

WHEN GLUCOCORTICOIDS CHANGE FROM PROTECTIVE TO HARMFUL LESSONS FROM A TYPE 1 DIABETES ANIMAL MODEL*

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Abstract A fundamental question in the neuroendocrinology of stress and adaptation is how stress mediators that are crucial for resilience and health can change into harmful signals enhancing vulnerability to disease. To address this question we focus in the rodent on corticosterone as end product of the hypothalamic-pituitary-adrenal (HPA) axis, which coordinates the behavioural and physiological response to stressors. The action of corticosterone is mediated by mineralocorticoid (MR) and glucocorticoid receptors (GR). The receptors are transcription factors regulating gene transcription but recently these nuclear receptors were found to mediate also rapid non-genomic actions. MR participates in the initial stress reaction important for appraisal and coping processes, while management of the later adaptive phase primarily depends on GR. Imbalance in stress mediators is a characteristic feature of a phenotype vulnerable for stressors. This concept calls for recovery of the MR:GR balance as a therapeutic strategy to promote resilience still present in the diseased brain. As an example, we discuss in this article, how the impact of excessive levels of corticosterone in a pharmacological model of type 1 diabetes can be ameliorated after a brief treatment with a GR antagonist.

Key words: diabetes, stress, stress hormones, glucocorticoids, glucocorticoid receptors, molecular markers

Resumen *Cuando los glucocorticoides cambian de protectores a dañinos en un modelo animal de diabetes tipo 1.* Una cuestión fundamental en la neuroendocrinología del estrés y la adaptación es saber cómo los mediadores del estrés cruciales para la resiliencia y la salud pueden convertirse en señales dañinas que aumentan la vulnerabilidad a las enfermedades. Para responder a esta pregunta nosotros nos centramos en la corticosterona como producto final del eje hipotálamo-hipófisis-glándula adrenal de los ratones, que coordina las respuestas fisiológicas y conductuales hacia los agentes estresantes. La acción de la corticosterona es mediada por los receptores de los mineralocorticoides (MR) y glucocorticoides (GR). Estos receptores son factores nucleares que regulan la transcripción de los genes aunque también se ha encontrado, hace poco, que pueden ser mediadores de acciones extra genómicas rápidas. Los MR participan en la respuesta inicial al estrés con relevancia en la evaluación y enfrentamiento al agente estresante, mientras que la fase adaptativa tardía depende de los GR. El desequilibrio de estos mediadores del estrés es rasgo característico de un fenotipo vulnerable a los agentes estresantes. La idea es recuperar el equilibrio MR:GR como una estrategia terapéutica para promover la resiliencia aún presente en el cerebro enfermo. Como ejemplo discutimos en este artículo cómo, en un modelo farmacológico de diabetes tipo 1, el impacto de niveles excesivos de corticosterona puede ser atenuado por un breve tratamiento con antagonistas de los GR.

Palabras clave: diabetes, estrés, hormonas del estrés, glucocorticoides, receptores para glucocorticoides, marcadores moleculares

Any stressor –either real or imagined– that threatens to disturb homeostasis triggers a physiological and behavioural response aimed to promote adaptation. The

adaptation to the stressor is coordinated in body and brain by humoral and nervous systems of which the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (adrenaline) are the primary mediators (Fig. 1). The end product of the HPA axis: cortisol (human) and corticosterone (human, rodents), collectively called CORT here, does not act alone but operates in concert with the humoral and nervous signals that mediate the stress response¹. While CORT is essential for health, the hormone becomes damaging if its circulating levels are either excessive or inadequate to deal with the stressor. A fundamental question in the biology of stress

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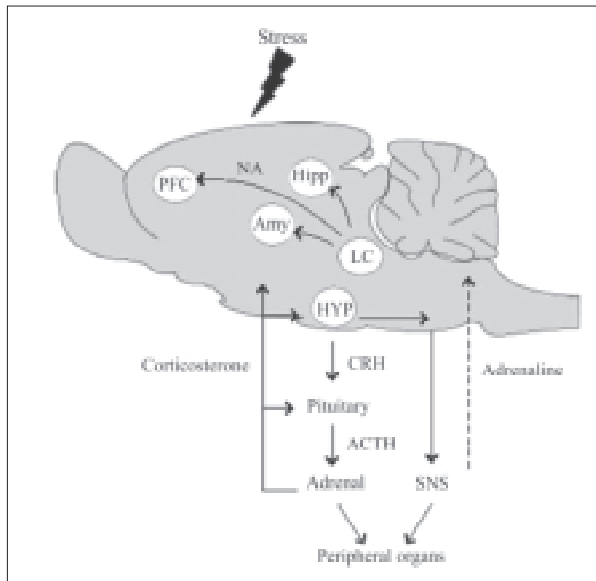


