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MINERALOCORTICOID EFFECTOR MECHANISM IN HUMAN MONONUCLEAR LEUKOCYTES

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Summary—Mineralocorticoid receptors and mineralocorticoid effector mechanism were determined in mononuclear leukocytes (MNL) from normal subjects. The hierarchy of affinities of competitors for the receptor was similar to that described in other non-classical target tissues for aldosterone. In spite of the relative high affinity of cortisol for the receptor, these binding sites are occupied *in vivo* by aldosterone and play a mineralocorticoid effect in terms of electrolyte content of the cells. The effect of aldosterone is to prevent the loss of electrolytes due to incubation in medium alone and this action is reversed by addition of actinomycin D. In addition, the incubation of the MNL with aldosterone plus human αANP leads to complete block of the action of aldosterone alone. This effect is not mediated by binding of αANP to mineralocorticoid receptors but is probably related to a some postereceptorial effect of aldosterone at the level of plasma membrane. We conclude that the model of MNL is a good tool for studying mineralocorticoid receptors regulation and consequent effector mechanism in humans.

INTRODUCTION

We recently characterized aldosterone receptors in human mononuclear leukocytes (MNL) [1]. In the presence of excess of RU-26988, aldosterone binds to a single class of receptors with high affinity and low capacity. The specificity studies showed that the hierarchy of affinities of competitors for the receptor is consistent with Type I binding sites. In addition, in a subsequent study it was demonstrated that mineralocorticoid receptors are absent or deficient in patients with pseudohypoaldosteronism [2]. The main characteristic of these patients is the inability of kidneys to respond to endogenous or exogenous mineralocorticoids, in terms of electrolyte balance. The clinical picture of pseudohypoaldosteronism stresses the possibility that the deficiency of mineralocorticoid receptors is present also in kidneys.

If mineralocorticoid receptors are present in MNL, they should play a physiological role in regulating the electrolyte balance in these cells. In a subsequent study we in effect demonstrated that the incubation of MNL for 1 h at 37°C in the presence of medium alone leads to a loss of both sodium and potassium from the cell. When the same cells are incubated at the same conditions in presence of physiological concentrations of aldosterone the loss of electrolytes is prevented [3]. The action of aldosterone is blocked by addition of canrenone, the active metabolyte of spironolactone, while addition of cortisol is followed by a loss of Na^+ and K^+.

In the present study we show data on additional factors which are involved in the modulation of mineralocorticoid effector mechanism in MNL.

MATERIALS AND METHODS

[^{3}H]Aldosterone (sp. act. 80 Ci/mmol) and [^{3}H]-corticosterone (sp. act 89 Ci/mmol) were obtained from the New England Nuclear Corp (Boston, MA). Unlabelled steroids and actinomycin D were purchased from Sigma Chemical Co. (St Louis, MO). RU-26988 was kindly supplied by Roussel (Romainville, France). RPMI-1640 medium was purchased from Serva (Heidelberg, Federal Republic of Germany). Percoll was obtained from Pharmacia (Uppsala, Sweden) and atrial natriuretic peptide (αANP) from Peninsula (Belmont, CA). All chemicals were supplied by Merck (Darmastadt, Federal Republic of Germany). The method for measuring mineralocorticoid receptors in MNL has already been described in detail [1, 2]. Briefly, the cells were obtained by a Percoll gradient and washed several times in order to eliminate contamination by plasma proteins and endogenous steroids. An aliquot of cells was then incubated in RPMI-1640 medium with a tracer amount of [^{3}H]aldosterone plus a 100-fold excess of RU-26988, to blocking glucocorticoid receptors. Incubations were done with tracer alone or with different competitors. After 1 h of incubation at 37°C, the cells were washed with cold saline and their intracellular radioactivity measured. Specific binding of [^{3}H]aldosterone was set at 100% and specific binding in presence of competitors was expressed as a percentage of the binding of tracer alone. The lack of contamination by transcortin was evaluated by incubation of blood with 5 nM of [^{3}H]corticosterone and by subsequent separation and washing procedures. The radioactivity measured in the supernatant of the final resus-

pension of the cells was not different from the values obtained in a control tube processed in the same way and containing tracer and medium alone. In addition, the binding of tracer to the cells was completely abolished after performing the same purification and washing steps which are usually performed in the normal assay before incubation with the tracer. The method for measuring the intracellular content of Na^+ and K^+ in MNL has already been described in detail [3]. Briefly, an aliquot of the cells was incubated for 1 h at 37°C in the presence of medium alone, aldosterone, αANP or actinomycin D. After the incubation the cells were washed in a MgCl-buffer, weighed and their content of Na^+ and K^+ measured by flame photometer. In parallel the extracellular space was determined for expressing the results as mmol of Na^+ and K^+ per kg of wet cells.

