their quantity in target cell nuclei; the functional target tissue response to the hormone is directly related to the amount of nuclear hormone–receptor complexes [8]. Estradiol induces the synthesis of specific receptors in target cells [9].

The amount and distribution of specific aldosterone receptors in target-cells could presumably depend on aldosterone content in the organism.

This paper presents a study of [^{3}H]aldosterone receptors in rat kidney cells differing in the content of aldosterone in the organism.

EXPERIMENTAL

Steroids

Labelled steroids 1,2-[^{3}H]aldosterone, sp. act. 53 Ci/mmol and 1,2,6,7-[^{3}H]aldosterone, sp. act. 125 Ci/mmol (Amersham, England) [labelled aldosterone was purified before use by thin layer chromatography], unlabelled steroids: aldosterone, corticosterone, deoxycorticosterone, estradiol (Koch-Light, England).

Animals

Male albino rats (180–240 g) were used in the experiments. Decrease or increase in adrenal aldosterone secretion was achieved by changing sodium content in the ration. The surplus of sodium in animals was attained by feeding them a high sodium diet (270–300 mEq/kg b.wt per day) for 9–11 days with water *ad libitum*. The deficiency of sodium in animals was caused by feeding them with a low sodium diet (0.05–0.1 mEq of Na/kg b.wt per day) with water *ad libitum* at the same time. The controls were: (1) rats fed a standard diet; (2) rats subjected to bilateral adrenalectomy and sacrificed at different intervals after the operation (these animals received instead of water a 0.9% NaCl solution after the operation); (3) adrenalectomized rats (12 days after the operation) injected with aldosterone, 5 h before sacrifice in a dose of 20 μg per 100 g of a body weight.

Isolation of nuclei and cytosol: kidneys were excised in the cold (0–4°C) immediately after sacrifice. Cytosol and nuclei were obtained from the kidneys of 3 rats. Renal tissue was homogenized in Tris–sucrose buffer (0.25 M sucrose, 0.003 M of $MgCl_2$, 0.01 M of

Tris–HCl, pH 8.0) and the homogenate was centrifuged at 900 *g* for 10 min. To produce cytosol the supernatant was recentrifuged for 60 min at 10,500 *g*. After the first centrifugation the residue was resuspended in dense sucrose ($d = 1.293$) and centrifugated for 60 min at 22,000 *g*. The sedimented nuclei were washed with Tris–sucrose buffer.

Determination of [^{3}H]aldosterone binding in cytosol

The determination of [^{3}H]aldosterone binding in cytosol was carried out by an exchange method in our modification for aldosterone [10]. This method permits to establish the total number of binding sites for the hormone, i.e. both the number of sites which are free at the time of cytosol isolation and those which are occupied by endogenous hormone. It has been previously shown that the cytosol fraction of renal cells has two types of aldosterone binding receptors: there are mineralocorticoid receptors with high affinity for aldosterone, and glucocorticoid receptors with lower affinity for aldosterone [6, 7, 11]. In the present work we studied [^{3}H]aldosterone binding to the first type of receptors only.

Cytosol from rat kidneys was incubated with unlabelled aldosterone (0.55 nmol of the hormone per ml of cytosol) for 1 h at 0°C, a 2% suspension of charcoal Norit A with 0.2% dextran-15 in Tris–sucrose buffer were added to the suspension (2 mg of charcoal per ml of cytosol). The suspension was shaken in the cold for 10 min and centrifuged at 4000 *g* for 10 min. The supernatant fraction was recentrifuged to remove all the charcoal.

Cytosol samples were saturated with unlabelled aldosterone, incubated with [^{3}H]aldosterone for 2 h at 0°C (amount of [^{3}H]aldosterone was increased from 0.18 up to 7.3 nmol for 0.5 ml of cytosol).

To remove the labelled aldosterone which remained unbound to cytosol protein, the samples were passed through fine Sephadex G-50 columns; radioactivity in the macromolecular fraction was determined in a liquid scintillation spectrometer Mark-II (Nuclear Chicago, U.S.A.) and protein content was measured according to Lowry[13]. The dissociation constant of the aldosterone–receptor complex and the number of binding sites for aldosterone were determined by Scatchard's method [14]. The two components of the specific binding on the plot corresponded to types I and II receptors (separation of the components was done as suggested by Rosenthal[15]. Thus, the quantity of the nonlabelled aldosterone complexed with the mineralocorticoid receptor formed after 2 h of incubation at 0°C was estimated in cytosol.

