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MINERALOCORTICOSTEROID RECEPTORS IN THE FOETAL COMPARTMENT

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SUMMARY

Mineralocorticosteroid receptors have been found in the foetal kidney of the guinea pig (at 25–40 days of gestation) in experiments carried out both *in vivo* and *in vitro*. More than 50% of the total d-aldosterone receptors were found in the nucleus. 20% of the total nuclear radioactivity was extracted by the 0·1 M Tris-HCl solution and 50% by the 1 M NaCl −0·01 M Tris solution, suggesting that at this period of the foetal evolution the aldosterone receptors are localized principally in the chromatin fraction. The formation of these [^{3}H]-aldosterone-macromolecule complexes is very rapid, maximum values being found at 4 min of incubation at 37°C. The nuclear receptors, but not those of the cytosol fraction, are temperature-dependent. d-Aldosterone and deoxycorticosterone compete with the [^{3}H]-aldosterone complex. On the other hand, d-aldosterone has no effect on the [^{3}H]-aldosterone-macromolecule complex if reincubation is carried out with the nuclear extracts (at 4°C) after extraction. Also, the 0·1 M Tris and 0·01 M Tris-1 M NaCl nuclear extracts do not form [^{3}H]-aldosterone complexes after incubation (separately or combined) at 4°C or 37°C, suggesting that the configuration necessary for the formation of the [^{3}H]-aldosterone complexes is altered by the extraction procedure. Incubation of the purified nucleus produces more [^{3}H]-aldosterone-macromolecule complexes (per mg of protein) than incubation with the total cell or with the crude nuclear fraction. These data suggest that unbound aldosterone can cross the nuclear membrane and form the aldosterone complexes without the participation of a cytosol intermediate.

INTRODUCTION

IT IS WELL known that in human pregnancy the secretion and excretion rates of aldosterone increase to many times their basal values, particularly in the third trimester of gestation[1–4]. After perfusion of [^{3}H]-corticosterone to the human foetus, it has been demonstrated that this steroid is converted into aldosterone in the foetal adrenals[5], suggesting that this increase could be, in part, due to the foetal contribution.

Recently, after subcutaneous *in vivo* and *in situ* perfusion of [^{3}H]-progesterone to guinea pig foetuses at 25–35 days gestation, it was found that the foetal adrenals converted this steroid into [^{3}H]-corticosterone and [^{3}H]-cortisol; *in vitro* studies with the adrenals of the same animal species showed that both [^{3}H]-corticosterone and [^{3}H]-progesterone are converted into [^{3}H]-aldosterone[6], indicating that the foetal compartment of this animal can biosynthesize both gluco- and mineralocorticosteroids.

In a recent preliminary note[7], the formation of the [^{3}H]-aldosterone-macromolecule complexes was demonstrated in experiments carried out *in vivo* and *in vitro* with guinea pig foetal kidney. In the present paper the conditions of the formation of these [^{3}H]-aldosterone-macromolecule complexes, as well as the interrelations of these complexes between the cytosol and the nucleus, are described.

EXPERIMENTAL

Biological material

Foetuses of Hartley albino guinea pigs at between 25 and 40 days of gestation were used.

Radioactive material

[1,2-^{3}H]-d-Aldosterone (S.A. 50 Ci/mmol), [1,2-^{3}H]-deoxycorticosterone (S.A. 39 Ci/mmol) and [1,2-^{3}H]-tetrahydroaldosterone (S.A. 50 Ci/mmol) were all purchased from New England Nuclear Corp., Boston, Massachusetts, U.S.A. and purified in different chromatographic systems before use.

Method

In the *in vivo* studies, the radioactive material was injected subcutaneously into the foetuses *in situ*, in a lightly ether-anesthesized mother, and 15, 30, 60 or 90 min after the administration, the foetuses were removed and the foetal tissues to be studied were separated into the different subcellular fractions. The *in vitro* studies were carried out using an intact cell homogenate incubated with the labelled steroids in Krebs–Henseleit buffer (pH: 7·4)[8]. The pellet obtained after centrifugation of the incubated tissues was homogenized in 0·25M sucrose in a 0·01 M Tris-HCl −0·003 M $CaCl_2$ solution and centrifuged at 900 *g* for 10 min. The supernatant was centrifuged at 250,000*g* for 30 min to obtain the cytosol fraction. The pellet from the 900*g* centrifugation was washed with a 0·4M sucrose −0·01M Tris-HCl −0·003M $CaCl_2$ solution to obtain the crude nuclear fraction, which was homogenized again in 2·0M sucrose −0·01M Tris-HCl −0·003M $CaCl_2$, layered in an equal volume of the same solution and centrifuged for 60 min at 250,000 *g* to obtain the purified nucleus. The purity of the nucleus was controlled by visual examination with a light microscope. The preparation consisted of more than 90% pure nucleus.*

The successive nuclear extractions as well as the percentage distribution of proteins and DNA among the different nuclear extracts are indicated in Table 1. The cytosol and the different nuclear extracts were chromatographed in Sephadex G-15, and the radioactive macromolecule complexes were submitted to density gradient ultracentrifugation in a 5–20 or 10–30 (w/v) sucrose solution in 0·01M Tris-HCl −0·0015M EDTA (pH 7·4) with or without 0·5M NaCl. All of these operations were performed at 0–2°C. Proteins were detected (at 280 nm) with

