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Central actions of glucocorticoids in the control of body fluid homeostasis: Review

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The involvement of the hypothalamic-pituitary-adrenal axis in the control of body fluid homeostasis has been extensively investigated in the past few years. In the present study, we reviewed the recent results obtained using different approaches to investigate the effects of glucocorticoids on the mechanisms of oxytocin and vasopressin synthesis and secretion in response to acute and chronic plasma volume and osmolality changes. The data presented here suggest that glucocorticoids are not only involved in the mechanisms underlying the fast release but also in the transcriptional events that lead to decreased synthesis and secretion of these neuropeptides, particularly oxytocin, under diverse experimental conditions of altered fluid volume and tonicity. The endocannabinoid system, through its effects on glutamatergic neurotransmission within the hypothalamus and the nuclear factor κB-mediated transcriptional activity, seems to be also involved in the specific mechanisms by which glucocorticoids exert their central effects on neurohypophyseal hormone synthesis and secretion.

Key words: Corticosterone; Vasopressin; Oxytocin; Endocannabinoid; Glutamate; NFκB

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Introduction

The control of body fluid homeostasis involves complex mechanisms that regulate water and electrolyte ingestion and excretion. It is well established that plasma osmolality is mainly regulated by water balance, while sodium, which is one of the major components of the extracellular compartment, is a determinant factor for the maintenance of blood volume. Specific areas in the central nervous system (CNS) implicated in the control of hydromineral homeostasis receive information from specialized systems that detect peripheral changes in both volume and tonicity of extracellular fluid. The baroreceptors, located in the cardiovascular system, are activated in response to changes in blood volume or blood pressure and the nucleus of the solitary tract (NTS) plays a key role in the integrative responses that reach the forebrain, particularly the hypothalamus. On the other hand, the osmosensitive areas are located in the circumventricular organs and the presence of both osmo- and sodium-sensitive neurons in these nuclei has been well characterized. Increases of 1-2% in plasma osmolality stimulate water drinking and also enhance neurohypophyseal secretion of vasopressin (AVP) and oxytocin (OT). Conversely, a 10% decrease in blood volume is known to increase AVP secretion.

The control of volume and osmolality of mammalian body fluids occurs in response to stimuli that arise from both the intracellular and extracellular compartments. This information is conveyed to specific areas of the CNS responsible for an integrated response, which is depend-

ent on the integrity of the third ventricle anteroventral region, which comprises the organum vasculosum of lamina terminalis, the ventral portion of the median preoptic nucleus (MnPO) and the subfornical organ (SFO). These structures, once stimulated, can determine responses that involve 1) the behavioral induction of thirst or salt appetite, or both, 2) changes in sympathetic and renal nerve activity, 3) activation of the renin-angiotensin-aldosterone system, or 4) secretion of AVP and OT from the neurohypophysis, and 5) secretion of natriuretic peptides from the heart.

Both AVP and OT are produced by magnocellular neurons of the paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus and stored in terminals located in the neurohypophysis. AVP is mostly known for its antidiuretic effects, but it is also involved in the thermoregulatory and cardiovascular responses (1). The activation of the V_2 vasopressinergic receptors in the kidneys leads to increased permeability to water and consequent fluid reabsorption along the distal nephron. A stimulatory action of AVP on atrial natriuretic peptide (ANP) release has also been proposed (2). OT was shown to act either directly on the kidneys to promote sodium excretion or by an indirect pathway, stimulating ANP release from the heart (3,4). The participation of the natriuretic peptidergic system in response to hypervolemia and hyperosmolality has been well established also in the brain (5, for a review, see Ref. 6).

The prototype of the natriuretic peptides is the circulating peptide containing 28 amino acids (ANP 99-126 amino acids), which is processed from the atrial prohormone (1-126 amino acids). Other members of the natriuretic peptide family are BNP, CNP and urodilatin. The natriuretic peptides act at the cell membrane level through three types of receptors (NPR-A, NPR-B, and NPR-C). NPR-A and NPR-B, but not NPR-C, have an intracellular guanylate cyclase domain that generates cyclic guanosine monophosphate (cGMP) from guanosine triphosphate, which in turn activates protein kinase G (7). In contrast, NPR-C is likely to act as a clearance receptor and to remove natriuretic peptides from the circulation (8). Recently, it has also been shown to have physiological effects on the heart and vasculature (9). The expression of NPR-A, analyzed by RT-PCR, was detected in all kidney layers (6).

