CNS & Neurological Disorders - Drug Targets, **2013**, *12*, **1163-1174**

Molecular Basis of Mineralocorticoid Receptor Action in the Nervous System

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Abstract: The most relevant biological action of aldosterone in epithelial tissues is the regulation of sodium reabsorption through binding to the mineralocorticoid receptor (MR). Glucocorticoids also bind with high affinity to MR, which is usually protected by the enzyme 11β-hydroxysteroid dehydrogenase. This activity prevents MR activation by cortisol despite the large prevalence of this steroid in plasma. Nonetheless, there are some aspects of the mechanism of action of MR that are not entirely explained by this competitive metabolic mechanism of protection. The picture is even more complicated in those tissues such as the nervous system where the enzyme is expressed at very low levels or is directly absent in various areas of the brain. Therefore, other cellular and molecular mechanisms must also intervene to allow specific aldosterone biological effects in the presence of overwhelming concentrations of glucocorticoids. In this article, we discuss some possible mechanisms that permit the specificity of action for each type of steroid, including those related to the recently discovered novel molecular mechanism of activation of corticosteroid receptors and the structural requirements of a given ligand to favor the mineralocorticoid action *via* MR. The relative contribution of these mechanisms may vary in different target cells allowing the fine tuning of cellular functions depending on the degree of cooperation between steroids, receptors, chaperones associated to receptors, and other factors. All these regulatory interactions can be altered in some pathophysiological situations, most of them related to stressing situations.

Keywords: Aldosterone, cortisol, glucocorticoid receptor, heat-shock protein of 90-kDa, immunophilins, mineralocorticoid receptor, stress disorders, steroid conformation.

INTRODUCTION

Corticosteroids are a family of steroid hormones produced by the adrenal cortex. They are secreted according to a highly regulated signalling control commanded by the hypothalamic-pituitary-adrenal (HPA) axis, where the adrenocorticotrophic hormone (ACTH) plays a cardinal role in the regulation of this cycle [1, 2]. From the quantitative point of view, the foremost steroids produced by the adrenal gland belong to either subfamily of steroids, glucocorticoids or mineralocorticoids. Aldosterone is the most important mineralocorticoid, whereas cortisol in humans, and corticosterone in rodents (except guinea pigs), are the main glucocorticoids.

Under basal conditions, there is pronounced circadian activity in the HPA axis characterized by circadian ACTH release and the consequent production and secretion of corticosteroids [3]. In the man, cortisol reaches its maximum plasma level at the end of the resting period in preparation of the increased metabolic demands of the active phase. Nocturnal animals such as rodents, peak in corticosterone levels towards the end of the afternoon when the dark cycle begins.

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ACTH is also a good stimulus for the production and secretion of aldosterone, although the effect is relatively transient because this rise in aldosterone produces hypervolemia (which inhibits angiotensin-II production) and also hypokalemia [4]. The most relevant biological action of mineralocorticoids involve the enhancement of unidirectional vectorial transport of Na^+ and water, and K^+ and H^+ in the opposite direction across epithelial barriers such as that of the renal tubule and choroid plexus. In the adrenal *zona glomerulosa*, synthesis of the primary mammalian mineralocorticoid aldosterone is favored by angiotensin II *via* the AT1 receptor, and is also increased by high K^+ levels and a low-salt intake, all three stimuli being the best physiologic controllers for aldosterone secretion. The best characterized biological effects of aldosterone are mediated by the soluble mineralocorticoid receptor (MR).

The renin-angiotensin-aldosterone system is also subject to a circadian rhythm that is largely in phase with the HPA axis rhythm. Nevertheless, the rhythm of urinary excretion of electrolytes is unlikely to be due to the rhythm of aldosterone secretion only since several other factors are also involved in the regulation of natriuresis [5]. Aldosterone, but no cortisol, is responsible for the central regulation of blood pressure and salt appetite [6, 7]. Thus, aldosterone infused intracerebroventricularly increases blood pressure, whereas an equivalent dose given peripherally is ineffective. This clearly showed that a specific central action. A small fraction of brain MR is related to this control of blood pressure, water and

1996-3181/13 $58.00+.00

electrolyte balance, sodium appetite, and sympathetic drive to the periphery, and it appears that circulating inflammatory cytokines modulate MR-mediated changes in sympatho-excitation (see [8] for a recent review).

As part of their general mediation of the stress response, glucocorticoids have been proposed to influence certain key cellular events within the central nervous system (CNS) such as synaptic plasticity [9], neurotransmitter receptor expression [10], the actions of neurotoxins [11] and protein processing [12]. Corticosteroids have also been proposed to play a role in psychiatric disorders including depression, Cushing syndrome and post-traumatic stress disorder [13] as well as age-associated diseases such as Alzheimer's [14, 15], in addition to having detrimental effects on brain development [16]. Glucocorticoids mediate their effects *via* two classes of corticosteroid receptors, MR and the glucocorticoid receptor (GR), which can be distinguished due to their differential ligand affinities. GR is widely expressed throughout the brain, while MR is found primarily in the hippocampus [17]. Both subtypes of receptor are, however, expressed in all brain cell types and under normal conditions, it appears that the GR expressed in the CNS is mostly unoccupied, although it becomes progressively activated under stressful conditions [18].

Both corticosteroids, aldosterone and cortisol, show similar affinity for the MR. Nonetheless, aldosterone binding occurs in the brain and specific mineralocorticoid responses are triggered despite of the fact that the concentrations of glucocorticoids could be three orders of magnitude higher than those of aldosterone. In this article we address this conundrum and will discuss some possible mechanisms for the selective activation of MR by aldosterone, the molecular mechanisms of action of MR-aldosterone complexes, the structural requirements for a given steroid to show mineralocorticoid action, and the effects of aldosterone in the brain.

STEROID RECEPTOR FAMILY

Steroid receptors are a subfamily of a large superfamily that comprises the most abundant classes of transcriptional regulators in metazoans, the nuclear receptor superfamily. Nuclear receptors are phylogenetically related proteins that have been clustered into a large superfamily [19], which includes receptors for hydrophobic molecules such as steroid hormones (estrogens, progesterone, androgens, glucocorticoids, mineralocorticoids, vitamin D, ecdysone, oxysterols, bile acids, etc.), retinoic acids (all-*trans* and 9-*cis* isoforms), thyroid hormones, dioxin, sterols, fatty acids, leukotrienes and prostaglandins.