Table 1. Sequence of the foetal kidney nuclear extractions and percentage of proteins and DNA in each of these fractions

Nuclear extractions*	Proteins (%)†	DNA (%)†
(A) 2 ml 0·1M Tris-HCl −0·0015M EDTA (pH: 7·4) (0·1M Tris)	3·6–13·5	0·8–1·2
(B) 2 ml 0·3M NaCl −0·01M Tris-HCl (pH: 7·4) (0·3M NaCl)	9·3–19	0·6–0·7
(C) 6 ml 1M NaCl −0·01M Tris-HCl (pH: 7·4) (1M NaCl)	62–83	91–98
(D) 3 ml 3M NaCl −0·01M Tris-HCl (pH: 7·4) (3M NaCl)	1·5–5·4	2·5–4·6
(E) Ethanol 90% v/v	–	–

*The volumes are indicated per g of tissue.

†Percentage of the nuclear protein or DNA extracted by the different solutions A, B, C and D. Range values of proteins correspond to 5 experiments and those of DNA to 3.

*DNA evaluation in the homogenate of the total cell and in nuclear extracts shows a recovery of 70–75% in the nucleus.

a Uvicord L.K.B. apparatus (Bromma, Sweden) or evaluated using the method of Lowry *et al.*[9]. DNA was evaluated by the method of Burton[10]. The radioactivity in the macromolecule complexes was evaluated after being dissolved in Instagel (Packard, Inc.) scintillation liquid (efficiency 20%), and unbound radioactivity was measured in the classical POPOP-PPO scintillation solution (efficiency 28%). Aldosterone and tetrahydroaldosterone contained in the macromolecule complexes were identified after precipitation of the protein with ethanol (80%, v/v). This solution had been left for 48 h at −5°C and then centrifuged. The supernatant was evaporated to dryness, dissolved in water, and the radioactive material was extracted with dichloromethane.

[^{3}H]-Aldosterone was identified after chromatography in chloroform/formamide and in ethylacetate-toluene-methanol-water (1:9:5:5, by vol.), and its 18,21-diacetate in isooctane-toluene-methanol-water (8:2:5:5, by vol.) systems. [^{3}H]-Tetrahydroaldosterone was identified in chloroform/formamide and isooctane-t-butanol-water (9:5:10, by vol.), and after acetylation in ligroin/propanediol and isooctane/propanediol systems.

RESULTS

To determine whether the aldosterone produced in the maternal compartment is transferred to the foetus, [^{3}H]-aldosterone was injected subcutaneously into the mother and the radioactivity was evaluated in the different foetal tissues. The results are indicated in Table 2. We can see that a minimal percentage of the radioactivity was transferred to the foetus and that this quantity was relatively small in the foetal kidney. After subcutaneous administration of [^{3}H]-aldosterone to the guinea pig foetus, it was observed that maximum values of the radioactivity in the foetal kidney were found about 60 min after administration (Fig. 1).

The *in vivo* studies on the formation of [^{3}H]-aldosterone-macromolecule complexes were carried out in the foetal kidney 30 min after subcutaneous administration of [^{3}H]-aldosterone to the foetus. The distribution of the radioactivity in the different subcellular fractions is indicated in Table 3, and the percentages of the radioactivity linked to macromolecules in Table 4. It is observed that the percentage of bound radioactivity in the cytosol fraction was small, but in the nuclear extracts most of the radioactivity was localized in the macromolecule complexes.

Table 2. Radioactivity in guinea pig foetal tissues 30 min after subcutaneous administration of 15 μCi ($3{\cdot}3 \times 10^{-10}$ mol) of [^{3}H]-aldosterone to the mother*

Foetal tissues	I 2 foetuses (%)	II 4 foetuses (%)
Kidney	0·02	0·09
Liver	0·04	0·30
Lungs	0·02	0·09
Brain	0·06	0·16
Intestines	0·09	0·10
Skin	–	0·38
Rest of the foetal tissues	1·00	2·10

*Values represent the percentage of the administered dose in the tissues of all the foetuses, of each mother, combined.

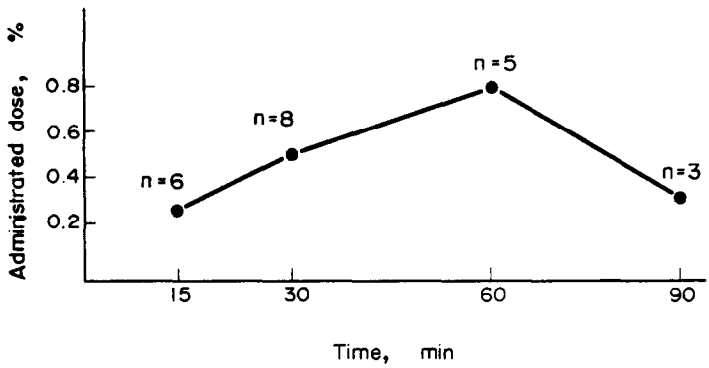

Fig. 1. Radioactivity in guinea pig foetal kidney after subcutaneous [^{3}H]-Aldosterone administration. (20 μCi($4{\cdot}4 \times 10^{-10}$ mol) of [1,2-^{3}H]-aldosterone/foetus). The data represent the average values of *n* foetuses at each time period.

