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Review

Analytical challenges for measuring steroid responses to stress, neurodegeneration and injury in the central nervous system

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ABSTRACT

Levels of steroids in the adult central nervous system (CNS) show marked changes in response to stress, degenerative disorders and injury. However, their analysis in complex matrices such as fatty brain and spinal cord tissues, and even in plasma, requires accurate and precise analytical methods. Radioimmunoassays (RIA) and enzyme-linked immunosorbent assays, even with prepurification steps, do not provide sufficient specificity, and they are at the origin of many inconsistent results in the literature. The analysis of steroids by mass spectrometric methods has become the gold standard for accurate and sensitive steroid analysis. However, these technologies involve multiple purification steps prone to errors, and they only provide accurate reference values when combined with careful sample workup. In addition, the interpretation of changes in CNS steroid levels is not an easy task because of their multiple sources: the endocrine glands and the local synthesis by neural cells. In the CNS, decreased steroid levels may reflect alterations of their biosynthesis, as observed in the case of chronic stress, post-traumatic stress disorders or depressive episodes. In such cases, return to normalization by administering exogenous hormones or by stimulating their endogenous production may have beneficial effects. On the other hand, increases in CNS steroids in response to acute stress, degenerative processes or injury may be part of endogenous protective or rescue programs, contributing to the resistance of neural cells to stress and insults. The aim of this review is to encourage a more critical reading of the literature reporting steroid measures, and to draw attention to the absolute need for well-validated methods. We discuss reported findings concerning changing steroid levels in the nervous system by insisting on methodological issues. An important message is that even recent mass spectrometric methods have their limits, and they only become reliable tools if combined with careful sample preparation.

Contents

Abbreviations: ACTH, adrenocorticotropic hormone; AD, Alzheimer's disease; BBB, blood–brain–barrier; CNS, central nervous system; CRH, corticotropin-releasing hormone; CSF, cerebrospinal fluid; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; EAE, experimental autoimmune encephalomyelitis; 5α-DHP, 5α-dihydroprogesterone; ELISA, enzyme-linked immunosorbent assays; ER, estrogen receptor; $GABA_A$ receptors, gamma-aminobutyric acid type A receptor; GC, gas chromatography; HPA, hypothalamo-pituitary–adrenal; HPLC, high-performance liquid chromatography; 3α-HSD, 3α-hydroxysteroid dehydrogenase; 3β-HSD, 3β-hydroxysteroid dehydrogenase; LC, liquid chromatography; MCAO, middle cerebral artery occlusion; MDR, multidrug-resistance transporters; MS, mass spectrometry or multiple sclerosis; MS/MS, tandem mass spectrometry; NMDA, N-methyl-D-aspartate; OPC, oligodendrocyte progenitor cells; PR, progesterone receptor; PREGS, pregnenolone sulfate; PTSD, posttraumatic stress disorder; RIA, radioimmunoassay; SPE, solid phase extraction; SSRI, selective serotonin reuptake inhibitors; TBI, traumatic brain injury; THDOC, tetrahydrodeoxycorticosterone; TSPO, Tanslocase 18 kDa.

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1. Introduction

It has taken time to free steroids from their confined roles as reproductive or stress hormones, and it is now well established that they exert multiple functions throughout the CNS. Steroids that are derived from cleavage of the cholesterol side chain by cytochrome P450scc in mitochondria regulate a wide range of neuronal and glial functions by using multiple signaling mechanisms. Over the past years, particular attention has been paid to the neuroprotective and rescue actions of steroids after injury or during disorders of the CNS [1–8].

Steroid hormones play a major role during development of the CNS. Estrogens, progestogens and androgens indeed promote the maturation of neuronal circuits and the elaboration of myelin [9–16]. They are also involved in the protection of the immature grey and white matters during early stages of development, when the CNS is particularly sensitive to oxygen and glucose deprivation, injury and other types of stressors [17–21]. In the adult CNS, endogenous steroid signaling has been proposed to be continuously part of protective, rescue and regenerative processes [1,4]. This concept is consistent with the observation that developmental processes are recapitulated, although not always entirely, when adult neural cells respond to injury or degeneration and during repair processes [22–24].