The half-life of the aldosterone–receptor complex was determined during the incubation of renal cytosol with labelled aldosterone and unlabelled corticosterone. Corticosterone was added to prevent the binding of [^{3}H]aldosterone to the receptors for glucocorticoids present in renal cytosol [12]. 0.5 ml Aliquots of cytosol were incubated with 7.3 nmol of [^{3}H]aldosterone and 36 nmol of corticosterone for 30 min at 25°C. The free hormones were removed by passage through a fine Sephadex G-50 column.

Protein content and radioactivity were first measured in the macromolecular fraction, then the content of [^{3}H]aldosterone remaining complexed with the receptors was determined by removing the free hormone with charcoal suspended in dextran (as described above).

The determination of the half-life of [^{3}H]aldosterone–receptor complex was based on the plot, where the time is the log concentration of the bound hormone. This time was found to be 4.5 h.

Having determined the half-life of the mineralocorticoid receptor–aldosterone complex, we calculated the total number of binding sites of type I.

Determination of [^{3}H]aldosterone binding in nuclei

The ability of renal cell nuclei to bind [^{3}H]aldosterone was determined by a method which provides exchange of endogenous hormone in the cell nucleus for the radioactivie one in the incubation medium [16]. One ml of Tris–sucrose buffer or 1 ml of cytosol (12–14 mg of protein) and 10.9 pmol of [^{3}H]aldosterone were added to 1 ml of the suspension, thoroughly shaken and incubated for 1 h at 25°C. The nuclear suspension after incubation with labelled aldosterone was layered upon 5% dextran-500 solution and centrifuged for 20 min at 3000 *g*. This procedure makes it possible to separate the free [^{3}H]aldosterone in the incubation medium from the hormone in the cell nuclei [17]. The precipitate was used for the fractionation of nuclear proteins [18]. DNA content in the samples was estimated according to Burton[19].

In all experiments, the nonspecific binding of [^{3}H]aldosterone by cytosol and renal cell nuclei was determined. To this end, together with the labelled aldosterone a 2000-fold excess of unlabelled hormone was added to the samples, and the subsequent treatment was performed as above. All data are presented after subtraction of nonspecific binding.

RESULTS

Content of aldosterone in rat plasma

Aldosterone in rat plasma was determined by the radioimmunoassay using standard kits (Aldosterone Radioimmunoassay Kit, "Aldok", CEA-IRE-SORIN).

Table 1 shows that plasma aldosterone concentration is 1.5 times lower in sodium loaded rats and

Table 1. Content of aldosterone in the rat plasma

Experimental conditions	*n*	Aldosterone (ng/100 ml)
Control	24	47.0 ± 3.8
Sodium load	12	30.3 ± 3.5
Sodium deficiency	30	284.1 ± 25.3
Adrenalectomy (5–14 days after operation)	16	<3.0

n—The number of animals.

Table 2. The number of aldosterone binding sites (estimated by the exchange method) in mineralocorticoid receptors of the cytosol fraction and [^{3}H]aldosterone binding by rat renal cell nuclei

Experimental conditions	Cytosol, number of binding sites 10^{-14} mol/mg of protein	Mean	Nuclei, [^{3}H]aldosterone binding 10^{-15} mol/mg of DNA	Mean
Sodium load	3.48		3.4	
	3.67	3.57	5.8	4.6
Sodium deficiency	1.13		13.2	
	1.46	1.30	7.2	10.2
Adrenalectomy	3.51		3.7	
(12 days after operation)	3.93	3.72	3.7	3.7
Aldosterone injection to	1.10		8.5	
adrenalectomized rats	0.43	0.77	10.3	9.4
Control	2.57		8.9	
	2.53	2.55	4.8	6.8

For every estimation the kidneys of three rats were pooled.

6 times higher in sodium deficient rats as compared with the control. Plasma aldosterone concentration in sodium-loaded and -deficient rats differ approx 10-fold.

[^{3}H]*Aldosterone binding in rat renal cytosol*

The results of this analysis of [^{3}H]aldosterone binding by the cytosol fraction of renal cells in rats are shown in Table 2. The dissociation constant of the mineralocorticoid receptor–aldosterone complex in renal cytosol of different experimental groups is 4.9×10^{-9} M. This is in agreement with the data in the literature [7].