Table 3. Distribution of radioactivity in the different subcellular fractions of foetal guinea pig kidney 30 min after subcutaneous administration of 20 μCi ($4{\cdot}4 \times 10^{-10}$ mol) of [^{3}H]-d-aldosterone to each foetus

Cytosol: 89%*			
Extracts	(%)*	Nucleus (mol $\times 10^{-14}$)	Mitochondria + microsomes (%)†
(A) 0·1M Tris	0·55	3·0	0·22
(B) 0·3M NaCl	0·4	2·2	0·04
(C) 1M NaCl	2·6	14·2	0·03
(D) 3M NaCl	0·35	2·1	0·04
(E) Ethanol	0·35	1·7	0·04

The values represent the average values of 7 foetuses.

*Percentage of the radioactivity in the total cell.

†The mitochondria-microsomal fraction is the pellet obtained from the 250,000 $\times g$ centrifugation. The extraction sequence of the mitochondria-microsomal fraction is identical to that of the nucleus (see Table 1).

Table 4. Percentage of the radioactivity linked to macromolecules in subcellular fractions of foetal guinea pig kidney 30 min after subcutaneous *in situ* administration of 20 μCi ($4{\cdot}4 \times 10^{-10}$ mol) of [^{3}H]-d-aldosterone to each foetus*

Subcellular fraction	% Linked	% [^{3}H]-Aldosterone macromolecule complexes	d.p.m./mg protein (in macromolecule complexes)
Cytosol	0·60–0·66	0·3	470
Nuclear extracts			
(A) 0·1M Tris	35–90	–	1015
(B) 1M NaCl	68–70	37–50	720

*The data represent the average values of 7 foetuses. Values are expressed as percentage of the total radioactivity of each subcellular fraction.

The *in vitro* experiments confirmed the *in vivo* results on the formation of [^{3}H]-aldosterone-macromolecule complexes in both the cytosol and nuclear fractions of the foetal kidney. The distribution of the radioactive material in the different subcellular fractions after incubation of [^{3}H]-aldosterone with the intact cell homogenate of the foetal kidney at 37°C is indicated in Table 5. It is observed that a significant quantity of [^{3}H]-aldosterone was localized in the 1 M NaCl nuclear extracts. Comparative studies after incubation of foetal and maternal kidney with [^{3}H]-aldosterone showed that the percentage of radioactive material (per g of kidney tissue) was 2–3 times higher in the foetal kidney than in that of the mother, and the radioactivity per mg of protein in the 0·3M NaCl and in the 1M NaCl nuclear extracts was 3–4 times higher in the foetal than in the maternal kidney (Table 6).

In Table 7 the percentages as well as the quantities of radioactivity bound in the different subcellular fractions are listed. As can be seen in this table,

Table 5. Distribution of the radioactivity in the subcellular fractions of foetal guinea pig kidney after incubation of 10 μCi of [^{3}H]-d-aldosterone ($2{\cdot}2 \times 10^{-10}$ mol) per g of tissue*

Cytosol: 94·7%†		($59{\cdot}9 \times 10^5$ d.p.m.)		(d.p.m./mg protein: $2{\cdot}56 \times 10^5$)
		Nucleus		Mitochondria
Extracts	(%)†	d.p.m. $\times 10^3$	d.p.m./mg protein	+ microsomes (%)†
(A) 0·1M Tris	0·40	25·3	15,300	1·5
(B) 0·3M NaCl	0·28	19·6	11,150	0·8
(C) 1M NaCl	0·92	60·1	7,400	0·6
(D) 3 M NaCl	0·14	8·4	16,050	0·1
(E) Ethanol	0·16	10·4	–	0·4

*Incubation was carried out in 4 ml Krebs–Henseleit buffer per g of tissue for 16 min at 37°C. The data represent the average values of 4 different experiments and correspond to 15 g of kidney from ~ 24 foetuses.

†Values are expressed as percentage of the total radioactivity in the cell.

Table 6. Formation of [^{3}H]-aldosterone macromolecule complexes in foetal and maternal kidney after incubation of 10 μCi ($2{\cdot}2 \times 10^{-10}$ mol) of [^{3}H]-aldosterone/g of tissue for 16 min at 37°C

	Foetal kidney		Maternal kidney	
		[^{3}H]-macromol.†		[^{3}H]-macromol.†
Fraction	d.p.m./mg protein	complex (%)	d.p.m./mg protein	complex (%)
Nuclear extracts				
(A) 0·1M Tris	19,000	32	15,100	15
(B) 0·3M NaCl	11,300	55	3,800	–
(C) 1M NaCl	6,700	53	1,800	26
Cytosol				
(in macromolecule complex)	7,250	2·7	6,650	1·7
after 24th.	1,100	0·41	2,800	0·7

*The data represent the average values of 3 experiments.

†Values are expressed as percentage of the total radioactivity in each subcellular fraction.