Fig. 1.— Brain regions activated after stress exposure (Amy; amygdala, Hipp: hippocampus, PFC: prefrontal cortex) and output of these areas through the hypothalamus (HYP). A resulting activation of the fast acting sympatho-adreno-medullary system (right) and the slower acting HPA axis system (left) will affect the function of peripheral organs and feedback to the brain via adrenaline and corticosterone, respectively. Adrenaline will eventually activate noradrenergic transmission from the locus coeruleus (LC), reaching the amygdala, prefrontal cortex and hippocampus among other areas. Corticosterone readily penetrates the blood-brain barrier and acts on receptor containing brain areas such as the limbic brain structures and the paraventricular nucleus in the hypothalamus. SNS: sympathetic nervous system; ACTH: adrenocorticotropin hormone; CRH: corticotrophin releasing hormone. Reprinted with permission from Joels and Krugers, *Neuronal Plasticity*, 2007.

is therefore how CORT can change from a protective into a harmful signal.

How does CORT act in the stress response? CORT dampens the initial stress (defence) reactions to the stressor, and prevents them from overshooting². This action is mediated by the receptors that control gene transcription and has as wide a diversity as the variety of stressors. However, the very same CORT actually also can boost the initial stress reaction via newly discovered membrane receptors for the hormone in the hippocampus that can enhance excitatory neurotransmission^{3, 4}. Moreover, CORT can mobilize the energy needed to perform these costly regulations of the stress response, and store energy for future use when coping with stress is successful.

The secretory pattern of CORT has been used widely as marker for health and disease. CORT is secreted under basal conditions in hourly pulses which are increased in amplitude during the activity period, i.e. night time in rodents and daytime in man. During stress-related dis-

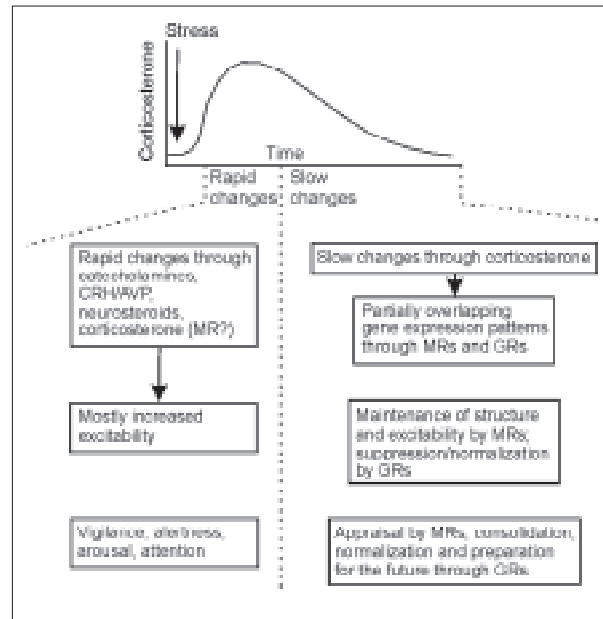


Fig. 2.— Time course of molecular, cellular and behavioral responses to stress hormones. MR: mineralocorticoid receptors, GR: glucocorticoid receptors. AVP: arginine vasopressin. Reprinted with permission from de Kloet, Joels, and Holsboer, *Nature Reviews Neuroscience*, 2005.

ease and aging the patterns become disordered, a sign that the HPA axis pulsatility has become dysregulated⁵. CORT secretory bursts can be triggered by a stressor any time. Like the basal pattern also the stress-induced CORT pattern serves as a biomarker for breakdown of adaptation and stress-related disease. A healthy resilient organism is characterized by a rapid activation of the CORT as long as the secretion of the hormone is also turned off efficiently.