Steroid receptors are not only receptors of a given hormone, but they are ligand-activated transcriptional regulators. Based on the alignment of the DNA-binding domain and the ligand binding domain, and due to phylogenetic analysis, the members of this subfamily were classified in a consensus branch of the superfamily tree [20] where those receptors with steroid-binding ability are comprised in the same subfamily of highly homologous members, i.e. estrogen receptor (ER), estrogen-related receptor (ERR), androgen receptor (AR), progesterone receptor (PR), GR, and MR. In turn, based on their binding to a consensus DNA sequence, ERR and ER belong to the so called ER-subgroup (they bind to an AGGTCA P-box sequence), whereas the GR, MR, AR and PR belong to the GR-subgroup (they bind to an AGAACA P-box sequence). The other members of the superfamily are more distant from those related to the adrenal and sex steroid receptors [21]. An important observation is that the human sequences of steroid receptors are close related to their homologous forms in amphibian and fish. Thus, it is possible that divergence of this subfamily from other nuclear receptors occurred before the appearance of fishes. Even though there is no expression of any nuclear receptors in yeasts or plants, nuclear receptors can function nicely when they are cotransfected along with a reporter gene. This indicates that the basic machinery for transcriptional activation by nuclear receptors evolved in the common ancestor of fungi and metazoans, even when this ancestor did not contain nuclear receptors.

To understand better the molecular mechanism of action of the steroid-receptor functional unit, we have to realize the context in which both components of the signalling pathway have evolved. The irruption of steroid receptors as transcriptional transducers of physiological responses mediated by adrenal and sex steroids provided early vertebrates with an advantage in competing with the diverse organisms that evolved during the Cambrian explosion and lacked either some or all of these receptors. It is thought that the members of the GR-subgroup are derived from an ancestral receptor that underwent genome duplication to give GR/MR and AR/PR ancestors [20]. This process was then followed by a new diversification step to give the four separate receptors. Steroidogenesis studies from fish interrenal tissues (an organ equivalent to the adrenal cortex of mammals) could not demonstrate that aldosterone can be synthesized, so the general consensus is that most fish do not produce this steroid, and mineralocorticoid effects are mainly managed by glucocorticoids [22].

There is a general agreement that both receptors, GR and MR, have diverged through gene duplication and divergence of a common ancestor [20, 23, 24]. Recent studies by Bridgham *et al.* [25] suggested that the capacity to bind aldosterone is previous to the acquisition of the capacity to synthesize the steroid itself. Using ancestral gene resurrection technique, these authors demonstrated that, long before the hormone evolved, the affinity of the common ancestral receptor for aldosterone was present as a structural by-product of its partnership with chemically similar, more ancient ligands. Introducing two amino acid changes into the ancestral sequence recapitulated the evolution of present-day receptor specificity, indicating that tight interactions can evolve by molecular exploitation or recruitment of an older molecule, previously constrained for a different role, into a new functional complex. In short, the ancestral receptor of GR and MR and its descendant genes were structurally preadapted for activation by aldosterone when that hormone evolved millions of years later. After the duplication that produced GR and MR, only two substitutions in the GR lineage were required to yield two receptors with distinct hormone-response profiles [25]. The evolution of an MR that could be independently regulated by aldosterone enabled a more specific endocrine response, because it allowed electrolyte homeostasis to be also controlled without triggering the GR stress response, and vice versa. This

evolutionary scenario that implies the recruitment of an ancient receptor into partnership with a novel ligand would be the obverse of the case of AR and PR, where duplication of an ancient estrogen-responsive receptor evolved affinity for steroids that previously served as intermediates in estrogen synthesis [26]. On the other hand, convergent evolution is also a possible explanation in other cases [27], for example, in the case of the high affinity binding for estradiol found in vertebrate ERs, α-fetoprotein, sex steroid binding globulin, and yeast enzymes (which do not express steroid receptors).

THE MINERALOCORTICOID RECEPTOR

As a direct consequence of the previously described evolutionary model, it may be predicted that MR and GR should share considerable homology and must show considerable cross-reactions at many levels. That this is the case is exemplified by the ability of glucocorticoids to bind both receptors, because MR can occupy glucocorticoid-response elements, and the fact that, to date, the putative mineralocorticoid-response element is unknown. This adds to the difficulty in separating GR and MR function in many tissues, particularly in the brain. The conundrum was partially answered after the discovery of the protective action of the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2) [28], an enzyme that converts cortisol and corticosterone to cortisone and 11-dehydrocorticosterone, both steroids being weak MR ligands. This rapid conversion of these potentially active glucocorticoids creates a microenvironment where aldosterone can effectively bind to the MR. This effect is particularly notorious in epithelial tissues, although there are tissue-specific differences. Even though the MR-mediated response in some tissues, including the brain, depends on whether the ligand is aldosterone or cortisol [7, 29], electrolyte transport in epithelial cells could be equally mediated by any of these steroids if the activity of 11βHSD2 is inhibited. Similarly, the elevated concentrations of cortisol attained in ectopic ACTH syndrome are thought to saturate the 11βHSD2 capacity to protect MR from glucocorticoids, resulting in inappropriate activation of MR by cortisol [30].

It is now well established that the MR forms heterocomplexes with the 90-kDa and 70-kDa heat shock proteins (Hsp90 and Hsp70, respectively), the acidic protein p23, and proteins that possess sequences of 34 amino acids repeated in tandems, the tetratricopeptide repeat (TPR) proteins [31, 32]. Some of these Hsp90-binding TPR proteins have peptidylprolyl-isomerase activity and are indeed intracellular receptors for immunosuppressant drugs such as FK506 and cyclosporine. Due to this reason, they have been called immunophilins (IMMs). The most important IMMs that have been recovered in steroid receptor-Hsp90 complexes are FKBP52, FKBP51, and CyP40, in addition to two IMM-like proteins, protein phosphatase 5 (PP5) and WISp39/FKBPL [31]. Even though the biological function of these proteins in the steroid receptor-Hsp90 heterocomplex remains poorly understood, it is clear that these IMMs are not related to the immunosuppressant effect, which depends on the low molecular weight family members of the IMM family [33]. To date, it has been documented the association of the MR-Hsp90 complex with FKBP51, FKBP52 and PP5 [32, 34], but not with CyP40 and WISp39/FKBPL.

