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Corticosteroid Receptors in Lymphocytes: a Possible Marker of Brain Involution?

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A similarity has recently been found between the regulation of corticosteroid receptors in brain and in lymphoid tissue. We have studied the regulation of corticosteroid receptors in human mononuclear leukocytes as a possible marker of brain involution. Type I corticosteroid receptors are down regulated by excess of mineralocorticoids (primary and secondary hyperaldosteronism, pseudohyperaldosteronism) and of glucocorticoids (Cushing's syndrome). Type II corticosteroid receptors are not reduced by excess of endogenous corticosteroids (Cushing's syndrome). In normal adults there is a direct significant correlation between plasma cortisol and Type I and between plasma cortisol and Type II receptors in mononuclear leukocytes, while in Cushing's syndrome the correlation is inverse between plasma cortisol at 8 a.m. and Type II receptors. In an aged population the mean numbers of Type I and of Type II receptors are lower and plasma cortisol is higher than in adult controls, but the increase of plasma cortisol is not followed by a clinical picture of hypercorticism. Corticosteroid Type I and Type II receptors are inversely correlated with age. After dexamethasone suppression (1 mg at 11 p.m.) Type I receptors always decrease in controls while the response of Type II is not homogeneous. In an aged group of patients, both receptors are reduced by dexamethasone. We conclude that the decrease with age of corticosteroid receptors is possibly related to a physiological involution of corticosteroid receptors and that this reduction does increase plasma cortisol concentration, without affecting the glucocorticoid effector mechanism.

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CORTICOSTEROID RECEPTORS

Corticosteroid receptors are of two types: Type I, or mineralocorticoid receptors, and Type II, or glucocorticoid receptors [1]. Type II receptors are ubiquitous while Type I receptors are the target of only a few tissues. Studies on Type I receptors have demonstrated that the specificity is not similar in all the targets [2]. In the kidney and in other classical targets, the main ligand is aldosterone, its affinity for the receptor being higher than the affinity of glucocorticoids. In other tissues, which are not classical targets of aldosterone, the hierarchy of affinity of steroids for Type I receptors is different, the affinity of glucocorticoids being similar to that of aldosterone. The difference seems not to be related to the receptor but to other factors, which allow the preferential binding of glucocorticoids to the receptor. The most important factor is the 11 hydroxysteroid-dehydrogenase /11 oxo-reductase enzymatic system, which catalyses the transformation of active cortisol into cortisone, an inactive compound, and vice versa. In tissues lacking the enzyme, the intracellular concentration of cortisol is much higher and this steroid can bind to Type I receptors [3].

CORTICOSTEROID RECEPTORS IN BRAIN AND IN MONONUCLEAR LEUKOCYTES

The distribution of Type I and Type II receptors in brain differs markedly. Type I receptors are mainly located in hippocampus and lateral septum, while Type II receptors are located in all areas of brain [4]. The preferential location of MR receptors must have a physiological significance, probably related to the CRH-ACTH-cortisol secretion in humans. Arriza *et al.* [5] have found that occupancy of Type I and Type II receptors exert a similar biological effect and they have focused the concept that the contemporary presence in the hippocampus of Type I and Type II receptors can expand the range of sensitivity to corti-

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costeroids and control ACTH secretion better. Another possibility is that corticosterone in rat and cortisol in humans usually bind to Type I receptors in hippocampus and lateral septum [6], thus regulating the immediate response to glucocorticoids. Only when cortisol reaches the highest concentrations, as happens early in the morning or during stress, can it also bind to Type II receptors. All these studies have been performed in the animal. It is of great importance to find a human model which resembles brain for studying the regulation of corticosteroid receptors. Circulating lymphocytes have been used for the study of the physiological and pathophysiological regulation of corticosteroid receptors [7–9]. A comparison between the Type I and Type II receptors in brain and lymphocytes has been done by Lowy [10], who showed that the regulation of corticosteroid receptors by corticosterone does not occur in all tissues consistently with a complex regulation of these receptors *in vivo*. He also found a simultaneous reduction of both hippocampal and lymphocyte glucocorticoid receptors by administration of corticosterone in adrenalectomized rat. From these data the same author emphasized that circulating lymphocytes do reflect some aspects of brain corticosteroid receptor regulation.