The quantity of aldosterone binding sites estimated by the exchange method is 3-fold higher for the first type in the kidney cytosol of sodium loaded rats than in renal cytosol of sodium deficient animals. We also evaluated the quantity of binding sites for [^{3}H]aldosterone in renal cytosol of control and adrenalectomized rats and also in adrenalectomized animals injected with a large dose of aldosterone (20 μg per 100 g b.wt) 5 h before sacrifice. It was found that, when the quantity of aldosterone binding sites in the renal cytosol fraction in adrenalectomized rats is taken for 100%, this quantity is virtually unchanged in the renal cytosol fraction of rats with suppressed aldosterone synthesis (sodium load). In the case of aldosterone surplus in the organism (sodium deficiency of aldosterone injection), the quantity of binding sites in cytosol decreases to 35 and 20%, respectively (Table 2). Thus, judging by these results, the higher the concentration of aldosterone in the blood, the lower the quantity of specific mineralocorticoid receptors in renal cytosol.

[^{3}H]*Aldosterone binding by rat renal cell nuclei*

[^{3}H]Aldosterone binding by rat renal cell nuclei was estimated by the exchange method. Endogenous aldosterone in the cell nucleus is exchanged for the radioactive hormone in the incubation medium [16].

As seen in Table 2, [^{3}H]aldosterone binding by renal cell nuclei of rats with low aldosterone content in the blood (sodium load or adrenalectomy) is not high; on the contrary, [^{3}H]aldosterone binding by renal cell nuclei of adrenalectomized rats injected with aldosterone and by those of sodium deficient rats, when the content of aldosterone is high, is considerably elevated.

[^{3}H]*Aldosterone binding by renal cell nuclear proteins of rats*

There are two main forms of nuclear receptors for aldosterone in renal cell nuclei: receptors extractable with 0.1 M Tris–HCl buffer and receptors extractable with 0.4 M KCl solution. The 0.1 M Tris–HCl buffer extracts soluble nuclear proteins and 0.4 M KCl solution-nonhistone proteins of chromatin [6, 20].

When cell nuclei were treated with 0.1 M Tris–HCl buffer, a part of intranuclear [^{3}H]aldosterone was extracted too: this radioactivity was about 50% in rats with sodium load and it was about 30% of total radioactivity in rats with sodium deficiency. While

Table 3. Binding of [^{3}H]aldosterone *in vitro* by renal cell nuclei and nuclear proteins of rats fed as high or low sodium diet

Experimental conditions	Bound [^{3}H]aldosterone 10^{-15} mol/mg DNA: Nuclei	0.1 M Tris-extract of nuclei	0.1 M Tris-extract of nuclei after treatment with CDS	0.4 M KCl extract of nuclei	0.4 M KCl extract of nuclei after CDS treatment
Sodium load	5.2 ± 0.7	2.5 ± 0.5	0	1.1 ± 0.2	0.2 ± 0.1
(hypoaldosteronemia)	(6)	(6)	(6)	(6)	(5)
Sodium deficiency	10.0 ± 2.6	2.8 ± 0.8	0	2.1 ± 0.1	1.7 ± 0.2
(hyperaldosteronemia)	(6)	(6)	(6)	(4)	(4)

The number of experiments is shown in parentheses; CDS = charcoal–dextran suspension.
Cytosol was not added to the incubation medium.

Table 4. [^{3}H]Aldosterone binding *in vitro* by nuclei and nuclear proteins of renal rat cells in the presence of cytosol extracted from the kidneys of adrenalectomized rats

Experimental conditions	Bound [^{3}H]aldosterone, 10^{-15} mol/mg DNA				
	Nuclei	0.1 M Tris-extract of nuclei	0.1 M Tris-extract of nuclei after CDS treatment	0.4 M KCl extract of nuclei	0.4 M KCl extract of nuclei after CDS treatment
Sodium load (hypoaldosteronemia)	3.5 ± 7.9	5.0 ± 0.9	0	5.6 ± 1.2	5.2 ± 0.9
Sodium deficiency (hyperaldosteronemia)	36.6 ± 10.2	3.7 ± 1.1	0	5.9 ± 2.0	5.2 ± 1.1

The means of three experiments are presented in the table CDS = charcoal–dextran suspension. Renal cell cytosol of adrenalectomized rats (5 days after the operation) was added to the incubation medium. Conditions were standard (See Experimental). One ml of renal cytosol from adrenalectomized rats (protein concentration 12–14 mg/ml) was added instead of the buffer to the incubation medium.

treating these extracts with the charcoal–dextran suspension to separate free [^{3}H]aldosterone, almost all the labelled hormone were separated from the proteins; this suggests that it is not stably bound with them and that this interaction may be of low specificity (Table 3).