RESULTS

In Table 1 are shown the data on the ability of different competitors to displace a tracer amount of [^{3}H]aldosterone from the receptor. The affinities of desoxycorticosterone, corticosterone and aldosterone are nearly equivalent. The affinity of cortisol is 1 : 2 and the affinity of dexamethasone 1 : 10 as compared with the affinity of aldosterone. No displacement of tracer aldosterone from receptor was found by addition of ANP at physiological and supraphysiological concentrations.

In Fig. 1 are presented experiments which demonstrate that the action of aldosterone on intracellular electrolytes is mediated by protein synthesis. Incubation of MNL with actinomycin D produces a complete block of the ability of aldosterone to prevent loss of electrolytes from the cells. The data on action and interaction of aldosterone and ANP in regulating the Na^+ and K^+ in MNL are shown in Fig. 2. Aldosterone is able to prevent the loss of electrolytes as previously demonstrated, while ANP alone has the same effect as medium alone. When aldosterone and ANP are added together in the cell preparation, the final effect is a block of the usual mineralocorticoid effector mechanism of aldosterone. The inhibition of the action of aldosterone on the electrolyte content of MNL is present also when ANP is added after 1 h of incubation with aldosterone (Fig. 3). In the same figure, the incubation of the cells for 1 h in the presence of ANP and subsequent washing out of the peptide restores the full reactivity of the cells to aldosterone in terms of electrolyte content.

Table 1. Per cent binding of [^{3}H]aldosterone to Type I receptors in the presence of increasing concentrations of different compounds (the binding of tracer alone is set at 100%)

Fold competitor	1	10	100
Aldosterone	72	28	0
Desoxycorticosterone	65	25	0
Corticosterone	68	25	0
Dexamethasone	100	75	35
Cortisol	85	45	0
αANP	100	100	100

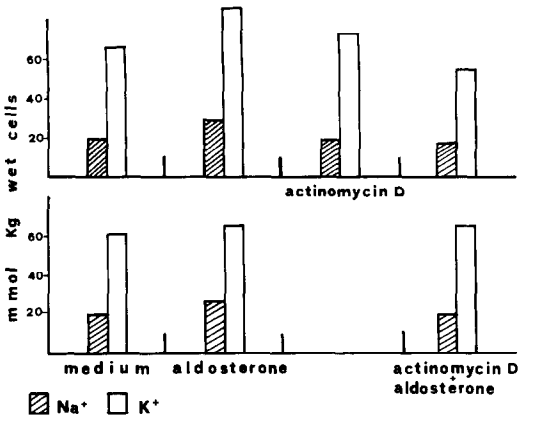

Fig. 1. Inhibition of the action of aldosterone on electrolyte content of mononuclear leukocytes by actinomycin D. An aliquot of cells (5×10^6) was incubated for 1 h at 37°C with RPMI alone or with added aldosterone (1.4 nM, 20 μg actinomycin D), and actinomycin (20 μg, 1.4 nM aldosterone). After incubation, cells were washed with cold MgCl buffer and their content of electrolytes expressed as mmol/kg wet cells.

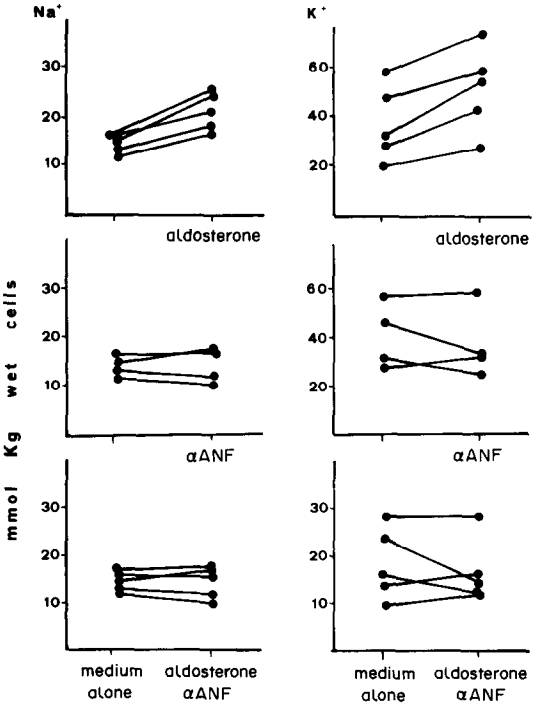

Fig. 2. Effect of aldosterone (1.4 nM), ANP (20 pM), and aldosterone (1.4 nM) plus αANP (20 pM) on the MNL content of Na^+ and K^+. The values are compared with the data obtained by incubation in medium alone. The experimental procedure is described in Fig. 1.