Table 7. Aldosterone and tetrahydroaldosterone linked to macromolecules in the subcellular fractions, of foetal guinea pig kidney after incubation of 10 μCi ($2{\cdot}2 \times 10^{-10}$ mol) [^{3}H]-aldosterone per g of tissue, for 16 min at 37°C

	Total bound		Aldosterone bound		Tetrahydro-aldosterone bound	
	(%)*	(mol $\times 10^{-14}$)	(%)	(mol $\times 10^{-14}$)	(%)	(mol $\times 10^{-14}$)
Cytosol	1	60	0·36	21	0·22	12·8
Nuclear extracts						
(A) 0·1M Tris	32	8·2	23	6	4	1·1
(B) 0·3M NaCl	55	11	25	6	5	1·2
(C) 1M NaCl	53	32	32	22	3·5	2·0

*% of the total radioactive material in each fraction bound to macromolecules. The data represent the average values of 7 experiments.

Table 8. Distribution of the radioactivity in the subcellular fractions of foetal guinea pig kidney after incubation of 10 μCi ($2{\cdot}0 \times 10^{-10}$ mol) of [^{3}H]-tetrahydroaldosterone per g of tissue, 16 min at 37°C

Nuclear extracts	(%)*	(mol $\times 10^{-14}$)
(A) 0·1M Tris	0·05	3·5
(B) 0·3M NaCl	0·02	1·4
(C) 1M NaCl	0·06	4·2
Cytosol fraction (in macromolecule complexes)	0·02	1·4

*% of the radioactivity in the total cell.

a significant part of the radioactive material of the macromolecule complexes in both the cytosol and the nuclear extracts consisted of non-transformed aldosterone, this percentage being higher in the nuclear extracts, particularly in the 1M NaCl extract. On the other hand, the quantity of tetrahydroaldosterone was higher in the cytosol complex. To investigate the uptake of this metabolite by the foetal kidney nucleus or the formation of a complex with a receptor in the cytosol, [1,2-^{3}H]-tetrahydroaldosterone was incubated with foetal kidney tissues. The distribution of the radioactivity in the different nuclear extracts as well as in the cytosol macromolecule complexes is indicated in Table 8. Comparison of this data with incubation of the same tissue with [^{3}H]-aldosterone (Table 5) shows that 10–15 times less radioactive material was found in the nuclear extracts, and 50 times less in the macromolecule complexes of the cytosol after [^{3}H]-tetrahydroaldosterone than after [^{3}H]-aldosterone incubation.

Incubation of the foetal kidney with another mineralocorticosteroid, [^{3}H]-deoxycorticosterone, revealed a significant percentage of the total radioactivity in the subcellular fractions localized in the nucleus, and 23–60% of the nuclear radioactivity in macromolecule complexes (Table 9).

The effect of non-labelled d-aldosterone, deoxycorticosterone and oestradiol-17β on the formation of the [^{3}H]-aldosterone-macromolecule complexes was studied after simultaneous incubation of [^{3}H]-aldosterone with these different steroids. As is indicated in Table 10, d-aldosterone and deoxycorticosterone

Table 9. Distribution of radioactivity in the subcellular fractions of foetal guinea pig kidney after incubation of 10 μCi of [^{3}H]-deoxycorticosterone ($2{\cdot}7 \times 10^{-10}$ mol) per g of tissue, for 16 min at 37°C

Nuclear extracts	(%)*	(% linked)	mol $\times 10^{-14}$
(A) 0·1M Tris	0·4	–	–
(B) 0·3M NaCl	0·2	23	5
(C) 1M NaCl	1·2	55	75

*% of the radioactivity in the total cell.

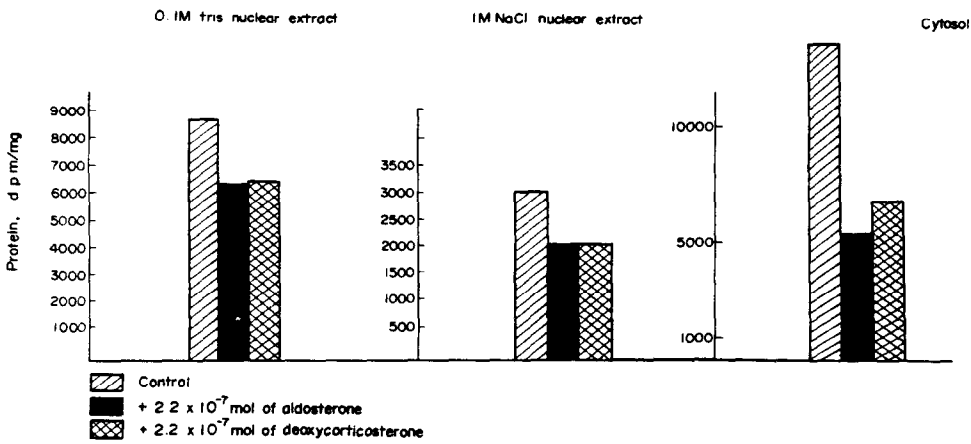

Fig. 2. Competitive effect of d-Aldosterone and deoxycorticosterone on the formation of [^{3}H]-d-Aldosterone macromolecule complexes in cytosol fraction and in the nuclear extracts of foetal guinea pig kidney.

significantly decreased the amount of radioactivity found in the nuclear extracts, while oestradiol-17β had no significant effect. Figure 2 shows the competitive effect of d-aldosterone and deoxycorticosterone on the formation of the macromolecule complexes of the 0·1 M Tris 1 M NaCl nuclear and cytosol extracts after chromatography on Sephadex G-15.