During the stress response CORT and the other HPA axis hormones mediate the ability to cope. For this purpose CORT targets in the brain precisely those circuits that have perceived the initial trigger of the stress response; the action exerted by the hormone can be divided in two temporal domains: a fast activating and a slower suppressive phase (Fig. 2). These actions are mediated by two types of corticosteroid receptors: mineralocorticoid receptors (MR) and glucocorticoid receptors (GR)^{6, 7}. MR and GR are nuclear receptors operating as transcription factors. MR is abundantly expressed in limbic structures involved in mood, affect and memory processes. GR has is expressed widely in neurons and glial cells with highest abundance in centers involved in regulating the stress response and also in limbic structures. Thus, in limbic structures such as e.g. the neuronal circuits of hippocampus (CA1-2 and dentate gyrus), the amygdala nuclei and areas of the prefrontal cortex MR and GR are co-localized and expressed in large amounts⁶.

An important target of glucocorticoids is the hippocampus, where GR and MR exert in a co-ordinate manner complementary actions of the glucocorticoids. It is thought that the activating MR and the suppressing GR need to operate in balance to control homeostasis and health. Thus, imbalance in the MR:GR ratio enhances susceptibility to disease for which the individual is predisposed. Currently, drugs are being developed that restore MR:GR imbalance with the purpose to correct stress-related dysregulations causal to disease. Here we will illustrate this concept with an animal model for diabetes, which is characterized by aberrant actions of CORT that can be corrected by a CORT antagonist.

Diabetes as a disease model for aberrant CORT

Type 1 diabetes (T1D) complications, such as peripheral and autonomous neuropathy, are well-recognized. However, T1D can also impact the integrity of the central nervous system (CNS) as appeared from the pioneering studies of De Nicola and colleagues at the *Instituto de Biología y Medicina Experimental* in Buenos Aires (IBYME-CONICET)⁸. How T1D affects CNS integrity remains to be elucidated. High association of T1D and psychiatric disorders, notably depression, were reported in cross-sectional studies⁹. The underlying mechanism and the causality behind this association still need to be identified.

A direct adverse effect of diabetes leading to psychiatric disorders, especially depression, was reported by Lustman et al¹⁰ and alterations in the activity of the HPA axis were reported in diabetic patients¹¹. In addition, studies on diabetic patients demonstrated mild to moderate slowing of mental speed and diminished mental flexibility¹². Although the alterations in cognitive functions under normal conditions are not severe, mild cognitive defects can influence everyday activities in more demanding situations. While the mechanism that underlies this cognitive impairment is poorly understood, Sandeep et al reported in 2004¹³ that hypercortisolism in diabetic patients may contribute to the dysfunction of the hippocampus, a brain area that belongs to the limbic system and plays major roles in memory processes and spatial navigation.

To investigate disease initiation, progression, and treatments without exposing humans to unnecessary and potentially unethical risks, animal models have been developed. They have contributed important knowledge regarding the study of diabetes. The physiology of mice, rats, and other animals is remarkably conserved in comparison to the human condition, and over the last 40 years several models have become available. For our studies we have used two animal models, i.e. a pharmacological model, the streptozotocin (STZ)-treated mouse, and a genetic model, the non-obese diabetic (NOD) mouse,

which spontaneously develops the disease. Like type 1 diabetic patients, these animal models show high circulating levels of glucocorticoids, increased sensitivity to stressors, and morphological alterations in various brain areas¹⁴⁻¹⁶. Our results obtained from experiments with the STZ model will be described here.

When T1D develops, the lack of insulin causes both hyperglycemia and cellular starvation, a condition known as metabolic stress. The metabolic stressor, as any other kind of stress condition, activates the HPA axis leading to increased corticosterone levels and occupancy of GRs in brain and pituitary. Previous reports in T1D animal models showed GR downregulation in the hippocampus¹⁷ and HPA axis hyperactivity¹⁸. In addition, elevated glucocorticoids further enhance the hyperglycemic effect of insulin by inhibiting cellular glucose uptake, also in neurons and glial cells in the brain. Therefore, hyperglycemia will become chronic. In this way, continuous metabolic alterations will contribute to the defective shut-off of the stress response. During chronic hypercorticism various pathophysiologicals are promoted including dysfunction of the hippocampus^{19, 20}. Remodelling of the structure of the hippocampus is accelerated and its vulnerability to cognitive dysfunctions is enhanced.