The natriuretic effects of OT occur through a dual mechanism: generation of nitric oxide (NO) in the kidney, leading to increased cGMP and, at higher doses, induction of ANP release from the heart that, in turn, also increases cGMP. Oxytocin-induced natriuresis occurs mainly through a cGMP-mediated decrease in tubular Na^+ reabsorption. In contrast to ANP, which increases cGMP in the renal vessels as well as in the tubules, OT acts through its receptors located in NOergic cells identified in the macula densa and proximal tubules, increasing cGMP production and closing Na channels. Thus, both ANP- and OT-induced natriuresis and kaliuresis appear to be mediated by cGMP (6).

The renin-angiotensin-aldosterone system also plays an important role in the regulation of body fluid homeostasis. An interaction between the angiotensinergic pathways and neurohypophyseal secretion has been described (6). Lauand et al. (10) recently showed that intracerebroventricular administration of angiotensin II (ANG II) increases AVP and OT secretion and also activates neurons in hypothalamic areas related to the control of fluid homeostasis. Several hormones (ANG II, AVP, OT, ANP, and mineralocorticoids) injected into the anterior hypothalamus of the rat modify neuronal activity and appear to be involved in the regulation of fluid and electrolyte balance. ANG II induces a delayed sodium appetite following water intake (11). It has been suggested that an active inhibitory system may exist to restrain NaCl intake. Peptides and hormones with the opposite effect to that of ANG II on fluid and electrolyte balance, such as ANP, may attenuate ANG II-induced salt appetite (6). It has also been demonstrated that pre-treatment with an OT receptor antagonist increases NaCl intake induced by intracerebroventricular injection of ANG II, without significant changes in water intake, suggesting an inhibitory action of OT on salt appetite (12). Taken together, these data provided a link between the central mechanisms controlling body fluid homeostasis and the peripheral adjustments of renal and cardiovascular systems in response to acute increases in plasma volume and osmolality.

Significant progress has been made to identify the neural circuits involved in the physiological and behavioral osmoregulatory responses. There is growing evidence for the participation of the hypothalamic-pituitary-adrenal (HPA) axis in the adaptive responses following changes in blood volume and osmolality. The presence of the cytoplasmic glucocorticoid receptor (GR) has already been reported in the SON (13) and also in the parvocellular portion of the PVN, where this receptor is predominantly co-expressed with corticotrophin-releasing factor (CRF) (14). Furthermore, AVP and OT are weak secretagogues of adrenocorticotropic hormone (15-17), strongly suggesting an interaction between the HPA axis and the secretion of neurohypophyseal hormones.

Glucocorticoids - general aspects

Glucocorticoids are essential for normal development and to regulate not only stress responses but also metabolic and immunologic mechanisms (18,19). The main actions of the glucocorticoids are mediated by the cytoso-

lic GR, which, in the absence of the endogenous ligand, is assembled into a multiprotein complex (20). Although retained in the cytoplasm, this conformation enables high-affinity ligand binding.

Produced by the adrenal glands under the stimulation of the HPA axis, the glucocorticoids act as ligand-dependent transcription factors that positively regulate genes through interaction with DNA enhancer sequences, called glucocorticoid response elements (GREs) (21-23). The activated GR was also shown to negatively regulate the expression of inflammatory genes through direct protein-protein interaction with proinflammatory transcription factors, without DNA binding (24,25).

The genomic and long-term mechanisms that follow glucocorticoid binding to the cytoplasmic GR and nuclear entry are also known as the classic pathway, but recent evidence suggests that fast glucocorticoid actions are mediated by membrane receptors and activation of nongenomic signaling events. In fact, glucocorticoids have been reported to bind specifically to cell membrane sites and to induce electrolytic movement changes (26,27). Although the molecular characterization of these receptors remains to be established in humans, a functional corticosterone membrane-associated receptor was identified in the amphibian brain (28).