In another series of experiments, [^{3}H]-d-aldosterone was first incubated with foetal kidney (10 μCi [$2{\cdot}2 \times 10^{-10}$ mol] per g of tissue) for 16 min, after which the 0·1M Tris and 1M NaCl nuclear extracts were re-incubated at 4°C with increasing quantities of d-aldosterone (Table 11). As can be seen in this table, no displacement of the [^{3}H]-aldosterone was observed from the [^{3}H]-aldosterone-macromolecule complexes. Also little or no [^{3}H]-aldosterone-macromolecule complexes were obtained when the nuclear extracts (immediately after extraction) from foetal kidney not previously exposed to [^{3}H]-aldosterone were incubated (separately or combined) with [^{3}H]-d-aldosterone at 4° or 37°C.

The time of penetration into the nucleus and formation of these complexes was studied at different periods. As indicated in Figure 3, the nuclear penetration of the [^{3}H]-aldosterone proceeded very rapidly: maximum values were obtained after 4 min of incubation.

The penetration of the [^{3}H]-aldosterone into the nuclear extracts was found

Table 10. Effect of d-aldosterone and deoxycorticosterone on the penetration of radioactivity in the different subcellular fractions (Experiment A) and effect of oestradiol-17β in the penetration and the specific activity (dpm/mg protein) in the macromolecule complex (Experiment B) after simultaneous incubation with 10 μCi ($2{\cdot}2 \times 10^{-10}$ mol) of [^{3}H]-aldosterone for 16 min at 37° per g of foetal guinea pig kidney

	Experiment A (dpm/mg protein)			Experiment B (dpm/mg protein in the total subcellular fraction)		Experiment B (dpm/mg protein in the macromolecule complex)	
Nuclear extracts	Control ^{3}H-Aldo alone	$+2{\cdot}2 \times 10^{-7}$ mol of d-aldo	$+2{\cdot}2 \times 10^{-7}$ mol of DOC	Control ^{3}H-Aldo alone	$+1{\cdot}1 \times 10^{-7}$ mol E_2	Control ^{3}H-Aldo	$+1{\cdot}1 \times 10^{-7}$ mol E_2
(A) 0·1 M Tris	24,800	19,300	17,500	28,100	27,000	6565	5540
(B) 0·3 M NaCl	14,100	9330	9315	11,650	12,500	—	—
(C) 1 M NaCl	6530	4975	3470	5340	4625	2100	2115
Cytosol (in macromolecule complex)	13,800	5570	6800	—	—	—	—

Aldo = Aldosterone; E_2 = Oestradiol-17β.

to be temperature-dependent: an experiment carried out at 4°, 20° and 37°C showed that the specific activity (d.p.m./mg protein) was 1·5–3 times higher in the nuclear extracts after incubation at 37°C than in the nuclear extracts incubated at 4°C. On the other hand, the activities per mg of protein in the macromolecule complexes of the cytosol fraction were similar at 4°C and at 37°C (Table 12).

Comparative studies after incubation of the intact cell, the crude nucleus (pellet from $900 \times g$ centrifugation, see Methods) or the purified nucleus with [^{3}H] aldosterone show that the activity per mg of protein (in the macromolecule peak) is 1·5–2·0 times higher in the nuclear extracts (0·1M Tris and 1M NaCl) of the incubation with purified nucleus than in those of the nuclear extracts of the incubation of the intact cell or the crude nucleus (Table 13).

The density gradient analysis by centrifugation in sucrose solution of the different subcellular fractions shows the presence of 3 principal components with sedimentation coefficients of 2·5–3·5, 4–5 and 8–9S, respectively. In the cytosol the 8–9S component is predominant. The 2·5–3·5 and 4–5S components are

Table 11. Nuclear [^{3}H]-d-aldosterone-macromolecule complexes reincubated for 2 h at 4°C with different concentrations of d-aldosterone

	Nuclear extracts			
	0·1M Tris*		1M NaCl†	
d-aldosterone added	% bound	d.p.m./mg protein	% bound‡	d.p.m./mg protein
Control	30	3000	21	1400
$2{\cdot}2 \times 10^{-12}$ mol	21	2750	26	1480
$2{\cdot}2 \times 10^{-11}$ mol	26	3000	20	1300
$2{\cdot}2 \times 10^{-10}$ mol	20	3060	26	1850
$2{\cdot}2 \times 10^{-9}$ mol	31	3750	20	1850

*Volume of incubation 0·75 ml, corresponding to 0·4 g of kidney tissue.

†Volume of incubation 1·00 ml, corresponding to 0·3 g of kidney tissue.

‡In this table the values of the percentage of the bound radioactivity are less than in Tables 6 and 7, since this experiment was carried out 20–24 h after extraction.

