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INTERACTION OF ALDOSTERONE AND CORTICOSTERONE WITH CELL NUCLEI OF TARGET ORGANS UNDER DIFFERENT FUNCTIONAL STATES

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SUMMARY

The effect of changes in the aldosterone secretion by adrenals and the functional state of target organs on the ^{3}H-aldosterone binding by isolated cell nuclei of kidney and brain have been studied.

The least amount of binding of ^{3}H-aldosterone in cell nuclei of kidney and brain has been found under sodium load when the secretion of aldosterone by adrenals was greatly suppressed. In animals with high aldosterone secretion under sodium deficiency, ^{3}H-aldosterone binding in cell nuclei was many times higher than in sodium loaded animals. However, if sodium load was combined with a restricted water intake, the aldosterone secretion was not completely suppressed, despite the excess of sodium. In this case, the level of ^{3}H-aldosterone binding in cell nuclei of kidneys and brain appeared high. The level of ^{3}H-corticosterone binding by cell nuclei of kidney and brain did not show such a dependence on experimental conditions which changed the mineralocorticoid status.

It is suggested that the presence of aldosterone in the blood could possibly influence the content of mineralocorticoid receptors in target organs. It is possible that aldosterone induces the formation of mineralocorticoid receptors in target cell nuclei. It is also probable that the synthesis of receptors can be influenced by some other factors participating in the regulation of target organ function.

INTRODUCTION

Physiological effects of steroid hormones are mediated through an induction in target cells of DNA-dependent synthesis of RNA molecules which program the synthesis of enzymes and other proteins responsible for these effects[1–5].

In recent years it has been found that in target cells there is a system of receptors which "recognize" steroid hormones and transport them from the cytoplasm into the nucleus towards acceptor sites on chromatin [6–7]. Aldosterone receptors have been found in cytoplasm and in cell nuclei of kidney, salivary glands, brain and some other organs [8–13]. The change of receptor content in cells can alter their sensitivity to steroids and serve as a means of modulation of hormonal action and regulation of transcription. It has been shown recently that it is only with the appearance, in the process of ontogenesis, of glucocorticoid receptors in liver cells that the latter become sensitive to cortisol action [14]. Mineralocorticoid receptors of the kidney appear just at the period of foetal life, and 50% of all aldosterone receptors are localized in cell nuclei [15].

It has been suggested that the content of corticosteroid receptors in target cells of an adult organism can change under different functional states[16].

We have studied the interaction of ^{3}H-aldosterone and ^{3}H-corticosterone with isolated nuclei of rat kidney and brain under different experimental conditions during which the adrenal aldosterone secretion and the functional state of target organs were varied. Some results of this work have been published elsewhere[17].

EXPERIMENTAL

Biological material

Male albino rats weighing 240–280 g were used. The animals were divided into three experimental groups. The first group (Na load) received a diet enriched with sodium (270–300 mEq/kg body weight in a day) and water *ad libitum*. The second group (Na deficiency) received a diet with low sodium content (0·05–0·1 mEq/kg b.w. a day). The third group (Na load + water restriction) received a diet enriched with sodium and 2% NaCl solution as drinking fluid. All animals

received the above-mentioned diets for 7–10 days after which they were decapitated during the first half of the day[18].

Radioactive material

[1,2-^{3}H]-aldosterone (specific activity 0·81 Ci/mmol) and [1,2-^{3}H]-corticosterone (specific activity 4·6 Ci/mmol) were purchased from the Radiochemical Centre, Amersham (England) and from Richter (Hungary), respectively. Labelled steroids were chromatographed before use.

Isolation of nuclei and cytosol

Brain and kidneys were excised and washed in cold saline solution. All procedures were carried out at 0–4°C. Nuclei were isolated from cells according to a modification of Chauveau's method[19]. The organs were homogenized in 10 vol. of 0·32 M sucrose with 0·002 M $MgCl_2$. Homogenates were filtered through nylon and centrifuged for 10 min at 900 *g*. The supernatants were centrifuged for 90 min at 105,000 *g* to obtain the cytosol. The sediment after the first centrifugation was resuspended in 2·2 M sucrose containing 0·002 M $MgCl_2$ and centrifuged for 10 min at 900 *g*. The nuclei were then resuspended in 2 ml of Tris–sucrose buffer containing 0·32 M sucrose, 0·002 M $MgCl_2$, 0·025 M KCl in 0·05 M Tris–HCl buffer, pH 8·0. The purity of isolation of nuclei was controlled under the light microscope.

Incubation of nuclei with labelled corticosteroids.