Adaptive responses of neural cells to environmental challenges, neurodegenerative processes and injury are indeed reflected by changes in steroid levels within the CNS. However, their interpretation is complicated by the multiple sources of steroids: the steroidogenic endocrine glands and the local synthesis of neurosteroids by neurons and glial cells. Another difficulty is the accurate measure of low levels of the lipophilic steroid molecules, which only differ by the presence or absence of functional groups and double bonds in their ring structures, together with the unequivocal distinction between multiple stereoisomers. This is particularly challenging for complex matrices such as the very fatty brain and spinal cord tissues. The appraisal of changes in plasma and CNS steroid levels reported in the scientific literature thus requires an understanding of the principles and limitations of the available analytical methods. The lack of specificity of immunological methods for steroid analysis has always been a major problem and has contributed to the accumulation of inconsistent results in the literature. Currently, gas or liquid chromatography (GC or LC) coupled to mass spectrometry (MS) or tandem mass spectrometry (MS/MS) are considered as the gold standards for accurate and sensitive steroid analysis. Although it is generally believed that steroid measures obtained by mass spectrometric methods are always accurate and precise, an appeal to caution has to be made. It is important to be aware that these sophisticated analytical technologies involve many purification steps, which can become major sources of errors. Only when combined with carefully validated sample workup, mass spectrometric methods become robust analytical tools, providing accurate reference values for steroids in different tissues and under various pathophysiological conditions [25]. We thus urge a more critical awareness of analytical procedures for steroids used in experimental research and clinical studies.

2. Analytical methods for the determination of steroid levels: radioimmunoassays

Before discussing changes in CNS steroid levels in response to stress, injury and degenerative processes, challenges faced by accurate steroid analysis will be examined. Prior to the advent of radioimmunoassays (RIA), the analysis of hormones relied on colorimetric- and bioassays. The first use of a RIA in 1959 was for the measure of plasma insulin [26]. Only 10 years later, the first RIA of a steroid hormone, estradiol, was reported [27]. It was soon followed by the radioimmunological analysis of progesterone and pregnenolone [28,29]. One difficulty to the analysis of steroid hormones by RIA is their low molecular weight, which means that they are not immunogenic. The solution to this problem has been brought by linking covalently steroids to a protein. This combination then becomes immunogenic and behaves as haptens, allowing antibodies with a certain degree of specificity to be produced by active immunization [30].

Steroid RIA is based on the competition for a limited amount of antibody between the hormone to be quantified and a fixed amount of the corresponding hormone labeled with a radioisotope. After separation of the antibody-bound hormone from the free hormone, the amount of steroid in the biological sample is determined against a standard curve [30]. In the 1970s, most steroid RIA utilized an iodinated ligand, and a second antibody was often employed to separate antibody-bound ligand from unbound. Although the limited specificity of RIA for steroid analysis has always been a problem, it should be acknowledged that a great deal of our information concerning the physiologic and pathophysiologic roles of steroid hormones comes from studies that utilized validated RIA with preceding purification and separation steps (indirect RIA). In the past, many journals were strict about the careful validation of RIA procedures. Unfortunately, the requirement for properly validated RIA has become less severe. Even reference journals now publish steroid measures performed by direct commercial RIA or enzyme immunoassay kits "according to the instructions provided by the manufacturer". Although commercial kits are easy to use and less time consuming than well validated assays, they cannot guarantee accurate and robust analysis of steroids in biological samples, and in particular in complex matrices such as nervous tissues. It is indeed important to pay attention to the rigorous validation of RIA procedures and to the sample workup procedures [31–33].

As steroid hormones are converted in their target tissues to large numbers of metabolites, it is absolutely necessary to use separation steps by chromatography prior to RIA [33]. It is worth noting that even for the first reported steroid RIA, a pre-purification and