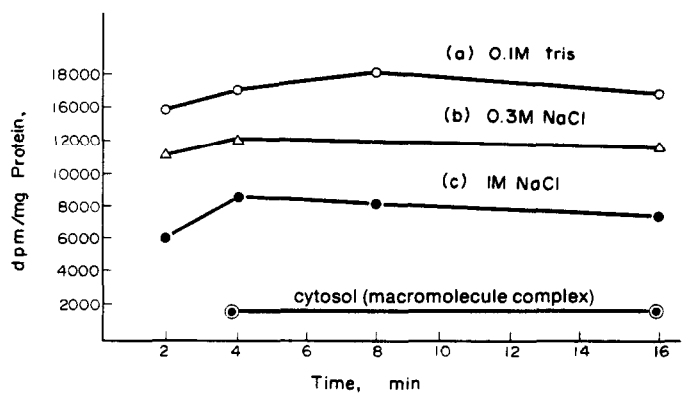

Fig. 3. Effect of incubation time on radioactivity per mg of protein in the different nuclear extracts and in the cytosol (macromolecule complex) after incubation of 10 μCi ($2{\cdot}2 \times 10^{-10}$ mol) [^{3}H]-Aldosterone/g of foetal kidney tissue at 37°C.

Table 12. Effect of temperature on the nuclear penetration of [^{3}H]-d-aldosterone. Incubation (16 min) of 10 μCi [^{3}H]-d-aldosterone ($2{\cdot}2 \times 10^{-10}$ mol)/g of tissue

	d.p.m./mg protein		
	(4°C)	(20°C)	(37°C)
Nuclear extracts			
(A) 0·1M Tris	12,800	17,450	14,000
(B) 0·3M NaCl	4,360	7,720	10,100
(C) 1M NaCl	3,100	5,790	9,580
(D) 3M NaCl	4,000	4,630	12,900
Cytosol			
(in macromolecule complexes)	2,700	–	2,500

Table 13. Formation of [^{3}H]-aldosterone macromolecule complexes in the nucleus of foetal guinea pig kidney at different steps of purification (d.p.m./mg protein in macromolecule complexes)

	Total cell		Crude nucleus		Purified nucleus	
Nuclear extracts	d.p.m. linked	d.p.m./mg protein	d.p.m. linked	d.p.m./mg protein	d.p.m. linked	d.p.m./mg protein
(A) 0·1M Tris	10,300	6,600	5,480	15,000	4,890	16,900
(B) 1M NaCl	29,200	3,015	19,350	3,400	33,300	6,060

In all of the experiments the material from 1g of foetal kidney was incubated with 10 μCi ($2{\cdot}2 \times 10^{-10}$ mol) of [^{3}H]-aldosterone for 16 min at 37°C. The data correspond to the average values of 4 experiments.

found in the 0·1M Tris and the 1 M NaCl nuclear extracts; the 2·5–3·5S is predominant in the 1 M NaCl nuclear extract.

DISCUSSION

The data found in the present investigation indicate that during foetal life the guinea pig kidney can form specific receptors for d-aldosterone at least starting at 25 days of gestation. These macromolecular aldosterone receptors are found in both the cytosol and the nucleus, and quantitative data show that more than 50% of all the aldosterone receptors are localized in the nucleus. Within this subcellular fraction most of these receptors are found in the 1M NaCl extract. Aldosterone receptors have been found in the kidney of adult adrenalectomized rats[11] and in toad bladder[12], but in these cases, the values of the aldosterone complex were higher in the cytosol fraction than in the nucleus[13, 14].

The [^{3}H]-aldosterone-macromolecule complexes are formed very rapidly, maximum values being found at 4 min of incubation (Fig. 3), and incubation at different temperatures shows that the nuclear penetration of the [^{3}H]-aldosterone is temperature-dependent, particularly in the 1M and 3M NaCl nuclear extracts (Table 12). The fact that 50–70% of the total radioactivity in the nucleus was extracted in the 1M and 3M NaCl-Tris solutions suggests that at this stage of development most of the aldosterone receptors are localized in the chromatin (Tables 3 and 5). These results are in agreement with the data on the nuclear localization of aldosterone in the kidney of adult rats[14] or in the bladder of the toad[14, 15].

The finding that d-aldosterone and deoxycorticosterone had a competitive effect on the formation of [^{3}H]-aldosterone-macromolecule complexes of both the cytosol and nuclear fractions (Fig. 2 and Table 10) indicates that the receptors formed in this tissue are specific for mineralocorticosteroids.

It is interesting to note that d-aldosterone reincubated with the nuclear extracts after extraction (Table 11) did not displace any [^{3}H]-aldosterone from the complex, a similar phenomenon was observed in the nuclear [^{3}H]-aldosterone complex formed with toad bladder tissues[14]. Furthermore, since incubation, at 4° or 37°C, of [^{3}H]-aldosterone with the different nuclear extracts immediately after extraction did not result in the formation of the [^{3}H]-aldosterone-macromolecule complex, it can be concluded that after extraction the macromolecules do not present the same conditions for the formation of the aldosterone receptor.

The data from the density gradient ultracentrifugation in 5–20% and 10–30% w/v sucrose solution demonstrate the presence of a principal macromolecule complex with a 8–9S sedimentation coefficient in the cytosol and with principal components of 2·5–3·5 and 4–5S in nuclear extracts[7], but the correlation of these components with the specific receptors of aldosterone is still to be explained. Studies in this problem are in progress.