Table 3 shows that the quantity of [^{3}H]aldosterone is about twice higher in 0.4 M KCl-extracts of cell nuclei (non-histone chromatin proteins) from the kidneys of rats fed a sodium deficient diet (hyperaldosteronemia) than in the extracts from renal cell nuclei of sodium loaded rats (hypoaldosteronemia). This difference increases after the unstably bound [^{3}H]aldosterone is separated from the extracted by 0.4 M KCl solution non-histone proteins of chromatin with the aid of the charcoal–dextran suspension. It is evident that in hyperaldosteronemic rats the quantity of [^{3}H]aldosterone stably bound to the non-histone proteins of renal nuclei is 6 times higher in hypoaldosteronemic rats (1.7 and 0.2, respectively).

[^{3}H]Aldosterone binding in vitro *by nuclei. Renal cytosol of adrenalectomized rats is added to the incubation medium*

It seemed important to determine whether the difference between the nuclei of hyper- and hypoaldosteronemic rats is due to changes in characteristics of DNP (deoxyribonucleoprotein) itself and in the content of the corresponding nuclear proteins or the [^{3}H]aldosterone binding by nuclei depends mainly on the quantity of cytoplasmic receptors.

The results of experiments with the incubation of renal cell nuclei with labelled aldosterone in the presence of cytosol fraction from kidneys of adrenalectomized rats are shown in Table 4. The binding of labelled aldosterone by renal cell nuclei of hypo- and hyperaldosteronemic rats was found to increase equally sharply when the renal cytosol fraction of adrenalectomized rats was added to the incubation medium.

Incubation of nuclear DNP with hydrolytic enzymes

In experiment consisting in the addition of renal cytosol of adrenalectomized rats to nuclei (Table 4) the quantity of [^{3}H]aldosterone tightly bound to DNP, nonextractable with 0.1 M Tris–HCl buffer and 0.4 M KCl solution (the residual fraction forms) made up about 70% of all the labelled hormone bound by renal cell nuclei of hyper- and hypoaldosteronemic rats. The nature of these chromatin acceptor sites and the role of proteins and DNA in the interaction with the hormone are of interest.

The incubation of DNP with hydrolytic enzymes shows that pronase and chymotrypsin release almost all the radioactivity of the residue fraction into the soluble state. Trypsin, the enzyme of high specificity relative to alkaline proteins, release about 70% of the label into the solution; with ribonuclease (RNAse) and deoxyribonuclease (DNAse), the enzymes hydrolysing nucleic acids, radioactivity is not so readily tranferred into the solution and the label remains in this residual fraction of nuclei (Table 5).

The nuclei treatment with DNAse

In subsequent experiments, the previous treatment of cell nuclei with DNAse was found to decrease sharply the nuclear binding ability for the [^{3}H]aldosterone–receptor complex (Table 6); almost all the labelled hormone bound with cell nuclei, preincubated with DNAse, were extracted with a 0.1 M Tris–HCl buffer and 0.4 M KCl solution.

DISCUSSION

In this study of the [^{3}H]aldosterone binding in the cytosol of rat kidney by the exchange method [10], it was possible to estimate the total quantity of the

Table 5. The results of treatment of DNP with hydrolytic enzymes of rat renal nuclei

Enzymes	% Of [^{3}H]aldosterone transferred into the solution from nuclear residue after incubation with an enzyme
RNAse	6.2 ± 1.6
DNAse	5.0 ± 1.0
Trypsin	69.0 ± 8.7
Chymotrypsin	90.0 ± 5.0
Pronase	93.8 ± 4.2

The quantity of [^{3}H]aldosterone in the residual fraction of renal cell nuclei is accepted as 100%. The time of enzyme incubation with DNP is 30 min at 25°C, the final enzyme concentration of RNAse, trypsin, chymotrypsin is 500 μg/ml, that of DNAse and pronase 250 μg/ml. After incubation the nuclear suspension was centrifugated in the cold for 30 min at 22,000 *g*. Radioactivity was determined in the supernatant.