The study of corticosteroid binding in nuclei was carried out according to the method described previously [17]. The incubation medium contained 2 ml of Tris–sucrose buffer, 0·2 ml of nuclear suspension and 0·2 μg of labelled hormone in 0·02 ml of 10% ethanol. During incubation of nuclei with ^{3}H-aldosterone we also added 0·2 ml of cytosol isolated from cells of the respective organs. Preliminary experiments had shown that the addition of cytosol to the system did not significantly influence the extent of ^{3}H-corticosterone incorporation in nuclei.

The mixture was incubated for 5, 15, and 30 min. at 37°C. The reaction was stopped by the addition of a cold solution containing 0·075 M NaCl and 0·024 M EDTA. After centrifugation for ten minutes at 3000 *g*, sediments were suspended in 5 ml of 0·075 M NaCl, 0·024 M EDTA, applied on VUFS microfilters (0·1–0·3 μm, Chemapol, CSSR) and washed five times with 5% solution of trichloroacetic acid, in 5 ml portions. The radioactivity of samples was measured with a liquid scintillation spectrometer MARK-I (Nuclear Chicago, U.S.A.) with the counting efficiency of 40% in toluene scintillator. The quantity of hormone bound by nuclei was expressed in pmol/mg of DNA. The DNA content in nuclei was determined according to Burton [20]. The competitive ability was determined in nuclei isolated from kidneys and brains of intact animals. Aliquots of 0·2 ml of nuclear suspension were incubated for 10 min at 37°C in a medium containing 2·0 ml of Tris–sucrose buffer, 0·2 ml of cytosol and 16 μg of unlabelled steroid in 0·02 ml of 10% ethanol. Then, 0·2 μg of ^{3}H-aldosterone in 0·02 ml of ethanol were added to the incubation medium and the incubation continued for ten more min. The reaction was stopped and the following procedures were as described above. Aldosterone and corticosterone secretions were determined *in vitro* [21] with subsequent use of thin-layer chromatography [22].

RESULTS

The in vitro *corticosteroid secretion by adrenals*

The levels of *in vitro* aldosterone and corticosterone secretion by adrenals under different intake of sodium and water are shown in Table 1. Under sodium load with an adequate water intake (1st series) the adrenal aldosterone secretion was practically decreased to zero. The adrenals of rats with sodium deficiency (2nd series) secreted a significant quantity of aldosterone. The level of aldosterone secretion in rats with sodium load and relative restriction of water (3rd series) occupied an intermediate position between secretions in animals under sodium load and in those with sodium deficiency.

Changes in *in vitro* corticosterone secretion differ from those in aldosterone secretion. The adrenals of rats under sodium load with an adequate water intake (1st series) secreted more corticosterone than the adrenals of rats with sodium deficiency. The level of corticosterone secretion by adrenals of rats under sodium load and water restriction (3rd series) was somewhat higher than in the other experimental series, however, because of a great scatter of data, the difference was not significant.

Table 1. The *in vitro* corticosteroid secretion by adrenals of experimental animals (μg/100 mg tissue/h)

Experimental series	Number of animals (n)	Aldosterone (M ± SD)*	Corticosterone (M ± SD)*
Na load	12	<0·1	3·9 ± 0·12
Na deficiency	12	1·5 ± 0·12	2·3 ± 0·06
Na load + water deficit	12	0·70 ± 0·14	4·3 ± 1·4

* M ± SD—mean ± standard deviation.

Table 2. ^{3}H-Aldosterone binding by isolated cell nuclei of kidney and brain (pmol/mg DNA) under different salt-water regimens (M ± SD)

Series	Experimental conditions	Kidney			Brain		
		Time of incubation			Time of incubation		
		5 min	15 min	30 min	5 min	15 min	30 min
1	Na load	1·0 ± 0·4 (6)	2·2 ± 1·2 (6)	1·6 ± 1·2 (6)	5·1 ± 0·8 (6)	3·6 ± 0·8 (6)	4·8 ± 1·0 (3)
2	Na deficiency	7·6 ± 0·4 (6)	7·9 ± 5·1 (6)	13·7 ± 5·1 (3)	17·9 ± 3·6 (6)	25·4 ± 5·8 (6)	16·8 ± 2·6 (3)
3	Na load + water restriction	11·1 ± 2·0 (3)	13·0 ± 3·0 (3)	11·9 ± 1·2 (3)	19·8 ± 10·0* (3)	8·0 ± 3·7* (3)	11·5 ± 2·3 (3)

M ± SD—mean ± standard deviation. The number of incubations is in parenthesis. Values of binding are compared with the 1st series (Na load). Differences, in all cases except those with an asterisk (*) are significant ($P < 0{\cdot}05$). Values of incorporation in Series 2 and 3 do not differ.