Some other similarities have been found between corticosteroid receptors in lymphocytes and in brain. Reserpine decreases the number of corticosteroid receptors in lymphocytes and in brain [11]. In patients with major depression [12] and anorexia nervosa, cortisol is increased and glucocorticoid receptors in mononuclear leukocytes are low. The therapy with antiglucocorticoids normalizes both cortisol and Type II receptors concentrations and ameliorates the clinical symptoms of depressed patients [12].

PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL REGULATION OF CORTICOSTEROID RECEPTORS IN HUMANS

Both Type I and Type II receptors in healthy adults are directly correlated with the values of plasma cortisol (Table 1) but not with plasma aldosterone [13, 14], which is probably more sensitive to the renin–angiotensin system. The correlation between cortisol and Type I receptors is consistent with preferential binding of cortisol rather than aldosterone to these receptors. It is possible that Type I receptors are bound by aldosterone in lymphocytes *in vivo* only when aldosterone is high, as in hyperaldosteronism. The mineralocorticoid effect of aldosterone has already been evaluated by measurement of intracellular electrolytes in lymphocytes *in vitro* [15]: cells incubated in medium alone lose both sodium and potassium, this loss is prevented by addition to the incubation medium of aldosterone at physiological concentrations. This effect of aldosterone is prevented by addition of canrenone, an aldosterone antagonist. Cortisol at physiological concentrations does not affect the intracellular concentration of electrolytes.

Table 1. Binding of tracers to steroid receptors in kidney cytosol after incubation of kidney cytosol with charcoal-stripped and unstripped plasma

		Bound nM	
	Added nM	Stripped	Unstripped
[^{3}H]dexamethasone	1.3	0.04	0.12
[^{3}H]aldosterone	0.9	0.06	0.04
[^{3}H]estradiol	1.05	0.04	0.04
[^{3}H]progesterone	2.6	0.04	0.03
[^{3}H]methyltrienolone	0.6	0.08	0.04

The effect of binding of cortisol to these receptors could indeed also involve the immunological function or the distribution of the subpopulations of lymphocytes. It is very interesting that the amount of Type I and Type II receptors in mononuclear leukocytes is similar in T- and B-lymphocytes [16] and for this reason a change in the extent of one subpopulation can not affect the receptor number in the whole lymphocyte population.

A lot of studies have been performed in clinical situations in which corticosteroids are involved. In particular it has been found that Type I receptors, but not Type II receptors, are down regulated by an excess of glucocorticoids [9]. In Cushing's syndrome however we found an inverse correlation between plasma cortisol in the morning and the number of Type II receptors in mononuclear leukocytes and this finding has been attributed to a subtle down regulation of these receptors. These data are not consistent with the finding of low Type II receptors and high plasma cortisol in chronic depression and anorexia nervosa [12]. It is possible that in these situations there is a partial insensitivity to glucocorticoids like in primary cortisol resistance. Some disease-related factors could reduce the number of Type II receptors or the access to the receptor and the subsequent increase of cortisol is a compensation for an adequate glucocorticoid effector mechanism.

A reduction of Type I, but not of Type II, receptors was always found in primary and secondary hypermineralocorticism, with or without hypertension [9, 17, 18], and in pseudohyperaldosteronism from chronic licorice consumption [18].

The down-regulation of Type I receptors by excess of mineralocorticoids in adult population was not present in normal pregnancy where plasma aldosterone is high and Type I receptors are normal [19]. These data are consistent with partial insensitivity to aldosterone in pregnancy as confirmed by our studies on measurement of subtractive potential difference [20] (rectal minus skin potential difference), which is an index of mineralocorticoid effect. In contrast, in preeclampsia, plasma aldosterone is high, as in normal pregnancy, but in this case Type I receptors are low and subtraction

potential difference is similar to that found in primary aldosteronism.

Some endogenous factors, different from the known corticosteroids can also be responsible for the increased mineralocorticoid effector mechanism in the pathogenesis of some cases of essential hypertension. In effect in 20% of patients with essential hypertension we have found a normal concentration of plasma aldosterone and cortisol and a reduced number of Type I receptors in mononuclear leukocytes, while Type II receptors are in the normal range [18].