The expression of GR in CRF-expressing neurons in the parvocellular PVN reinforces the direct feedback action of glucocorticoid in the control of CRF synthesis and release (14,29). Co-localization of mineralocorticoid receptor (MR) and GR was also observed in the parvocellular region, but not in the magnocellular region of the PVN (30). These results suggest that MR and GR may interact in the control of HPA axis activity.

There are several lines of evidence showing that glucorticoid action is modulated by the presence of 11ß-hydroxysteroid dehydrogenases (11ß-HSDs) in several tissues (31). 11ß-HSD1 activates cortisone to cortisol to facilitate GR-mediated action. In addition, 11ß-HSD2 plays an important role in aldosterone target tissues where it catalyzes the opposite reaction (i.e., inactivation of cortisol to cortisone) to prevent activation of MR by cortisol. Therefore, 11ß-HSD activity allows aldosterone occupancy of MR by inactivating endogenous glucocorticoids. In the kidney, 11ß-HSD2 is mainly expressed in collecting ducts, where it is co-localized with MR. Inhibition of 11ß-HSD2 can result in glucocorticoid-dependent mineralocorticoid excess and hypertension. This enzyme is also found in several tissues that are not classic targets for mineralocorticoids, such as peri- and circumventricular regions and NTS. Therefore, tissue-specific glucorticoid metabolism may be involved in the control of fluid balance and blood pressure.

Effects of glucocorticoids on neurohypophyseal hormone secretion

Several studies from our laboratory have demonstrated that isotonic (0.9% NaCl) blood volume expansion (BVE) increases plasma concentrations of OT and ANP and decreases plasma AVP levels, resulting in increased water and sodium excretion. On the other hand, rats submitted to hypertonic (1.8% NaCl) BVE presented increased plasma levels of AVP, OT and ANP in order to promote water reabsorption and renal sodium excretion (32-34).

In 2004, Durlo et al. (33) reported the first evidence for the participation of the HPA axis in hormone secretion induced by BVE in rats. These investigators reported that both hypervolemia and hyperosmolality increased plasma corticosterone concentrations, an event also known to occur in response to other types of stress, such as forced swimming, immobilization and hypoglycemia (35). This study also demonstrated that pre-treatment with dexamethasone, a synthetic glucocorticoid, blunted OT, but not ANP secretion, induced by BVE. These results were confirmed by Ruginsk et al. (34), who also showed that previous administration of dexamethasone did not alter AVP secretion in response to isotonic and hypertonic BVE. Pretreatment with dexamethasone also decreased the secretion of OT but not AVP induced by central angiotensinergic and cholinergic stimulation (10).

Taken together, these results suggest that, besides their genomic action on the modulation of transcriptional events, glucocorticoids are likely to present a fast negative modulation of OT but not AVP release from neurohypophyseal stores. In fact, Limbourg and Liao (36) reported that glucocorticoids could exert short-term effects on NO production in the heart, contributing to vasodilatatory responses during ischemic conditions. These data suggested that glucocorticoids may have actions that depend on non-genomic mechanisms, since the effects are rapidly observed.

Additionally, the HPA axis also seems to be involved in the mechanisms underlying chronic adjustments to osmotic challenges. Berghorn et al. (37) reported that long-term hyperosmolality induced an increase in the number of receptors for glucocorticoids in magnocellular vasopressinergic cells. Furthermore, increased plasma corticosterone levels were also reported in response to salt loading (38-40). These investigators also suggested that the HPA responsiveness to either CRF or AVP seems to be impaired after long-term osmotic stimulation.

One of the most studied neuromodulators that mediate the effects of the HPA axis on neurohypophyseal hormone secretion is NO, although the use of very distinct ap-

proaches has been generating conflicting results. Ventura et al. (40) demonstrated that the increase in plasma OT and AVP induced by salt loading was accompanied by an increased NO synthase activity in the SON and PVN, although the previous administration of NO synthase inhibitor produced different effects on the secretion of these peptides. These data indicate that NO differentially modulates the secretion of neurohypophyseal hormones in response to chronic salt loading.