The binding of ^{3}H-aldosterone by isolated cell nuclei of kidney and brain under different salt-water regimens

During the incubation of cell nuclei of kidney and brain with ^{3}H-aldosterone, an intensive binding of the hormone was seen (Table 2).

Nuclei isolated from renal tissue of animals under sodium deficiency (2nd series) bind ^{3}H-aldosterone 4–7 times as much as renal nuclei of rats under sodium load plus adequate water intake (1st series).

Nuclei isolated from kidneys of animals under sodium load and inadequate water intake (3rd series) bound ^{3}H-aldosterone with the same high intensity and showed the highest level of binding (Fig. 1).

^{3}H-aldosterone binding by cell nuclei of the brain was significantly higher than that of renal cell nuclei (Table 2). Saltwater regimen changes also significantly influence the level of ^{3}H-aldosterone binding by cell nuclei of the brain. Thus, the lowest level of ^{3}H-aldosterone binding by brain cell nuclei was seen in animals under sodium load (1st series). In animals of the two other series the aldosterone binding by brain cell nuclei rose more than twice (Fig. 1).

Competition for binding sites in the nucleus

The ability of unlabelled aldosterone to suppress the ^{3}H-aldosterone binding by cell nuclei of kidney and

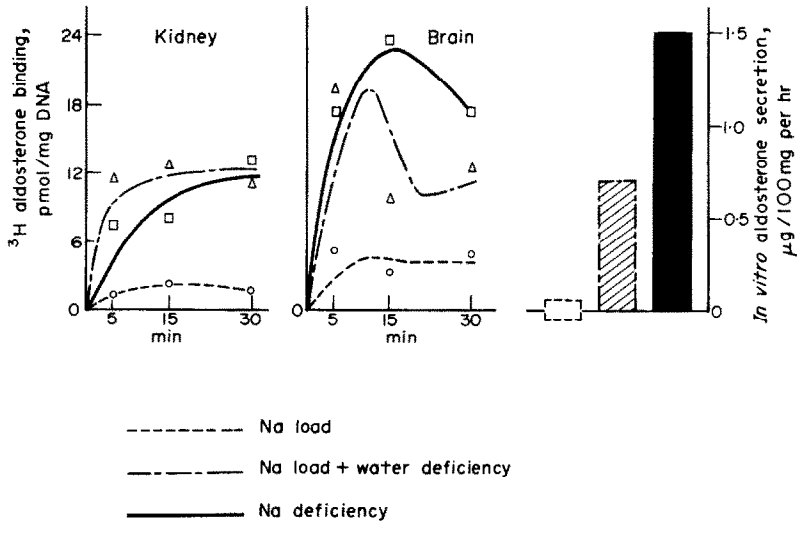

Fig. 1. [^{3}H]-aldosterone binding by isolated cell nuclei of kidney and brain (pmol/mg DNA) and *in vitro* aldosterone secretion (μg/100 mg/h) under different salt-water regimens.

Table 3. The ^{3}H-aldosterone and unlabelled steroid competition for sites of binding in isolated nuclei

Unlabelled steroids	Kidney	Brain
Aldosterone	30 ± 3	20 ± 7
Corticosterone	40 ± 8	45 ± 15
Progesterone	85 ± 5	88 ± 11

The table shows % of ^{3}H-aldosterone binding after incubation of isolated nuclei with unlabelled steroids. The ^{3}H-aldosterone binding without unlabelled steroids is taken for 100%.

brain is shown in Table 3. Unlabelled aldosterone in a concentration 80 times higher than that of labelled hormone suppresses the binding of ^{3}H-aldosterone by isolated nuclei by 70–80%. Under the same conditions, unlabelled corticosterone suppresses the ^{3}H-aldosterone binding by 55%, and progesterone by 12–15%.

^{3}H-Corticosterone binding by cell nuclei of kidney and brain under different salt-water regimens

The experiments on ^{3}H-corticosterone binding by isolated nuclei were carried out in incubation medium without any addition of cytosol. Despite this, the level of ^{3}H-corticosterone binding by isolated nuclei was high enough and in some series did not significantly differ from the level of ^{3}H-aldosterone binding (Table 4). This is true to a greater extent for the third series (sodium load + water restriction) and to a smaller extent—for the second series (sodium deficiency). In the first series (sodium load), the ^{3}H-corticosterone binding by isolated nuclei of kidney was higher than that of ^{3}H-aldosterone, and the binding of isolated nuclei of brain did not significantly differ (compare Tables 2 and 4).