From these findings one can suggest that the regulation of steroid effector mechanism is more complex, also involving factors which are not directly related to the receptor.

REGULATION OF THE BINDING OF CORTICOSTEROIDS TO TYPE I AND TYPE II RECEPTORS BY FACTORS WHICH ARE INDEPENDENT OF THE RECEPTOR ITSELF

In vivo some factors can affect the effector mechanism of steroids by regulating the access to the receptor itself. One factor is the 11 hydroxysteroid-dehydrogenase. This physiological regulator can also be involved in the pathogenesis of some pathological situations like apparent mineralocorticoid excess syndrome (AME) and the chronic consumption of licorice [21, 22]. In this latter situation, however, the effect seems temporary as the direct binding of glycyrrhetinic acid to the receptors is prevalent when the plasma concentration of the active compound is consistent with this binding [22]. The defect in the AME can be genetic or involve intracellular factors which regulate the activity of 11 hydroxysteroid-dehydrogenase itself.

Other factors have been described or hypothesized on the basis of unusual findings. Substances which hinder glucocorticoid receptors binding can regulate the effect of glucocorticoids. There are naturally occurring cellular factors which impede receptor binding. Khalimi [23] found that treatment of rat liver cytosol with dextran coated charcoal, greatly reduces glucocorticoid receptor binding. We observed the same effect by incubating kidney cytosol with plasma, treated with charcoal–dextrane [24]. Incubation with charcoal-stripped plasma produces a reduction by 70% of the binding of tracer to its receptor when compared with incubation of the same plasma untreated, and thus containing the endogenous steroids. This effect is not an artifact since incubation of the same charcoal-stripped plasma with the same cytosolic preparation and tracer aldosterone, estradiol and methyltrienolone (RU1881), produces a higher binding to the respective receptor than with the same plasma untreated (Table 1). Another interesting situation of altered binding to corticosteroid receptors is chronic depression [12] where Type II receptors in leukocytes are low and become normal after therapy with antiglucocorticoids.

A factor which reduces the binding of aldosterone to Type I receptors is also present in uncomplicated pregnancy as previously seen [19].

Another anomalous finding has been observed in pseudohypoaldosteronism syndrome [25] where Type I receptors, measured by radioreceptorassay, are lacking or extremely low, even when the clinical symptoms disappear. In this syndrome however no alteration has been found of the Type I receptor by molecular biology studies. A genetic factor should thus impair the binding of aldosterone to its receptor both *in vivo* and *in vitro*; the abnormality should remain when the clinical picture disappears; plasma aldosterone should still remain high and receptors absent or very low by radioreceptorassay.

There is another form of pseudohypoaldosteronism which is acquired usually due to obstructive uropathy in infancy. We recently measured Type I receptors in one case with this disease and found no receptors by radioreceptorassay. In the same case the receptors returned to normality after removal of the obstruction, thus hypotheses that some bacterial toxins or other factor related to the uropathy can block the binding of aldosterone to its receptor. From these observations we can argue that the radioreceptorassay *in vitro* can not always reflect the *in vivo* situation, factors being present which regulate the access of the steroid to the receptor. These factors can be produced by the foetus, placenta or by bacteria or they can be genetic. Finally other factors can amplify the action of aldosterone: for example carbenoxolone, a derivative of glycyrrhetinic acid, is able to amplify the action of amounts of aldosterone which exert a maximal effect at the level of urinary electrolyte excretion in adrenalectomized rat [26].

THE GLUCOCORTICOID HYPOTHESIS OF AGING

The glucocorticoid hypothesis of brain aging and neurodegeneration was proposed 20 years ago by Landfield [27] on the basis of reduced hippocampal neurons in young adrenalectomized rats treated with high amounts of corticosteroids and the same findings were reported in old stressed rats. Sapolski hypothesized [6] that in rat, the stress or age-related decrease of Type I receptors in hippocampus is the initial event which activates the CRF–ACTH system and increases plasma cortisol which can damage all brain areas which possess Type II receptors. If this theory is true, the reduction of receptors should be a protective factor while the loss of receptors increases with age and that is not consistent with such an hypothesis. In addition in Cushing's syndrome Type II receptors are not reduced.