Effects of glucocorticoids on hypothalamic neuronal activation

The detection of immediate-early gene products, such as Fos nuclear protein, has been used extensively as a marker of neuronal activation (41). The number of Fos-positive neurons in the PVN and SON was increased in rats submitted to hypertonic BVE and this effect was observed in parallel with enhanced neurohypophyseal secretion of AVP and OT (34). Furthermore, the use of specific antibodies has also allowed the identification of neuron phenotype, which is of interest to clarify the particular cellular mechanisms involved in response to a specific stimulus. Data obtained by Godino et al. (42) and extended by results from our laboratory (34) have shown that hypertonic BVE induced increased numbers of vasopressinergic and oxytocinergic neurons activated in both the PVN and SON. On the other hand, the number of oxytocinergic, but not vasopressinergic, magnocellular neurons activated in these nuclei was increased in rats submitted to isotonic BVE.

The responsiveness of magnocellular neurosecretory cells to plasma hyperosmolality is subject to inputs from the osmosensitive neurons (43), which are also activated by administration of hypertonic solution. A significant number of these neurons project to the hypothalamic magnocellular nuclei and other areas involved in the regulation of body fluid homeostasis, since hypertonicity also increases the number of Fos-positive neurons in the SFO, central nucleus of the amygdala, parabrachial nucleus, locus coeruleus, ventrolateral medulla, NTS, and area postrema (42). The activation of these areas depends on intact osmosensory pathways and mediates the autonomic, endocrine and behavioral responses involved in the osmoregulation (44).

The glucocorticoids seem to be also involved in the mechanisms underlying magnocellular neuronal activation following BVE. Ruginsk et al. (34) showed that, besides inhibiting hormone secretion, pre-treatment with dexamethasone decreased the number of oxytocinergic neurons activated in response to both isotonic and hypertonic BVE. This inhibitory effect induced by dexamethasone was observed on vasopressinergic neurons following hypertonic but not isotonic BVE. We also demonstrated that Fos expression and plasma AVP levels were both increased by osmotic, cholinergic and angiotensinergic central stimulation. However, dexamethasone pre-treatment induced a decrease of Fos expression in the MnPO, PVN and SON, but did not affect AVP secretion. Therefore, it appears that Fos expression and AVP secretion were parallel events that occurred in response to a given stimulus, rather than interacting with one another (10). The apparent divergence between unaltered AVP secretion and decreased neuronal activity observed in dexamethasone-pretreated rats subjected to hypertonic BVE or osmotic, cholinergic and angiotensinergic central stimulation suggests that the glucocorticoids may differentially modulate the AVP release from neurohypophyseal storages and the *de novo* hormone synthesis in the PVN and SON.

According to some other studies, glucocorticoid actions on magnocellular hypothalamic neurons seem to be predominantly inhibitory. Di et al. (45,46) proposed that corticosterone could act on transmembrane G-coupled receptors and stimulate the release of endocannabinoids in the hypothalamus. According to these investigators, the endocannabinoids would act as retrograde messengers and not only inhibit the release of glutamate, the main excitatory neurotransmitter in the CNS, but also stimulate the release of gamma-aminobutyric acid (GABA), contributing to the decreased activity of both parvocellular and magnocellular hypothalamic neurons.

Results obtained in our laboratory indicate that there is an increase in total glutamate content in the PVN and SON of rats submitted to BVE and this response is not altered by dexamethasone pre-treatment, suggesting that the balance between neurotransmitter production and release would not be affected in this condition (47). These results confirm the crucial role of glutamate in mediating the responses to hypervolemia and hyperosmolality.

Lopes da Silva et al. (48) also suggested that dexamethasone is likely to inhibit the expression of AVP and OT mRNA in the hypothalamic PVN and SON of rats submitted to water restriction and salt loading. The participation of the nuclear factor κB (NFκB) in the mediation of glucocorticoid actions in transcriptional events has also been investigated by our group. The presence of its phosphorylated p85 subunit in the PVN and SON after water deprivation and salt loading was also observed (Lopes da Silva A, unpublished data) and this evidence strongly supports the hypothesis that this pathway is involved in the effects of glucocorticoids on AVP and OT release. Therefore, these data indicate that glucocorticoids may act on different points of gene transcription and protein synthesis, from neuronal activation and neurotransmitter balance to hormone secretion.