A fundamental question in the neuropathophysiology of T1D is therefore whether glucocorticoids aggravate the functional and morphological signs of neurodegeneration and cognitive impairment. In our studies we tested the hypothesis that the onset of diabetes induces dysregulation of the HPA axis and hypersecretion of glucocorticoids which then renders the brain more vulnerable to metabolic insults causing damage and concomitant cognitive disturbances.

The STZ-induced diabetes mouse model showed that the primary event leading to hypercorticism is hyperresponsiveness of the adrenal gland²¹. To further investigate the underlying mechanism leading to the observed hypercorticism in diabetes we analyzed molecular markers of the HPA axis activity at different time points. The results showed a profound dysregulation of the HPA axis and a time-dependent adaptation to the new metabolic condition. Whether this adaptation in T1D leads to a more fragile state of the brain in which glucocorticoid excess may enhance the potential for damage and attenuate a protective mechanism, thus facilitating cognitive impairment was addressed in the next two experiments.

First, the hippocampus of the diabetic mice exhibited increased neuronal activation, signs of oxidative stress and astrogliosis. These data support the concept that uncontrolled diabetes produces signs of hippocampal pathology, which precedes together with changes in other brain structures such as hypothalamus and cerebral cortex^{16, 21}. Cognitive deficits were observed in the hippocampus-dependent novel object-placement recognition (NOPR) task. The NOPR task uses the spontaneous ex-

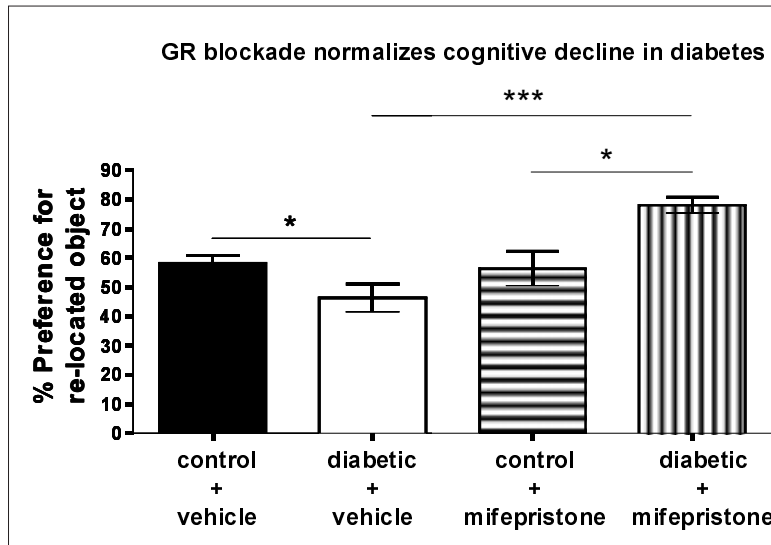


Fig. 3.— Mifepristone (a glucocorticoid receptor antagonist) effect on the novel object-placement recognition (NOPR) task. A) Photograph of the NOPR task: during the sample trial the two objects (water bottles) are equally explored (data not shown). After 50 minutes, during the test trial, one of the objects is re-located. B) Percentage of preference for the re-located object during the test trial for control and 11-days diabetic mice vehicle- and mifepristone-treated. Values expressed mean \pm SEM. * $p < 0.05$.

ploration of objects in a novel environment²² and thus allows the study of mild hippocampal alterations. Non-diabetic control mice preferred the exploration of the object placed in a novel location while the diabetic mice do not. This reduced exploration of the diabetic mice indicates impaired spatial object-placement memory^{23, 24} (Fig. 3). Interestingly, performance in the so-called Morris water maze, retention in the forced swim task and emotional parameters in the elevated plus maze were not affected in diabetic mice. These tasks possibly are insensitive to the moderate hippocampal disturbances observed at early stages of diabetes, while during a more prolonged disease state behavioral deficits were established²⁴.