It has been shown that episodes of stress or administration of high doses of corticosteroids produce a fall in

Table 2. Significant correlations between plasma aldosterone, plasma cortisol, age, Type I and Type II receptors, lymphocyte subpopulations in the aged group, in the controls and in all cases together

	All cases	Aged	Controls
Cortisol vs age	$r = 0.24$, $n = 61$ $P < 0.05$ D	—	—
Type I vs age	$r = 0.23$, $n = 63$ $P < 0.03$ D	—	—
Type II vs age	$r = 0.65$, $n = 54$ $P < 0.0001$ I	—	—
Type I vs cortisol	—	—	$r = 0.53$, $n = 21$ $P < 0.01$ D
Type II vs cortisol	—	—	$r = 0.56$, $n = 21$ $P < 0.01$ D
Type II vs aldosterone	—	$r = 0.49$, $n = 52$ $P < 0.03$ D	—
Type I vs Type I	$r = 0.54$, $n = 45$ $P < 0.0001$ D	$r = 0.51$, $n = 21$ $P < 0.01$ D	—
CD4 vs age	—	$r = 0.34$, $n = 36$ $P < 0.05$ I	—
CD4/CD8 vs age	—	$r = 0.38$, $n = 37$ $P < 0.05$ I	—

D = direct correlation; I = inverse correlation.

the receptor population in hippocampus of adrenalectomized rat. Landfield has later hypothesized the presence of an age related susceptibility factor which potentiates the neurotoxicity of glucocorticoids [27]. He demonstrated that in old hippocampus there is an enhanced influx and an elevation of intracellular calcium. The neurotoxicity of calcium can play a key role in the neurodegeneration and in the loss of corticosteroid receptors. Prolonged calcium current occurs with aging and both physiological and morphological changes, observed with stress or with aging could depend on altered calcium homeostasis in hippocampus and in other tissues.

CORTICOSTEROID RECEPTORS AND AGING

We have seen that corticosteroid Type I and Type II receptors in MNL have a different regulation in corticosteroid-related diseases. We never found a parallel reduction of both receptors, only mineralocorticoid receptors being feasible of down regulation [9, 17–19]. When Type I receptors are low, usually Type II receptors are normal. That happens in Conn's syndrome, Cushing's syndrome, preeclampsia, chronic licorice ingestion and even in pseudohypoaldosteronism [24].

Only in aging did we find a parallel pattern of decrease [13, 14], Type I and Type II receptors being directly correlated. It seems that the factors which produce the reduction of one receptor are also acting at the level of the other. We found an inverse correlation between Type I receptors and age and Type II receptors and age (Tables 2 and 3) [13, 14] and in addition in the same patients cortisol at 8 a.m. is higher than in controls [13]. The increase of glucocorticoids is not associated with an increased effector mechanism. In effect we could not find some clinical signs of increased glucocorticoid effector mechanism in aged population and on the other hand in Cushing's syndrome, where glucocorticoid effect is markedly high, the Type II receptors are not decreased. We believe that the involution of brain or other tissues is a constituent of aging and it develops earlier or later in relation to physiological, genetic and stressor situations. This phenomenon involves both Type I and Type II receptors and the loss of receptors disregulates the hypothalamic–pituitary–adrenal axis with an increase of corticosterone in animal or cortisol in man. The increase of these hormones is not followed by an increased effector mechanism, receptors being low, but it is simply a compensation for maintaining the normal glucocorticoid effector mechanism: in effect we did not find an alteration of the extent of lymphocyte subpopulations in the same cases where we found high cortisol and low corticosteroid receptors. The stable loss of corticosteroid receptors is responsible for the altered recovery of adrenal function after stress which has been found in aged rats. Another interesting finding of these studies is the direct correlation between Type II receptors and aldosterone in the aged group. Usually in the aged population the renin–angiotensin system is less reactive

Table 3. Mean ± SD of plasma cortisol, plasma aldosterone, Type I receptors, Type II receptors, CD4/CD8 ratio in aging and in controls