Discussion

In the experimental models of BVE, water restriction and salt loading, the autonomic, neuroendocrine, cardiovascular, renal and behavioral systems are recruited to restore body fluid homeostasis (6,49). A better understanding of these mechanisms has been obtained with the recent contributions of molecular studies, but many of the events at the ultrastructural level are still unknown. The participation of the HPA axis, particularly the glucocorticoids, in mediating the responses to plasma volume and osmolality changes seems to involve diverse mechanisms ranging from the control of molecular aspects within the CNS to systemic modulation of hormone release, as summarized in Figure 1.

The glucocorticoid interaction with DNA binding sites has been well characterized and may also be activated after the acute stimulus or under prolonged exposure to stress. A GRE was identified in target genes (50) and by DNA sequence analysis, several elements upstream to the transcriptional start point of the rat OT gene were identified, matching the consensus sequence of enhancers inducible by glucocorticoids (51). Therefore, the observed effect of prolonged dexamethasone pre-treatment, reducing OT mRNA content and the consequent hormone secretion, could be due to an inhibition of OT gene transcription. The presence of GREs is speculated in the AVP gene, although no relevant result has been reported thus far.

The participation of the NFκB cascade in the transcriptional events evoked by glucocorticoids is also a promising area of study, although many pathways are known to converge to this common point. Additionally, the involvement of the nitrergic system in the mediation of AVP and OT release represents a growing area of investigation, since NO seems to differentially regulate the release of both neuropeptides.

The study of the pharmacological benefits of cannabis-like substances was described very early in medical sciences, but it was only in 1988 that the cannabinoid CB_1 receptor was described in the CNS. The main endogenous ligands for this type of receptor are anandamide and 2-arachidonoylglycerol and a clear relationship between the predominant anatomical localization of CB_1 receptors in the CNS and the cognitive, affective and motor systems was then established (52). Since then, the endocannabinoids have been extensively studied in the mediation of homeostatic responses, such as those related to energy balance, pain and behavior.

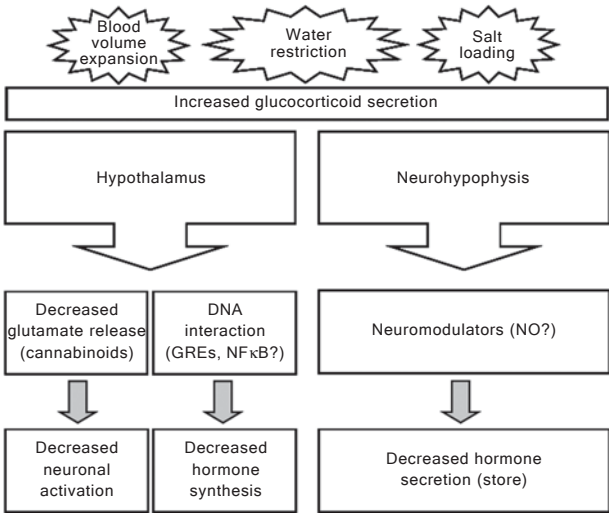

Figure 1. Schematic representation of glucocorticoid actions in the CNS in response to blood volume expansion, water restriction and salt loading. GREs = glucocorticoid response elements; NFκB = nuclear factor κB; NO = nitric oxide.

Hartman et al. (53) reported the first evidence for the *in vivo* participation of the cannabinoid system in the control of body fluid homeostasis. This study demonstrated that previous administration of naloxone, an opioid antagonist, to adult rats enhanced OT but not AVP secretion induced by hypertonic saline injection. Additionally, these investigators also showed that the central content of OT but not AVP was depleted in naloxone-pretreated mature rats. The most recent link between the endocannabinoids and the neuroendocrine system that controls body fluid homeostasis was obtained from *in vitro* manipulations of CB_1 receptors. The inhibition of the glutamatergic neurotransmission within the hypothalamus by endocannabinoids, as reported by Di et al. (45,46), provides a new insight into the molecular mechanisms that may be recruited in the presence of specific disturbances in body fluid homeostasis.

In conclusion, it has been suggested that the HPA axis might be involved in the integrative control of body fluid homeostasis, modulating neurohypophyseal hormone secretion, supporting the idea that the hypothalamus, particularly the PVN, contains a complex network with specific and integrative pathways. The advance of new techniques, such as interference RNA, blocking specific proteins, peptides or neurotransmitters, as well as the data on electrophysiological studies will provide in the near future useful tools for the understanding of mechanisms of glucocorticoid control of body fluid homeostasis.

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