In the absence of steroid, this MR-Hsp90-TPR protein oligomer resides predominantly in the cell cytoplasm [32, 35-38]. However, like other transcription factors, the MR is not confined statically to any particular compartment of the cells, but it continuously shuttles between the cytoplasm and the nucleus [31, 39]. Consequently, the primary accumulation of the MR in a given compartment (it is cytoplasmic in the absence of steroid, and nuclear in its presence) is the resulting average of this dynamic equilibrium displaced to one compartment or the other.

It has always been assumed that the driving force of movement for steroid-receptors is diffusion. This classical model was posited by general consensus during the 80's and supported the heuristic notion that the receptor-chaperone complex must be dissociated immediately after steroid binding (a process referred to as 'transformation'). Therefore, according to the classical model, transformation should be a requirement to favor the release of the receptor from the cytoplasmic anchoring sites, allowing its cytoplasmic diffusion and subsequent passage through the nuclear pore complex. Thus, the receptor reaches its nuclear sites of action. Nonetheless, the recent observation that the dynein/dynactin motor complex associates with the Hsp90-FKBP52 complex of both receptors GR and MR, indicates that this motor protein complex powers the active retrograde movement of steroid-receptors [32, 40]. Therefore, the Hsp90-FKBP52 complex plays a significant role when it is still associated to the receptor and should not be dissociated form the receptor during its early steps of activation. Recently, it was demonstrated that receptor transformation and receptor dimerization are indeed nuclear events [32, 40, 41].

While FKBP52, CyP40 and PP5 are redundant IMMs in their ability to interact with dynein/dynactin [39], FKBP51 is a poor interactor with the motor complex and is also an effective transcriptional inhibitor of both receptors [34]. Therefore, it is not surprising that upon steroid binding, FKBP51 is released from the receptor complex and replaced by FKBP52, which in turn recruits the dynein/dynactin complex (Fig. **1**). This IMM exchange assemblies the molecular machinery for the efficient retrotransport of the steroid-receptor complex. Further studies demonstrated that Hsp90 is still part of the heterocomplex when MR is nuclear [31]. Accordingly, the entire Hsp90-based heterocomplex cross-linked to either GR [40] or MR [32] translocates intact through the nuclear pore of digitonin-permeabilized cells in a hormone-dependent manner, suggesting that steroid-receptor transformation is a nuclear event. Accordingly, members of the chaperone heterocomplex are able to interact with structures of the nuclear pore such as nucleoporins and importins [40].

This novel mechanism of action differs from the classical model in that Hsp90 should not dissociate from the receptor to initiate its nuclear translocation. Actually, the experimental evidence shows that Hsp90 is required for this process [40]. If this model is correct, homodimerization should also be a nuclear event. Recent evidence with the GR