Table 6. *In vitro* [^{3}H]aldosterone binding to the nuclei and nuclear proteins of renal cells of intact rats in the presence of renal cytosol from adrenalectomized rats

Experimental conditions	Bound [^{3}H]aldosterone, 10^{-15} mol/mg of DNA					
	Nuclei		0.1 M Tris-extract of nuclei		0.4 M KCl-extract of nuclei	
		Mean		Mean		Mean
Nuclei without enzyme treatment	30.2		3.7		5.5	
	39.2	34.7	3.7	3.7	6.5	6.0
Nuclei treated with the enzyme	14.4		3.7		9.1	
	11.0	12.7	4.3	4.0	6.1	7.6

Treatment of nuclei DNAse was carried out for 30 min at 25°C (the final enzyme concentration was 500 μg/ml). After incubation the samples were placed on ice layered on 10 ml of 5% dextran solution 500 in Tris–sucrose buffer and centrifuged for 20 min at 2000 *g*. The residue of the nuclei was used for further incubation with [^{3}H]aldosterone.
Experiments were carried out without incubation or with preincubation of the nuclei with DNAse.

hormone binding sites (both occupied with endogenous steroids and free). As a result, it was shown that the content of the mineralocorticoid receptors in renal cytosol depends on endogenous aldosterone level, i.e. the higher the concentration of aldosterone in the blood, the lower the quantity of specific mineralocorticoid receptors in renal cytosol, and *vice versa*.

When studying [^{3}H]aldosterone binding in rat renal nuclei by the exchange method [16], an inverse relation between the labelled hormone in the nuclei and the level of endogenous aldosterone was found, i.e. the higher the concentration of aldosterone in the blood, the higher aldosterone binding in the nuclei.

The data obtained could be interpreted as follows. When aldosterone content is low (sodium load or adrenalectomy), mineralocorticoid receptors in the renal cell are located mainly in the cytoplasm; when the aldosterone content in the body increases (sodium deficiency or aldosterone injection), the majority of mineralocorticoid receptors, bind the hormone, pass from the cytoplasm to the target cell nuclei. A similar dependence between the endogenous hormone content and the distribution of the specific receptors between the nucleus and cytoplasm in the target cells was shown also for steroid hormones such as estradiol [8, 21], progesterone [22], glucocorticoids [23].

The difference between [^{3}H]aldosterone binding by renal cell nuclei in hypo- and hyperaldosteronemic rats varies about 2-fold (Table 3). Considering the balance of [^{3}H]aldosterone bound with nuclei it is easy to see that after [^{3}H]aldosterone extraction with 0.1 M Tris–HCl buffer and 0.5 M KCl solution, about 30% of all aldosterone remains firmly bound with the remaining DNP in the hypoaldosteronemic rats and about 50% in the hyperaldosteronemic ones (i.e. the absolute values are 1.6 and 5.2×10^{-15} mol/mg of DNA). These data support the idea that the physiological effect of steroid hormones in the target cells is accomplished during their interaction with the chromatin of cell nuclei [4, 5].

With the addition of renal cytosol of adrenalectomized rats to the incubation medium, [^{3}H]aldosterone binding by renal cell nuclei of rats with hypo- and hyperaldosteronemia increases sharply and becomes almost equal in these two groups of animals. If the quantity of acceptors for the aldosterone–receptor complex in the nuclear chromatin of these groups is different, then, possibly, these differences may also be evident under conditions of excess of [^{3}H]aldosterone–receptor complexes in the incubation medium. Therefore, the quantity of acceptor, i.e. binding sites for hormone–receptor complexes in nuclear chromatin of renal cells in these groups cannot be expected to be different.

From these data, as with those concerning other steroid hormones [24], the sensitivity of renal cell to aldosterone may presumably be due to the presence of specific protein-receptors in the cytosol fraction and not to nuclear acceptors, whose quantity is probably constant in different functional states of the target organ.

In experiments with the addition of renal cytosol of adrenalectomized rats to the nuclei, the quantity of [^{3}H]aldosterone tightly bound to DNP and non-extractable with 0.1 M Tris–HCl buffer and 0.4 M KCl solution is about 70% of all bound labelled hormone in hyper- and hypoaldosteronemic animals (Table 4) and also in intact animals (Table 6). While treating this DNP with proteolytic enzymes, it was found that almost all residual fraction radioactivity was turned into a soluble state due to pronase and chymotrypsin. It is clear that aldosterone (or the aldosterone–receptor complex) joins certain specific non-histone proteins of DNP.

The binding activity of rat renal cell nuclei relative to [^{3}H]aldosterone–receptor complex decreases sharply when the nuclei were preincubated with DNAse. Similar data were obtained by other investigators [20]. Based on the results obtained there is reason for suggesting the following. The structural integrity of DNA is needed to provide the interaction of the hormone–receptor complex with the acceptors of chromatin. This may be expected in spite of the fact that the hormone joins the proteins of DNP but not of DNA. When this interaction occurs, DNA can be hydrolyzed without releasing the hormone bound to the chromosomal proteins.