The fact that very little [^{3}H]-tetrahydroaldosterone was found in the cytosol macromolecule peak as well as in the different nuclear fractions after incubation of [1,2-^{3}H]-tetrahydroaldosterone (Table 8), but that a significant part of the steroid moiety of the macromolecule peak of the cytosol fraction after incubation of the foetal kidney with [^{3}H]-d-aldosterone consisted of tetrahydroaldosterone, suggests that the tetrahydroaldosterone found in this fraction can be directly metabolized from the aldosterone-macromolecule complex; the following pathways are thus suggested:

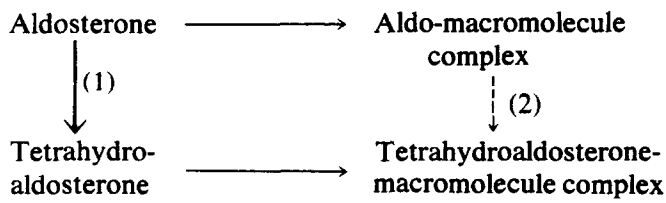

Fig. 4. Suggested aldosterone pathway in the foetal kidney of guinea pig. (1) Pathway demonstrate recently after [^{3}H]-aldosterone administration to guinea pig foetus (Pasqualini J. R., Bedin M. and Cogneville A. M., unpublished data), (2) Suggested conversion in agreement with the data of the present paper.

Another possible explanation for these findings is a selectivity in the nuclear penetration for aldosterone over its metabolite.

Finally, concerning the possible pathways in the nuclear formation of aldosterone receptors, the data that the nuclear [^{3}H]-aldosterone-macromolecule complexes can be obtained after direct incubation of [^{3}H]-aldosterone with purified nucleus and that the specific activity per mg of protein in the macromolecule complexes of the different nuclear extracts was higher than in the corresponding extracts after incubation of the total cell or of the crude nucleus (Table 13), suggest that the aldosterone receptors in the nucleus of foetal guinea pig kidney can be formed directly from the unbound aldosterone.

The data that the isolated nucleus can form aldosterone receptors is in

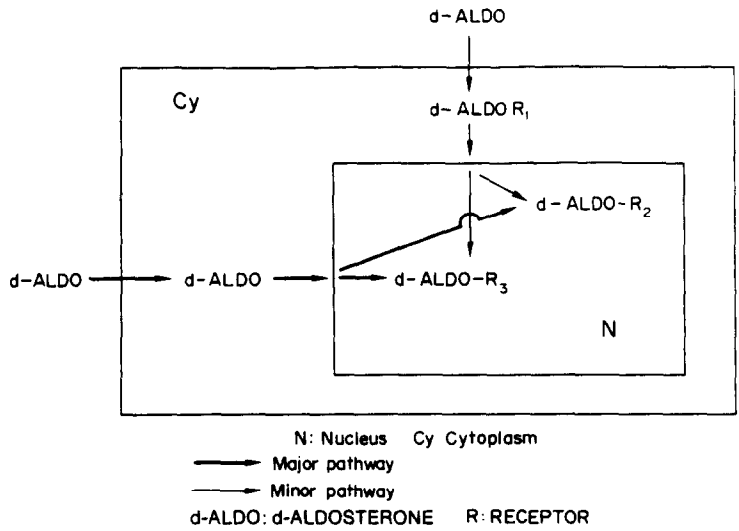

Fig. 5. Hypothetical model of Aldosterone interaction with macromolecules in the guinea pig foetal kidney cell.

opposition to the data obtained with kidney tissues of adult adrenalectomized rats, in which it was suggested that the formation of nuclear aldosterone receptors is obtained through the cytosol complex[16]; the formation of nuclear receptors from a cytosol intermediate was also demonstrated for such other hormones as oestradiol-17β in the rat uterus[17], testosterone in the rat ventral prostate[18] and progesterone in the chick oviduct[19].

In agreement with the present results, the pathways suggested for the nuclear formation of aldosterone receptors in the nucleus of foetal guinea pig kidney are indicated in Fig. 5.

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DISCUSSION

Crabbé: Dr. Pasqualini, if I'm not mistaken, before birth, mammals are known not to respond to aldosterone by sodium retention, and therefore the data you've presented raise a very interesting issue because they would indicate that the receptor protein system appears during ontogenic development before the effect of the hormone can be expressed physiologically. I wonder whether there are corresponding data available for other tissues and other steroid hormones?

Pasqualini: The mineralocorticosteroid activity during foetal life needs to be investigated in the different foetal tissues. I would like to mention that in recent experiments after incubation of [^{3}H]-d-aldosterone at 37°C with guinea pig foetal skin, a significant part of the nuclear radioactivity was associated with macromolecules.

As far as the second part of your question is concerned; the formation of receptors for other steroid hormones in foetal compartment, we found that cortisol can form macromolecule complexes in the foetal liver of guinea pigs (Pasqualini J. R., Costa Novaes S., Ito Y. VII Pan-Amer. Cong. of Endocrin. (Sao Paulo) 1970 P. *39*). Also, we found specific receptors for oestradiol in the foetal brain of the same animal (Pasqualini J. R. and Palmada M. N.; 53 rd Meeting Endocrin. Soc. 1971 p. A-242, and *C.R. Acad. (Paris)* **274** (1972) 1218).