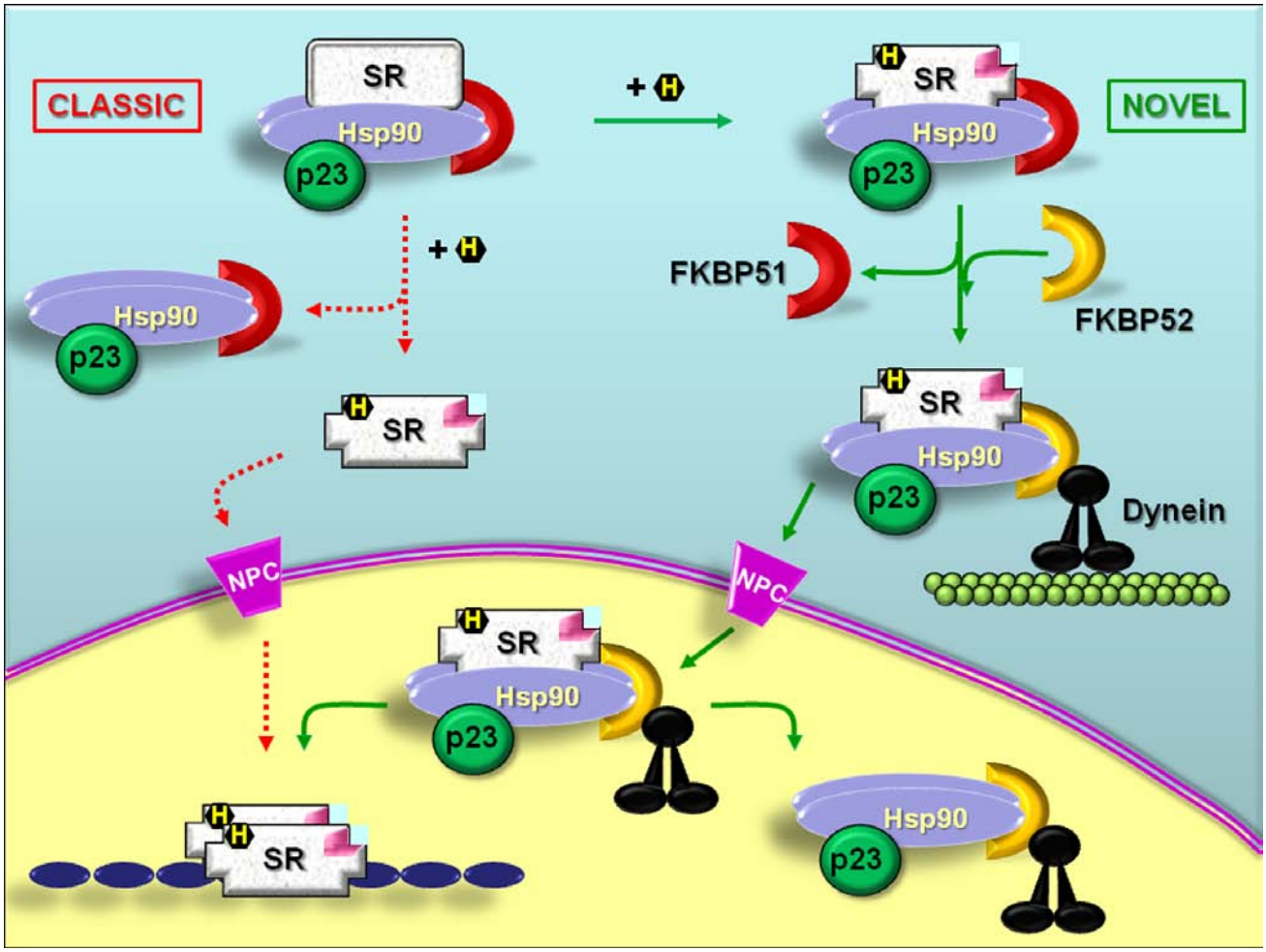

Fig. (1). Molecular mechanism of activation of corticosteroid receptors. According to the classical model for steroid receptor (SR) activation (depicted with red dashed lines), the Hsp90-based complex dissociates immediately after hormone (H) binding. This promotes both the release of the SR from the cytoplasmic anchoring sites and its free diffusion towards the nucleus. The passage of this transformed receptor through the nuclear pore complex (NPC) is facilitated by the exposure of its nuclear localization signal (pink area). In the nucleus, the dimer interacts with the promoter regions of the hormone response elements to induce transactivation of target genes. In this model, it results uncertain whether receptor dimerization takes place in the cytoplasm upon Hsp90 dissociation or in the nuclear compartment (either in the nucleoplasm or on the promoter sequences). According to the novel model (depicted with continuous green lines), the SR heterocomplex associated to the IMM FKBP51 (red crescent) exchanges this TPR-domain protein with its homologous FKBP52 (yellow crescent), which is able to interact with the dynein/dynactin motor complex (black). Therefore, the complex serves as a traction chain for the SR, whose movement towards the nucleus occurs on cytoskeletal tracts. The nuclear localization signal protrudes upon steroid binding and the whole complex (still associated to Hsp90) translocates through the nuclear pore. Receptor transformation and subsequent dimerization occurs in the nucleoplasm. The SR interacts with its specific nuclear sites of action and the chaperone complex is recycled.

agrees with this speculation [42]. Because the dimerization domain of the receptors is blocked by the Hsp90 complex, it is valid to wonder whether receptor dimerization is a nuclear event or whether it paves the way to favor the nuclear translocation of the receptor. Recently, Grossman *et al.* [41] have elegantly confirmed the above-described novel model of action of MR and have univocally demonstrated that MR homodimerization is a nuclear process. Moreover, previous DNA-binding experiments favor a model involving early dimerization prior to DNA-binding rather than consecutive binding of monomers [43], which is also in agreement with the lower affinity of monomers for DNA [44].

It should be kept in mind that receptors represent only a part of a complex biological mechanism. The other relevant character of this plot is the ligand. It should be emphasized that the information for hormonal regulation is written neither in the steroid nor in the receptor exclusively, but in both components of a complex functional unit. In turn, this functional unit may be subject of other kinds of non-hormonal- and/or non-receptor-dependent regulations.

MINERALOCORTICOID ACTIONS IN THE BRAIN

Pioneer studies in the rat brain [45, 46] demonstrated by autoradiography that aldosterone and corticosterone are mostly retained in the hippocampus, followed by circumventricular organs and select nuclei of the brainstem, aldosterone binding in the cortex, thalamus, and brainstem

being more widespread and greater than that of corticosterone. The importance of specific nuclei of the anterior hypothalamus, circumventricular organs, particularly the floor of the third ventricle and area postrema, and central sympathetic nervous system in renovascular and mineralocorticoid-salt induced hypertension were established by works in DOCA (deoxycorticosterone acetate)-resistant rats [47] and a large series of ablation studies (see [48-50] for complete reviews of this matter). The chronic intracerebroventricular infusion of aldosterone at rates that do not alter the blood pressure when infused systemically increases the blood pressure of rats and dogs, whereas infusions of MR antagonists or inhibitors of effectors of aldosterone such as the epithelial sodium channel (ENaC) prevent the onset and mitigate established systemic mineralocorticoid–salt-induced hypertension (reviewed in [51]). Interestingly, recent evidence indicates that ENaC is not exclusively expressed in epithelial tissues, but it is also present in the nervous system [52] in the choroid plexus and various circumventricular organs (see [53] for a recent review).

Recent evidence indicates that hypothalamic MR is also involved in responses to injury and inflammation in the cardiovascular and renal systems. Accordingly, the central blockade of the MR in models of mineralocorticoid excess or myocardial infarction decreases the severity of congestive heart failure, sympathetic drive and sympathetically mediated renal dysfunction [48, 54]. Moreover, both the intracerebroventricular administration of DOCA and myocardial infarction increase plasma inflammatory cytokines, including TNFα, an effect that is blocked by antimineralocorticoids such as spironolactone and eplerenone [55, 56]. Even though cytokines do not readily cross the blood brain barrier, they are able to influence neuronal activity by inducing brain microvascular COX2 activity, resulting in an increase in prostaglandin E2 (PGE2). In turn, PGE2 crosses the blood-brain barrier and activates neurons, including those of the AV3V area that project to the medial preoptic area and paraventricular nucleus of hypothalamus. Among other effects, this increases sympathetic nervous system drive to the vessels, heart and kidneys [57]. The oral administration of MR antagonists also significantly reduces hypothalamic COX-2 protein expression, followed by decrease in cerebrospinal fluid PGE2, plasma norepinephrine, TNFα, and interleukins 1β and -6. Importantly, these effects mimic those of anti-cytokine drugs [56].

CORTICOSTEROID LIGANDS

Even though the analysis of the structural requirements needed for an ideal mineralocorticoid agonist have always been extremely difficult to define, the observation that aldosterone possesses a poorly angled steroid nucleus at the A/B-ring junction led to postulate that mineralocorticoids may require a flat conformation for optimal activity *in vivo* [58-60]. In this regard, the conformational structure of aldosterone shows a C_3/D-ring angle equal to -14^o whereas cortisol shows an angle equal to -34^o (see schemes in Fig. **2A**). It is classically accepted that certain critical functional groups enhance mineralocorticoid potency, for example, a C_{21}-hydroxyl. Interestingly, the synthetic preganesteroid 11, 19-oxidoprogesterone (Fig. **2A**) lacks this functional group, its main characteristic being its overall conformational planarity. A similar statement can be made for the biological potency of other pairs of compounds such as the flat steroid 5*α-diH*-progesterone (a strong sodium-retainer) and its bent isomer 5*β-diH*-progesterone. Because these (and other) compounds possess exactly the same functional groups, but differ in their conformational properties, it was suggested that a flat conformation of a given ligand may be more important for the acquisition of mineralocorticoid activity than the presence of certain functional groups [60]. Such observation applies to most 21-deoxypregnanesteroids and might also be extended to 21-hydroxypregnanesteroids [59]. Note that 11,19-oxidoprogesterone is as a potent sodium retainer as 11-deoxycorticosterone, whereas the highly bent conformer 6,19-oxidoprogesterone is almost devoid of effect (Fig. **2B**). Like in the previous case of the dihydroderivatives of progesterone, these two steroids share exactly the same functional groups, but a totally different conformation.