It should be noted that ^{3}H-corticosterone binding by isolated nuclei of the kidney was the same under different salt-water regimens.

DISCUSSION

In what concerns the nature of ^{3}H-aldosterone binding by isolated nuclei of kidney and brain, can one suppose that the described phenomenon is based on the formation of a receptor–aldosterone complex with the subsequent binding of aldosterone on acceptor sites in nucleus?

According to the literature, the aldosterone binding by receptor proteins of the kidney has no absolute specificity with respect to other corticosteroids. Corticosteroids can compete with aldosterone for binding sites on the receptor protein, isolated from cytosol and chromatin in direct proportion to their mineralocorticoid activity [12]. The receptor proteins of cytosol and nuclear fractions of kidney are equally specific for aldosterone and deoxycorticosterone and, thus, authors [15] prefer to speak about mineralocorticoid receptors of kidney.

In our experiments, unlabelled aldosterone competes for binding sites, suppressing ^{3}H-aldosterone binding by 70–80% and that of corticosterone—by 55–60%.

Similar results have been obtained during the examination of the specificity of ^{3}H-aldosterone binding by receptors of cytosol, nuclei [8, 9, 23], and chromatin of the kidney [14, 24].

The ability of unlabelled hormone to compete for binding sites in nuclei isolated from target organs is also shown with oestradiol [25].

Differences in changes of ^{3}H-aldosterone and ^{3}H-corticosterone binding under different salt-water regimens gives evidence for the fact that the above-mentioned ^{3}H-aldosterone binding is ensured by a specific system of nuclear receptors and acceptors. Inhibition and activation of adrenal mineralocorticoid function has no significant effect on the level of ^{3}H-corticosterone binding by isolated cell nuclei of kidney and brain.

Another picture was found in ^{3}H-aldosterone binding. The least amount of binding of ^{3}H-aldosterone by

Table 4. ^{3}H-Corticosterone binding by isolated cell nuclei of kidney and brain (pmol/mg DNA) under different salt-water regimens (M ± SD)

Series	Experimental conditions	Kidney			Brain		
		Time of incubation			Time of incubation		
		5 min	15 min	30 min	5 min	15 min	30 min
1	Na load	3·5 ± 1·4 (5)	4·7 ± 1·2 (5)	7·6 ± 2·8 (5)	7·1 ± 2·2 (4)	4·3 ± 1·5 (4)	10·8 ± 1·6 (4)
2	Na deficiency	5·8 ± 2·9 (5)	4·2 ± 1·2 (5)	7·2 ± 2·1 (5)	11·2 ± 0·8 (6)	10·8 ± 1·4** (6)	11·8 ± 1·6 (6)
3	Na load + water restriction	9·1 ± 3·3 (3)	11·9 ± 1·6** (3)	11·9 ± 1·9 (3)	10·9 ± 1·9 (3)	8·0 (1)	8·0 (1)

M ± SD—mean ± standard deviation; the number of incubations is given in parenthesis. Differences, in all cases except those with asterisks (**) are not significant.

isolated nuclei of kidney and brain took place under sodium load, when adrenal aldosterone secretion was almost completely suppressed (Table 1). The ^{3}H-aldosterone binding by isolated nuclei of kidney and brain in rats under the condition of sodium deficiency was 3–4 times higher than in animals under sodium load. One could suppose that the intensity of ^{3}H-aldosterone binding by isolated nuclei is inversely related to the quantity of sodium. Indeed, during the study of the interaction between glucocorticoids and nuclei of rat kidney it was found that the NaCl concentration influences significantly the ^{3}H-dexamethasone binding by isolated nuclei [26]. As the NaCl concentration in the incubation medium increases within some limits, the glucocorticoid binding by isolated nuclei decreases significantly. Is this decrease in ability of isolated nuclei of kidney and brain to bind ^{3}H-aldosterone dependent on the excess of sodium or in the decrease in aldosterone secretion in response to sodium load? For the discrimination of these two factors additional experiments were carried out in which the sodium load was associated with restricted water intake. In these experiments, despite an excess of sodium, the aldosterone secretion was not suppressed and the level of ^{3}H-aldosterone binding was as high as in sodium-deficient rats (Fig. 1).

The explanation of the results should be looked for in the physiological peculiarities of the experimental models. The methods used for making the experimental models were aimed at changes of the aldosterone secretion level and functional states of target organs.