REFERENCES

1. Korner A.: Ribonucleic acid and hormonal control of protein synthesis. *Prog. biophys. Molec. Biol.* **17** (1967) 61–98.
2. Yudaev N. A.: Hormone action on the level of genetic information transfer. *Problems Endocr.* **13** (1967) 112–121.
3. Salganik R. I.: Role of genetic induction in normal and pathological processes. *Vestnik Akad. Med. Nauk SSSR.* **8** (1968) 3–11.
4. O'Malley B. W. and Means A. R.: Female steroid hormones and target cell nuclei. *Science* **183** (1974) 610–620.
5. Edelman I. S.: Mechanism of action of steroid hormones. *J. steroid Biochem.* **6** (1975) 147–159.
6. Edelman I. S.: Aldosterone binding proteins. *Adv. Biosci.* **7** (1971) 267–279.
7. Rousseau G. G., Baxter J. D., Funder J. M., Edelman I. S. and Tomkins G. M.: Glucocorticoid and mineralocorticoid receptors for aldosterone. *J. steroid Biochem.* **3** (1972) 219–227.
8. Anderson J. N., Clark J. H. and Peck E. J.: The relationship between nuclear receptor-estrogen binding and uterotrophic responses. *Biochem. biophys. Res. Commun.* **48** (1972) 1460–1468.
9. Gorski J., Sarff M. and Clark J.: Regulation of uterine concentration of estrogen binding protein. *Adv. Biosci.* **7** (1971) 5–20.
10. Daehnfeldt J. L.: Endogenously blocked high affinity estradiol receptors in the immature and mature rat uterus. *Proc. Soc. exp. Biol. Med.* **146** (1974) 159–162.
11. Funder J. W., Feldman D. and Edelman I. S.: The role of plasma binding and receptor specificity in the mineralocorticoid action of aldosterone. *Endocrinology* **92** (1973) 994–1003.
12. Funder J. W., Duval D., Meyer R. and Dahl K.: Mineralocorticoid receptors in salt-susceptible and salt-resistant rats. *Endocrinology* **94** (1974) 1739–1743.
13. Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J.: Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193** (1951) 265–275.
14. Scatchard G.: The attractions of proteins for small molecules and ions. *Ann. N.Y. Acad. Sci.* **51** (1949) 660–672.
15. Rosenthal H. E.: A graphic method for the determination and presentation of binding parameters in a complex system. *Analyt. Biochem.* **20** (1967) 525–532.
16. Anderson J. N., Clark J. H. and Peck E. J.: The relationship between nuclear receptor-estrogen binding and uterotrophic responses. *Biochem. biophys. Res. Commun.* **48** (1972) 1460–1468.
17. Kalimi M., Beato M. and Feigelson Ph.: Interaction of glucocorticoids with rat liver nuclei. I. Role of cytosol proteins. *Biochemistry* **12** (1973) 3365–3371.
18. Funder J. W., Feldman D. and Edelman I. S.: Specific aldosterone binding in rat kidney and parotid. *J. steroid Biochem.* **3** (1972) 209–218.
19. Burton J.: A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of the deoxyribonucleic acid. *Biochem. J.* **62** (1956) 315–323.
20. Marver D., Goodman D. and Edelman I. S.: Relationships between renal cytoplasmic and nuclear aldosterone receptors. *Kidney Int.* **1** (1972) 210–223.
21. Mester J. and Baulieu E.-E.: Dynamics of oestrogen-receptor distribution between the cytosol and nuclear fractions of immature rat uterus after oestradiol administration. *Biochem. J.* **146** (1975) 617–623.
22. O'Malley B. W., Sherman M. R. and Toft D. O.: Progesterone "receptors" in the cytoplasm and nucleus of chick oviduct target tissue. *Proc. natn. Acad. Sci., U.S.A.* **67** (1970) 501–508.
23. Beato M., Kalimi M., Beato W. and Feigelson Ph.: Interaction of glucocorticoids with rat liver nuclei: effect of adrenalectomy and cortisol administration. *Endocrinology* **94** (1974) 377–387.
24. Anderson J. N., Peck E. J. and Clark J. H.: Estrogen-induced uterine responses and growth: relationship to receptor oestrogen binding by uterine nuclei. *Endocrinology* **96** (1975) 160–167.