Dose-response curves in the large range of 0.01-500 μg/100 g of rat body weight for some natural and synthetic compounds, exhibit a parabolic function (Fig. **2B**), i.e., a maximal anti-natriuretic effect, which varies according to the steroid, is shown at certain doses, whereas a clear reversion of the effect is observed at higher doses. Although less evident than for weaker steroids, the tendency to reverse the Na^+-retaining effect can also be observed for the most active compounds, including aldosterone, at the highest doses. Such a biphasic function of the dose-response curves makes unsuitable the use of the ED_{50} value to quantify the biological effect. A classical ED_{50} does not consider the multiple parameters involved in the parabolic function, such as doses at which the maximal retention is achieved, the magnitude of this maximal response, the minimal active dose, and more importantly, the reversion of the effect observed at higher doses.

The problem could be partially solved by correlating the sodium retaining response with the second-order polynomial of the function defined by the equation $y = ax^2 + bx + c$. Thus, the second-order coefficient '*a*' is a direct measure of the concavity of the polynomials, which represent in turn the biopharmacological parameters of the dose-response curves obtained with each steroid. The best correlation was found for the C_3=O/D angle plotted against the coefficient '*a*' as representative of the biological action (Fig. **2C**). Accordingly, there is a tendency to increase the mineralocorticoid effect (i.e., lower coefficient '*a*' value) with a higher affinity for the MR [59].

Because the coefficient '*a*' is calculated from *in vivo* measurements, it includes all the variables that affect ligand availability for the MR *in vivo*. From this hypothesis, such a good correlation is not surprising, but it should not be confused with the oversimplified conclusion that affinity potency for the receptor *in vitro* can predict ligand potency. The analysis of the whole dose-response curve measured *in vivo* allows the calculation of the coefficient '*a*', which seems to be the most representative factor to semi-quantify, and perhaps predict, the mineralocorticoid effect for a given steroid according to its geometry. Nonetheless, one of the limitations of this model is that the second-order coefficient '*a*' cannot be measured for ligands that show no parabolic