In the first series of experiments, the rats received a diet enriched with sodium and water *ad libitum*. The adequately high intake of sodium and water increases the volume of extracellular fluid and filtration in kidney. The increase in these two parameters inhibits the secretion of aldosterone [27–29] and suppresses the main renal functions. Under these conditions the level of aldosterone binding in the kidney cell nuclei decreases.

A contrary situation takes place under sodium deficiency (2nd series) where the aldosterone secretion is increased. The increase in aldosterone secretion leads to the rise of one of the main renal functions—sodium reabsorption in renal tubules. As our study shows, under these conditions, the cell nuclei of the kidney bind a larger quantity of aldosterone. The high level of ^{3}H-aldosterone binding in animals with sodium load and relative water deficiency (3rd series), possibly reflects an increase in total mineralocorticoid (MC) activity. The total MC is at least the sum of the actions of aldosterone, 18-OH-DOC and DOC [30].

It was shown that aldosterone receptor proteins are present not only in cells of specialized organs managing the sodium balance in the body but also in some other cells, in particular, those of the brain. Possibly, this fact reflects the ability of aldosterone to regulate the ion transport at the level of cell membranes in different organs. Evidently, this regulatory effect of aldosterone could be of great importance for the brain where the maintenance of an electrolyte gradient is the base for the specific function of the nerve cell.

One can suppose that with the increase in the function of the target organ, "the mineralocorticoid capacity" of its nuclei enlarges. It is possible that in the process of intensification of target organ function, an increased production of a special "protein factor" takes place which promotes the penetration of the receptor–steroid complex into the nucleus. The existence of a special "protein factor" promoting the transport of glucocorticoids from cytoplasm into nucleus has been shown in recent work [31]. The second assumption supposes that the increase in aldosterone secretion induces the synthesis of receptors of this hormone in target cell nuclei. Note should be taken that receptor proteins of steroid hormones have a relatively short half-life period [32] and maintenance of a constant synthesis of these proteins in the absence of the hormone or with its low content should be disadvantageous. It should not be excluded that "mineralocorticoid capacity" of target cell nuclei under the state of an intensive function is due to an increase in nuclear chromatin of "new" acceptor sites. The last supposition is possibly more real, because the state of this link of the steroid macromolecular interaction determines, for the most part, the hormonal effect on the genetic apparatus of the cell and the target organ's function.

We have presented several hypotheses and additional studies are being undertaken to determine the most correct explanation. At present, we are carrying out investigations which are developing in two directions. In one of them we are studying the quantity of cytosol receptors, in the other—the quantity of nuclear ones and that of binding sites in chromatin.

Preliminary results confirm the main theoretical principle of this work. The changes in the functional state of target organs are followed by changes in "mineralocorticoid capacity" of their cells.

We hope to publish these data in the near future.

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DISCUSSION

Neher:

Have you any data on binding in adrenalectomized animals?

Kolpakov:

We have done these experiments, too, but it's part of another one of our investigations in this field. In this investigation we used only animals with adrenals. In our experiments we are looking at the differences between 2 series of animals, one with high aldosterone secretion where we find more [^{3}H]-aldosterone binding, the other with a decrease in aldosterone where we have less aldosterone binding. I think that depressed aldosterone secretion is a similar situation to that in adrenalectomized mice.

Neher:

Do you have no binding or very little binding in adrenalectomized animals?

Kolpakov:

I don't know exactly, because we didn't compare these animals. I think that after adrenalectomy we have 2 situations. *In vitro*, we found more corticosteroid binding in the cytosol but less in the nucleus.

Pasqualini:

Do you have some data on whether this nuclear receptor for aldosterone is temperature dependent?

Kolpakov:

Yes, I think that nuclear receptors are dependent on temperature. In our study we used only one temperature, 37°C.

Jensen:

Would you describe again the medium in which you carry out incubation of nuclei with [^{3}H]-aldosterone? What is the composition of the medium?

Kolpakov:

The incubation medium contained Tris-sucrose buffer, nuclear suspension with labelled hormone (0·2 μg per sample) and 0·2 ml of cytosol.

Müller:

Could you perhaps explain again the experimental conditions of simultaneous dehydration and sodium loading?

Kolpakov:

These animals have a diet with sodium load and water deficiency. It isn't an absolute water deficiency. It was only a relative deficiency because these animals have a diet enriched with sodium and 2% sodium chloride solution as drinking fluid instead of water.

Crabbé:

Do you get a higher labelling of your nuclear fraction if you

expose the whole tissue to the steroid before breaking it down into different fractions, than after injection of the radioactive steroid into the animal prior to handling tissue the way you did?

Kolpakov:

Our studies were only done *in vitro* and not *in vivo*.