	Aging	Controls
Type I receptors $n \times$ cell	198 ± 96	260 ± 120
Type II receptors $n \times$ cell	1745 ± 802	3339 ± 918
Plasma cortisol nmol/l	346 ± 140	260 ± 120
Plasma aldosterone nmol/l	270 ± 230	330 ± 170
CD4/CD8	1.9 ± 0.8	1.6 ± 0.5

Table 4. Type I receptors, Type II receptors, plasma cortisol and plasma aldosterone in aging (n = 7) and in controls (n = 10) before and after 1 mg of dexamethasone

	Controls		Aged	
	Baseline	Dexamethasone	Baseline	Dexamethasone
Plasma cortisol μg/dl	15.2 ± 3.9*	3.7 ± 3.6	19.1 ± 6.9*	1.4 ± 0.3
Plasma aldosterone ng/dl	19.8 ± 9.3	18.4 ± 9.8	8.8 ± 4.7	10.7 ± 6.7
Type I receptor $n \times$ cell	279 ± 68*	180 ± 74	209 ± 60	168 ± 83
Type II receptor $n \times$ cell	2745 ± 767	2342 ± 537	1796 ± 671*	720 ± 345

*$P < 0.05$.

and it is possible that in this situation the plasma concentration of aldosterone is dependent on the number of Type II receptors.

Probably the brain damage and the subsequent loss of corticosteroid receptors is due to a cellular alteration. A factor which is surely involved in this situation is the increased permeability of cells to calcium with disrangement of cell function and senescence. This loss could be due to a normal physiological process of alteration of cell membrane mechanisms which regulate the omeostasis of transmembrane exchanges. Probably this is ot a susceptibility factor for the increased damage by glucocorticoids but rather the increase of calcium itself is the primary damaging factor.

RAPID DEXAMETHASONE SUPPRESSION TEST AND CORTICOSTEROID RECEPTORS

In controls, overnight suppression with dexamethasone produced a clear reduction of Type I receptors in all patients (Table 4), which can be interpreted as a primary involvement of Type I receptors in the response to dexamethasone, although the affinity *in vitro* of dexamethasone for Type I receptors is very low. The reduction of Type II receptors was evident in 5 cases and in the other 4 subjects the receptor number increased. Plasma cortisol was suppressed in all the cases.

It is possible that the response to dexamethasone involves primarily Type I receptors and subsequently Type II receptors. We have studied the status of corticosteroid receptors also in uncomplicated aging after dexamethasone suppression. In these subjects, at difference with controls, the Type II receptors were also clearly reduced from pretreatment values. The explanation of this phenomenon is that in adult there is a possibility of up regulation of glucocorticoid receptors while in aging the reduction of receptors is consistent with submaximal occupancy and function and in this case the down regulation is evident.

CONCLUSIONS

The human mononuclear model is probably a feasible tool for studying the regulation of the CRF–ACTH–cortisol axis via the occupancy of Type I and Type II corticosteroid receptors. The mechanisms which regulate the receptor number and their function are complex and do not only involve the plasma concentration of corticosteroids and their affinity for the receptor. Factors are probably present which can regulate the binding of corticosteroids and affect the receptor concentration. These factors can be physiological or pathological, being involved in the pathogenesis of some diseases and particularly of essential hypertension and in some psychiatric diseases. The reduction of the number of corticosteroid receptors in aging is probably related to an involution of cell function and protein synthesis not only in lymphocytes but in all tissues and also in the brain. The Type I receptors seem to be more important in the regulation of the corticosteroid effector mechanism, these receptors being responsible for the immediate response to glucocorticoids, as demonstrated by their involvement in the response to dexamethasone administration. Type II receptors are probably involved when plasma concentration of corticosteroids is higher, like in the early morning or during stress.

From these studies we have suggested that: (i) the evaluation of corticosteroid receptors in MNL resembles that of brain; (ii) the increase of cortisol in aging is not a marker of increased glucocorticoid activity but a compensation to the reduced number of receptors also in the brain; and (iii) the decrease of receptors is an age-dependent phenomenon, but in some patients receptors remain normal as a possible genetic or acquired marker of brain involution. The measurement of corticosteroid receptors could thus be used as a marker of brain involution.

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