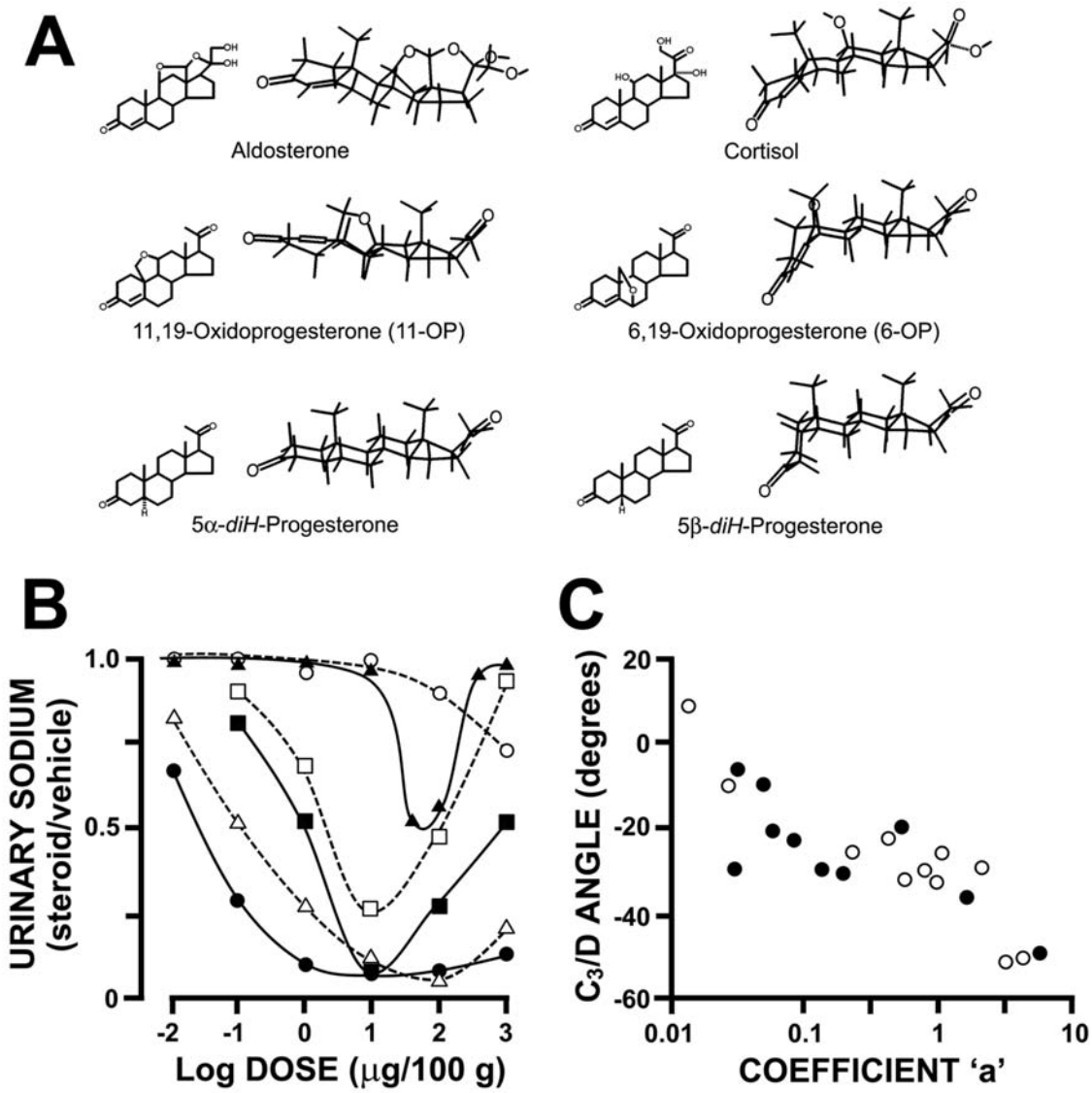

Fig. (2). Steroid structure-mineralocorticoid activity relationship. (**A**) Aldosterone shows a flat conformation compared to the highly bent angle of glucocorticoids such as cortisol. The steroids 11,19-oxidoprogesterone and 5α-diH-progesterone (left hand side) show similar flat conformations and are stronger mineralocorticoids than those bent conformers showing identical chemical groups (depicted on the right hand side). (**B**) Sodium elimination profiles show parabolic shapes that fit in a second-order polynomial functions defined by the equation $y = ax^2 + bx + c$. The second-order coefficient '*a*' is a direct measure of the concavity of the polynomial function and summarizes the biopharmacological variables related to the final biological response for each steroid. Lower '*a*' values indicate stronger mineralocorticoid action. Note that steroids with a greater maximum effect at a given dose also show narrower parabolic shapes of the whole function. Symbols for steroids are: aldosterone (–•–), cortisol (--○--), 6,19-oxidoprogesterone (–▲–), 11,19-oxidoprogesterone (--Δ--), 5α-diH-progesterone (--■--), 5β-diH-progesterone (–□–). (**C**) Steroids align in a linear function when the C_3= O/D angle of the ligand is plotted against the final biological effect (estimated as the coefficient '*a*'). Note that some steroids cannot be included in this plot because they do not show a counter-effect that permits the fitting into a parabolic function (for example, see the dose-response curve for cortisol in panel **B**). White circles represent 21-deoxysteroids and black circles are 21-hydroxysteroids. Panels **B** and **C** were adapted from reference [59].

function in the range of doses assayed, i.e. steroids like dexamethasone that exhibit a very weak mineralocorticoid effect *in vivo*.

Attempts to find correlations by using several other parameters were unsuccessful (i.e., steroid hydrophobicity, hydration sphere, length of the molecule, total surface area, van der Waals radius, electronic density, etc.). It is noteworthy that the structure-activity correlation can only be obtained when the *in vivo* biological effect is considered as a whole, regardless of the number and nature of the regulatory mechanisms involved in the resultant mineralocorticoid action.

Nonetheless, planarity alone does not explain different biological activities; the best example is the contrast between cortisol and aldosterone. Even in assaying what appears to be similar actions, different methods and different tissues may

yield varying results, and some of the effects of a steroid may be indirect. For example, while the 11-ketocorticosteroids are generally far less potent than the 11β-hydroxycorticosteroids, they may have a physiological role, perhaps in modulating responses to aldosterone [61]. Accordingly, cortisol blocks aldosterone action in cardiomyocytes, yet it is an aldosterone agonist in vascular smooth muscle or kidney. Comparisons of biological activity thus depend very much on the system that is being studied and represents a challenge. Even so, the ligand must possess some particular features that make it suitable for a given receptor. Recently, studies applying molecular dynamics methodology suggested that the dynamic flexibility of the three six-carbon atom rings A, B and C of the steroid varies substantially from molecule to molecule, these variations being correlated with specificity [62]. Thus, pure glucocorticoid activity was associated with a relatively rigid ring A, and to some extent ring B, but more flexible ring C. High mineralocorticoid potency, in contrast, is often (though not exclusively) reflected in a rigid ring C.

OTHER FACTORS RELATED TO ALDOSTERONE SPECIFICITY

In addition to the conformational requirements of the ligand to interact more efficiently with the MR, there are several other factors that confer specificity of action to corticosteroids. The previously commented regulation mediated by the protective action of the enzyme 11βHSD2 on the compiting action of the MR against cortisol is a relatively efficient mechanism in epithelial tissues where MR and GR coexist. Nonetheless, under normal steroid concentrations, most MR is at least partially occupied by non-stress levels of glucocorticoids, including in the brain [63, 64] where GR is occupied by cortisol or corticosterone only at the zenith of the circadian cycle and during stress.

The MR is also broadly expressed in the cardiovascular system in cardiomyocytes, endothelium, and smooth muscle cells of the vasculature. However, even though in the vasculature there is a co-expression of 11βHSD2, the enzyme is not expressed in cardiomyocytes [65]. Consequently, the MR is overwhelmingly occupied (although normally not activated) by endogenous glucocorticoids (see [66] for a recent perspective). Similarly, the MR is expressed extensively in the CNS, and the hippocampus arguably has the highest abundance of MR of any tissue in the body with the possible exception of the distal colon [67]. Nonetheless, only a small number of nuclei in the CNS show co-expression of 11βHSD2 and MR. Despite of this fact, there is compelling evidence that the role of MR does relate to sodium balance by regulation of salt appetite [68].

Even allowing for issues of plasma binding, the MR would be permanently occupied by cortisol in contexts where the activity of 11βHSD2 is low or absent. In other words, other mechanisms must also exist to confer specificity to aldosterone over cortisol. Among them, it can be postulated different efficiencies for homodimerization versus heterodimerization with other factors, for example, with the GR [69] or NFkB [70]. Similarly, the receptor can interact with different co-factors according to the nature of the bound ligand, which in turn affects the final biological response. There is strong evidence in favor of this speculation. Kitagawa *et al.* [71] convincingly demonstrated that aldosterone binding to the MR, but not cortisol, favors the binding of RNA-helicase A (RHA) and CREB-binding protein (CBP) complexes to the AF-1a region of the receptor, as well as to permit the cooperative potentiation of MR transcriptional activity by the RHA/CBP complex. Chromatin immunoprecipitation assays showed that aldosterone-bound MR, but not cortisol-bound MR, recruited RHA to native MR target gene promoters, which then recruited a complex with histone acetyltransferase activity that contained CBP. These observations imply that the conformation induced by aldosterone, but not by cortisol, determine the accessibility of the AF1a domain to RHA/CBP complexes. Therefore, even though both steroids show comparable affinities for the MR, aldosterone binding is not entirely equivalent to cortisol binding.