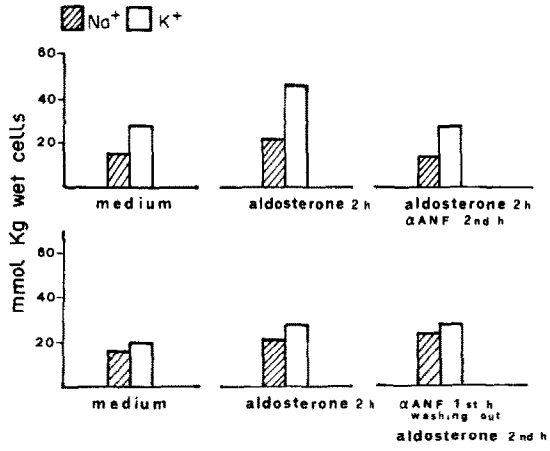

Fig. 3. Intracellular Na^+ and K^+ after incubation with medium alone, with aldosterone for 2 h and with addition of αANP during the 2nd hour of incubation (upper panel) and after incubation for 1 h with αANP (20 pM), washing and subsequent incubation with aldosterone (1.4 nM) (lower panel).

DISCUSSION

The action of aldosterone at the cellular level in the target tissues is to promote unidirectional flux of sodium across the plasma membranes [4]. In tissues with asymmetrical cells such as kidney, the final event is a reabsorption of Na^+ and an excretion of K^+. The real mechanism of aldosterone action at the cellular level can better be evaluated with symmetrical isolated cells. Mononuclear leukocytes possess mineralocorticoid receptors and respond to aldosterone in terms of electrolyte balance. It is important to note that the effect of aldosterone should be present also *in vivo* in spite of the high plasma concentration of free cortisol [3]. The finding that incubation with physiological concentrations of aldosterone and cortisol leads to the same effect as aldosterone alone, stresses the possibility that some intracellular mechanism promotes preferential binding of aldosterone to Type I receptors.

Since the effect of aldosterone in the electrolyte content of MNL is reversed by actinimycin D, it is likely that this action is really mediated by protein synthesis and is not merely a direct effect at the level of plasma membrane. The possible model of action of aldosterone could involve a binding to cytosolic receptors, activation and nuclear translocation of the steroid-receptor complex, binding to DNA, synthesis of mRNA and therefore protein synthesis. The protein should stimulate the unilateral flux of sodium across the plasma membrane probably by creating new sodium channels. In our study we found that aldosterone is able to affect in the same way both Na^+ and K^+ and this finding postulates a mechanism for explaining the entrance of K^+ into the cell. It is possible that increased intracellular Na^+ due to direct action of aldosterone stimulates the Na^+,K^+-ATPase at the level of the plasma membrane with a consequent entrance of K^+ into the cell in exchange with Na^+. Probably the regulation of intracellular content of Na^+ and K^+ is necessary for the usual immunological functions of MNL. In addition, the flux of Na^+ is usually accompanied by a parallel flux of Ca^{2+} in the same direction and this ion is very important for various cellular functions [5].

The model of MNL is also useful for pharmacological studies which investigate possible agonism or antagonism for the mineralocorticoid effector mechanism as previously demonstrated for canrenone and liquorice derivatives [6]. Another possible factor which could affect the mineralocorticoid effector mechanism *in vivo* is αANP. From our study we can deduce that the effect of αANP is not directly related to a possible antagonism at the level of mineralocorticoid receptors, but surely to the membrane effects of aldosterone. In effect we showed that ANP is able to block the usual effect of aldosterone, and previous studies *in vivo* have demonstrated that mineralocorticoids are involved in the expression of the full action of αANP [7]. In effect it was demonstrated that adrenalectomy abolishes the acute natriuretic effect of αANP, which is restored by combined *in vivo* therapy with mineralocorticoids and glucocorticoids. The action of αANP is probably directed to some postreceptorial events due to aldosterone, at the level of plasma membrane. Other drugs, for example amiloride, are able to reverse the mineralocorticoid effector mechanism by factors other than that of antagonism for mineralocorticoid receptors. This action of αANP suggests the presence of specific αANP-receptors at the level of plasma membrane of MNL, but studies on these receptors in these cells were not consistent. Only a binding to specific receptors in platelets was present [8, 9]. It is possible, however, that the method for measuring ANP-receptors in MNL is not sensitive enough, probably because of the fast reversal of binding during the washings after incubation.

From the experiments presented and from those previously described, we can conclude that the model of MNL is useful not only for studying the regulation of mineralocorticoid receptors and mineralocorticoid effector mechanism, but also for evaluating the possible involvement of factors other than aldosterone in the regulation of intracellular electrolytes and cellular functions.

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