O'Malley: Well, that's a very difficult experiment in mammals because during foetal life there is exposure to oestrogens and perhaps androgens. If the receptors are induced by these steroids (which are growth stimulators) then during foetal life they could be induced and present at birth. We have a better situation in the chick, in that the embryo grows outside the mother and is not exposed to external steroids. Since the chick ovary doesn't begin functioning till somewhere after 100 days of age, the new-born chick has never been exposed to any significant quantities of steroids. What's more, we know the progesterone receptor in the chick is induced by estrogens. However, somewhere during ontogeny and before it ever sees oestrogens, although it can be further induced with oestrogens, the receptor is already present. So it's geared to respond, or at least to combine with the steroid, and develops sometime during ontogeny. And what's even more interesting is that the nuclear acceptor sites can be changed depending on the growth, the state of differentiation of the tissue; but there is nuclear acceptor also at birth before any steroid stimulates the tissue. I also wanted to ask a question. Did you say there was no specificity for nuclear binding of the cytosol aldosterone complex between different nuclei of the foetus when you incubate the cytosol with them? Have you done experiments where you take purified nuclei from different tissues and incubate them with cytosol of aldosterone, target tissue. Is there a specificity for the binding of this complex between different nuclei?

Pasqualini: After [^{3}H]-d-aldosterone incubation with purified nucleus of guinea pig foetal kidney, a significant part of the [^{3}H]-aldosterone was localized in the macromolecules of the different nuclear extracts, and in recent experiments we found that the addition of the cytosol fraction to the incubation of [^{3}H]-aldosterone with purified nucleus does not increase the formation of these nuclear [^{3}H]-aldosterone complexes. We have not yet performed this experiment with other foetal tissues.

Munck: In partial answer to Dr. Crabbé's question, we have measured thymus cortisol receptors in neonatal and one-day prenatal rats – that's the furthest back

we've got—and find a more-or-less simultaneous appearance of the receptors or just before birth. Both begin to appear after a day or two. There is a complication, however, and that is the stress that the rats are exposed to when they are born or when they're removed by Caesarean delivery. We haven't been able to convince ourselves that the high endogenous cortisterone levels resulting from stress may not be influencing our results. I don't think they are, but we still have reservations.

Spät: You said that complex formation of aldosterone with the macromolecule is irreversible at 4°C. Don't you think that the irreversibility depends on the temperature?

Pasqualini: The word reversibility used here for the formation of the [^{3}H]-aldosterone-macromolecule complexes concerned only the particular experimental conditions: d-aldosterone did not displace the [^{3}H]-aldosterone from the complex when d-aldosterone was added into the nuclear extracts after the extraction of the nucleus which had previously been incubated with [^{3}H]aldosterone at 37°C, and reincubated. The negative effect was observed in the incubation at 4°C or 37°C, but when the d-aldosterone was incubated simultaneously with [^{3}H]-aldosterone in the intact cell, a significant competitive effect was observed on the formation of the [^{3}H]-aldosterone complex. Furthermore, these nuclear aldosterone complexes are temperature-dependent, and studies carried out by dialysis at 4°C show that the dissociation of [^{3}H]-aldosterone complexes in the 0·1M Tris nuclear extracts was many times more rapid than for the [^{3}H]-aldosterone complex of the 1M NaCl nuclear extracts.

Fazekas (A. G.): Dr. Pasqualini, did you examine the formation of the aldosterone-macromolecule complexes in the liver of these foetuses? I ask this question because recently we obtained solid proof that aldosterone has a definite extrarenal effect on the biosynthesis of flavin coenzymes in the liver.

Pasqualini: Studies of the formation of [^{3}H]-aldosterone complexes in the foetal liver of guinea pig relative to the foetal kidney show that in the nuclear extracts the radioactivity per mg of protein is 5–10 times less in the liver than in the kidney. Also, after foetal perfusion with [^{3}H]-aldosterone, it was observed that in the foetal liver 70–80% of this hormone was metabolized principally into tetrahydroaldosterone.

Fazekas (A. G.): I asked the original question because during foetal development the adequate formation of flavin coenzymes is extremely important and it might be influenced by aldosterone.

Slater: I wonder whether you could explain why you think the binding to nuclei is specific in view of the fact that added aldosterone does not displace it at all?

Pasqualini: At the moment we have no explanation of these results, but since d-aldosterone competes with the formation of [^{3}H]-aldosterone-macromolecule complexes of the nuclear extracts after simultaneous incubation with the intact cell, but not if d-aldosterone is added after extraction, it is suggested that the configuration or the conditions in the macromolecule moiety for the formation of the complex have been altered by the extraction procedure.

Martini: In connection with the question asked by Dr. Crabbé have you tried to see what would be the influence on your macromolecule of adrenalectomy of the mother?

Pasqualini: This is an interesting question. We haven't carried out this experiment yet, but we think there is a great risk of spontaneous abortion after adrenalectomy of the mother.