Similarly, it has been demonstrated that limited proteolysis of the steroid-MR complex generates different proteolytic fragments [34] upon binding of the synthetic mineralocorticoid agonist 11,19-oxidoprogesterone regarding to aldosterone binding [72]. This is compatible with the induction of a different conformational change of the MR. Accordingly, each steroid-MR complex recruits different TPR-domain co-chaperones and shows different nuclear distribution pattern [34]. In turn, this raises the possibility that the relative expression level of a given TPR-protein could also regulate MR activity in a given tissue or cell type. In line with those studies, in a recent work [73] ligand-selective MR-interacting peptides were analyzed, and it was shown that LxxLL-containing peptides bind the MR in the presence of aldosterone, whereas non-LxxLL containing peptides bind MR preferentially in the presence of cortisol, suggesting that aldosterone and cortisol induce unique MR conformations.

Two additional mechanisms should also be considered — the existence of plasma membrane receptors and the local extra-adrenal synthesis of aldosterone. Aldosterone modulates the expression of membrane targets such as the subunits of the ENaC, in combination with important signalling intermediates such as serum and glucocorticoid-regulated kinase-1. These actions appear to be mediated by the classical cytoplasmic MR and are antagonized by antimineralocorticoids (see [74] for a recent review). In addition, there are rapid 'non-genomic' activation of protein kinases and secondary messenger signalling cascades, that are not affected by MR antagonists, which appear to be mediated by plasma membrane receptors. To date, the identity of this alternative aldosterone receptor remains elusive. Nevertheless, aldosterone promotes the activation of multiple secondary messenger responses including a rise of intracellular calcium, cyclic AMP, and nitric oxide release in a matter of a few seconds [74-77]. Electrophysiological evidence suggests the presence of a rapid membrane MR and GR at both pre- and post-synaptic locations [78, 79], which rapidly induce changes to synaptic transmission. Moreover, behavioral and cognitive evidence suggests the presence of both receptors throughout the limbic system, including hippocampus and amygdale [80]. The rapidly activated signalling cascades add a level of fine-tuning to the activity of aldosterone-responsive membrane transporters and also modulate the aldosterone induced changes in gene

expression through the conventional receptor and transcription factor phosphorylations. All these events can modulate a given biological response per se, or they can regulate the classical MR-mediated biological action.

Aldosterone shows one of the lowest coefficients of permeability of the blood-brain barrier. When aldosterone uptake and its subsequent binding to the MR in the brain are compared with the same properties in other organs, the extremely low permeability of aldosterone is readily noticeable [68, 81]. This marginal brain penetration of the steroid is due to a protein transporter situated within the barrier, a P-glycoprotein named mdr1, which pumps certain substrates back across the cerebral vascular endothelium and into the blood stream [82, 83]. A direct consequence of the limited permeability of aldosterone is that the vast majority of cells in the brain are exposed to only a very small fraction of its typically subnanomolar concentration in the blood plasma. In a cell-free system, aldosterone binds avidly to the MR in brain tissue homogenates [84, 85], and all enzymes necessary for the synthesis of aldosterone from cholesterol have been demonstrated in the brain of rats [86, 87] and humans [88]. Thus, brain minces from adrenalectomized rats synthesized aldosterone from endogenous substrate and converted deoxycorticosterone to aldosterone *in vitro* [87, 89], and aldosterone is synthesized in the normal rat brain *in vivo* [90].

MR AND DEPRESSIVE DISORDERS

All these previously discussed factors that are capable to provide biological specificity to steroid hormone-dependent responses have an excellent experimental model to analyze such selectivity in stress-related disorders. As it was advanced in the introduction section, the brain activates a comprehensive stress system that engages the organism in an adaptive response to the threatening circumstance in response to stressful situations. This stress system acts on multiple peripheral tissues and feeds-back to the brain, in particular thanks to the key role of corticosteroid hormones. Stress has two faces since it is a highly adaptive response to disturbances in homeostasis. On the other hand, it is also a potential risk factor for a large number of diseases, ranging from peripheral illnesses such as obesity and heart and vascular problems to several psychiatric disorders including major depression, schizophrenia, drug addiction, and post-traumatic stress disorders (PTSD) [91-93].

It has recently been postulated that prenatal stress increases the risk of those depressive disorders in adult offspring, mainly due to hippocampal dysfunctions [94]; how this attenuates the development and function of hippocampal networks is not well understood, although a recent study linked this pathophysiology to impaired morphological and functional maturation of hippocampal granule cells in adult offspring *via* the down-regulated expression of MR [95]. Interestingly, prenatal stress reduces the dendritic complexity and spine density of neonatal-generated granule cells, which persists into adulthood. Importantly, these granule cells exhibit depressed synaptic responses to stimulation of the medial perforant path. The study demonstrates that the proper dendritic maturation requires MR function, which is significantly impaired in granule cells by down-regulation. The pharmacological activation of MR rescued the stress-induced dendritic impairment, suggesting that MR could be a possible molecular determinant of detrimental effects of prenatal stress on granule cell maturation, which could prevent the attenuation of neuronal maturation and the subsequent dysfunction of the neuronal network in adulthood [95].

One possible cause for this phenomenon is the high glucocorticoid levels produced by the pregnant mothers during the prenatal stress period, which can be transferred to fetuses through the placenta [96-99] and may consequently down-regulate the expression of MR. Nonetheless, there are studies suggesting that cortisol levels in the fetus are mainly originated in its own adrenal gland [100, 101], whereas other studies were not conclusive in this regard [102, 103].

The matter is even more complex since a similar and broad question can be extended to other aspects of the individual's life. It is quite striking the high vulnerability of some individuals to affective disorders such as depression, anxiety or PTSD, while others are resilient under similar stressful experiences. Clearly, the mechanisms underlying these inter-individual differences in coping with stress depend on the secretion and action of stress hormones shaped by gene-environment interactions throughout life. The failure to manage chronic stress situations may cause a sort of 'vicious cycle' that increases the already high levels of glucocorticoids, leading to down-regulation of the GR in the hippocampus. This can trigger a feed-forward cascade of degeneration and further disease. On the other hand, the limbic MR activated by the high level of production of glucocorticoids could balance those effects favoring the processing of information in circuits underlying fear, reward, social behavior and resilience, even the dysregulation of the HPA axis, which would favor behavioral adaptation to negative situations. Therefore, the functional balance between both receptors becomes critical. However, polymorphic gene variants can induce lasting epigenetic changes in the expression of both receptors, the maternal environment being a particular potent epigenetic stimulus, as it was described above.