Second, to clarify the role of hypercorticism in the hippocampus of STZ-diabetic mice we have used mifepristone (RU38486), an antagonist of the action of glucocorticoids at the GR. We showed that the continuous blockade of glucocorticoid action by treatment with mifepristone for 4 consecutive days (from day 6 to 10 of after onset of diabetes in the STZ model) prevented some of the hippocampal aberrations and reversed others²⁴. The prevention of astrogliosis and excessive neuronal activation in the hippocampus showed that mifepristone treatment can interfere directly with the progression of hippocampal alterations observed in diabetic mice^{24, 25}. Cognitive deficits observed at day 11 of diabetes were ameliorated. Surprisingly, the diabetic animals treated with

mifepristone performed even better than the untreated controls. A rationale behind this observation is that the blockade of the GR would allow a more prominent function of neuroprotective MR-mediated actions²⁶. Hence this would predict that during GR blockade in the face of high circulating glucocorticoids the maintenance of hippocampal integrity is a necessary condition for improved performance in the NOPR task²⁷.

In addition to this role of glucocorticoids in diabetes neuropathology other factors altered in T1D might also be involved. For instance, the imbalance of glucose metabolism may be of importance in modulating brain disturbances induced by diabetes. Some studies have described that hyper- and hypoglycemic episodes can cause acute cerebral dysfunction in diabetic animals^{14, 28}. In the STZ-induced mouse model of T1D, GR antagonist normalized hippocampal functions regardless the hyperglycemic state, which is not altered in diabetic mice treated with mifepristone. Therefore, it is unlikely that hyperglycemia *per se* accounts for the hippocampal disturbances observed. These findings are in agreement with a recently published report²², in which corticosterone replacement of adrenalectomized rats at physiological concentrations restored hippocampal functions, while glycemia remained elevated. However, the possibility of an additional effect of glucocorticoid excess on hippocampal glucose metabolism in T1D can not be discarded.

Furthermore, cerebral dysfunction in T1D can also be a result of insulin deficiency. Although several studies describe a central role of insulin in animal models of diabetes²⁹⁻³¹, a recent report revealed that hippocampal impairments are not determined by changes in insulin production²². Nevertheless, it is likely that the negative effect of diabetes on hippocampal plasticity may be attributable to an interaction between elevated glucocorticoids and insulin receptor signaling.

In conclusion, the results described in this article provide evidence that glucocorticoid excess and the concomitant continuous activation of the GR are responsible for molecular changes and hippocampal-associated behavioural dysfunction at the early stages of diabetes. They indicate that in T1D excess glucocorticoids have lost their function in restoring homeostasis and have become damaging to the brain. The receptors for glucocorticoids may therefore be an excellent target for a therapy aimed to normalize the disturbed hippocampal functions characteristic for diabetes neuropathology.

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Conflicts of interests: E.R. de Kloet is member of the Scientific Advisory Board of Corcept Therapeutics Inc.

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My work is now (1858) nearly finished; but as it will take me many more years to complete it, and as my health is far from strong, I have been urged to publish this abstract. I have more especially induced to do this, as Mr. Wallace [Alfred Russell Wallace], who is now studying the natural history of the Malay Archipelago, has arrived at almost exactly the same general conclusions that I have on the origin of species. In 1858 he sent me a memoir on this subject, with a request that I would forward it to Sir Charles Lyell and Dr. Hooker, who sent it to the Linnean Society, and it is published in the third volume of the Journal of that society. Sir C. Lyell and Dr. Hooker, who both knew of my work –the latter having read my sketch of 1844– honoured me by thinking it advisable to publish, with Mr. Wallace's excellent memoir, some brief extracts from my manuscripts.

Mi trabajo está ahora (1859) casi terminado; pero completarlo me llevará muchos más años, y como mi salud dista de ser robusta, me han apurado a publicar este resumen. He sido especialmente inducido a hacer esto, porque Mr. Wallace, quien está ahora estudiando la historia natural del Archipiélago Malayo, ha arribado a casi exactamente las mismas conclusiones generales que yo tengo acerca del origen de las especies. En 1858 me mandó una memoria sobre el tema con el pedido de pasarla a Sir Charles Lyell y al Dr. Hooker, quienes la mandaron al *Linnean Society* y está publicada en el tercer volumen del *Journal* de esa sociedad. Sir C. Lyell y el Dr. Hooker, quienes conocían mi trabajo, –el último leyó mi borrador de 1844– me honraron al juzgar recomendable publicar, con la excelente memoria de Mr. Wallace, algunos breves extractos de mis manuscritos.

Charles Robert Darwin (1809-1882)

The Origin of Species by Means of Natural Selection (First edition: November 24th, 1859). Chicago: Encyclopaedia Britannica, 1952. Introduction, p 6