It is interesting to point out that mice with a point mutation (at A458T) in the GR promoter cannot generate GR homodimers, and the resultant monomer cannot bind to DNA. Nonetheless, protein-protein interactions could still take place [104]. This mutation was found to cause a selective impairment of spatial memory in the water maze, which could not be rescued by glucocorticoids [105]. Importantly, MR-related behaviors were left intact as demonstrated by similar exploration patterns in the novel environment of open field, light/dark box, and during the first exposure to the Morris water maze. Thus, this unique experimental model suggests that DNA binding and transactivation of the GR homodimer is required for glucocorticoid effects on spatial memory in the face of unaltered functioning of the MR.

Therefore, the functional balance between MR and GR is strongly related to disease or adaptation to the deleterious environment. This can be modified by early-life programming events such as prenatal stress and mother-infant interactions, as described above. Additionally, stressors during adult life (i.e., acute traumatic events) and/or repeated or chronic stresses can serve as triggers for the

development of psychopathologies by setting into motion a pathophysiological cascade in predisposed individuals [106, 107]. A third variable is the existence of gene variants for both receptors [108, 109]. A representative example of this situation is the 'loss of the function' MR gene variant, which enhances the neuroendocrine and autonomic responsiveness to psychosocial stressors, and is associated with feelings of depression in the elderly.

Finally, the fourth leg of this plot comprises the members of the Hsp90-based heterocomplex associated to steroid receptors. Recent studies have assigned to the immunophilin FKBP51 (FK506-binding protein of 51-kDa, gene name *fkbp5*) a particular role in the development of stress disorders. As it was mentioned above, this IMM shows inhibitory action on corticosteroid receptors and, upon steroid binding, is normally replaced by the dynein-interacting partner, FKBP52. In Binder's pioneer study [110], a C/T single nucleotide polymorphism in the intron 2 of the *fkbp5* gene (rs1360780) encoding for FKBP51 was reported. The T allele of this polymorphism is associated with higher levels of FKBP51 protein and with less suppression of cortisol to the dexamethasone test, as well as to slower recovery of cortisol response to a psychological stress test in healthy subjects [111]. Given the fact that these polymorphisms are associated with GR resistance and impaired negative feedback, it could be speculated that FKBP51 alleles associated with a slower return to baseline of stress-induced cortisol levels also increase the risk for stress-related psychiatric disorders. Currently, there is evidence for the impact of *fkbp5* in both mood and anxiety disorders (see [93] for a recent review). In this respect, it is intriguing that *fkbp5* is relevant for the development of stress-related mental disorders only in combination with traumatic events [112]. Clearly, GR is related to the termination process of stressing stimuli. As it was discussed before, its balance with the MR plays a cardinal role to become resistant to deleterious stimuli. Nonetheless, it is unclear how *fkbp5* variants may differentially affect one or another receptor. Actually, most of the studies related to depression and trauma have been focused on the effects of FKBP51 isoforms on the GR only. The rapid up-regulation of cellular FKBP51 potentially creates ultra-short feed-back loops in steroid signaling that can also curb the activity of MR and PR, and it is able to strengthen the signaling by the AR. To date, it is uncertain the possible interrelation between all these signalling cascades in the brain. Moreover, although agonistic ligands generally up-regulate the FKBP51, their efficacies in the up-regulation have not been systematically compared yet.

CONCLUDING REMARKS

It is still unclear the exact mechanism by which glucocorticoids and mineralocorticoids exert their specific actions in the nervous system. We have explored a number of reasons, each of one is not suffice by itself to be conclusive. Then, the question is whether or not all these pre-receptor and receptor-dependent factors work together in a sort of biological symphony that is responsible for a given effect and not the other. Clearly, during stressing situations, glucocorticoids mediate the protective action against the stressor *via* the MR, whereas GR-mediated effects facilitate processing of the stressor and storage of stressful events into memory. For reasons still unknown, the imbalance of MR-activating and GR-suppressing components of the stress reaction can enhance vulnerability to disease. As a result of this, the onset of the stress reaction or its termination is consequently impaired or delayed.

Nevertheless, MR- and GR-mediated actions are not always in balance at the cellular level. For example, while in the CA1 area of the hippocampus a dose-response relationship of glucocorticoids may account for both MR- and GR-mediated action, this is not the case in the dentate gyrus and the amygdala. Accordingly, the selective deletion of GR in dopaminoceptive neurons showed that the neuronal population seems the key factor that resolve various behavioral aspects [113].

That key question related to how these disparate cellular MR- and GR-mediated actions translate at the physiological and behavioral level, where both receptors seem to exert even opposite influences on behavioral adaptation. This is still an unresolved conundrum. Moreover, limbic structures are prominent areas where MR and GR are abundantly co-expressed whereas peripheral tissues and some brain regions related to electrolyte homeostasis and blood pressure express a classical 'protected' MR thanks to the action of 11βHSD2. While MR appears to be involved in the initial stress reaction allowing glucocorticoids to modulate the initial stress reaction by promoting the ability to switch to coping strategies, given to steroid ligand and its cognate receptor the control of the stress response and, consequently, providing the key to resilience and health. The picture is even more complicated when early experiences in life and genetic variances are also considered. In this sense, the emerging importance of chaperones and co-chaperones associated to steroid receptors, as well as novel epigenetic mechanisms able to modulate promoter activity in the receptor genes complete a very intricate physiological network that we are still far to elucidate in a conclusive manner, in particular when these mechanisms operate from birth to senescence following an apparent similar biological principle in all living beings of the same species.

LIST OF ABBREVIATIONS

ACTH	=	Adrenocorticotrophic hormone
AR	=	Androgen receptor
CNS	=	Central nervous system
ER	=	Estrogen receptor
GR	=	Glucocorticoid receptor
11βHSD	=	11β-hydroxysteroid dehydrogenase
HPA	=	Hypothalamic-pituitary-adrenal axis
Hsp90	=	90-kDa heat-shock protein
IMM	=	Immunophilin
PP5	=	Protein phosphatase 5
PR	=	Progesterone receptor
PTSD	=	Post-traumatic stress disorder
TPR	=	Tetratricopeptide repeats

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

This work was supported by funding from The University of Buenos Aires (UBACYT 2011-2014) and ANPCyT (PICT 2010-1170 and PICT 2011-1715).

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Received: July 24, 2012 **Revised: September 20, 2012** **Accepted: October 10, 2012**

PMID: 24040819