General Characteristics and Measurement of the Main Glucocorticoids

Subjects: Veterinary Sciences

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The main glucocorticoids involved in the stress response are cortisol and cortisone in most mammals and corticosterone in birds and rodents. Therefore, these analytes are the biomarkers more frequently used to evaluate the physiological response to a stressful situation. In addition, “total glucocorticoids”, which refers to the quantification of various glucocorticoids by immunoassays showing cross-reactivity with different types of glucocorticoids or related metabolites, can be measured.

glucocorticoids cortisol cortisone

1. Introduction

Glucocorticoid, also known as glucocorticoid (English: glucocorticoid), is an adrenocortical hormone, a steroid hormone secreted by the fascicular zone of the middle layer of the adrenal cortex, which can also be synthesized by chemical methods. Cortisone and cortisol in human body belong to glucocorticoids. Because it can be used for diseases that are not matched by ordinary antibiotics or anti-inflammatory drugs, such as SARS, septicemia, etc., it has the function of regulating the biosynthesis and metabolism of sugar, fat, and protein, and also has the anti-inflammatory effect. It is called "glucocorticoid" because its activity of regulating carbohydrate metabolism is first known by people.

Currently, stress is defined as a state of threat to homeostasis [1]. Glucocorticoids are presently the group of biomarkers most frequently used to evaluate the physiological response to stress [2]. The reason is that from a neuroendocrinological point of view, any stressful stimulus triggers the release of the adrenocorticotropic hormone (ACTH), which leads to the secretion of these molecules (**Figure 1**) [3][4][5][6].

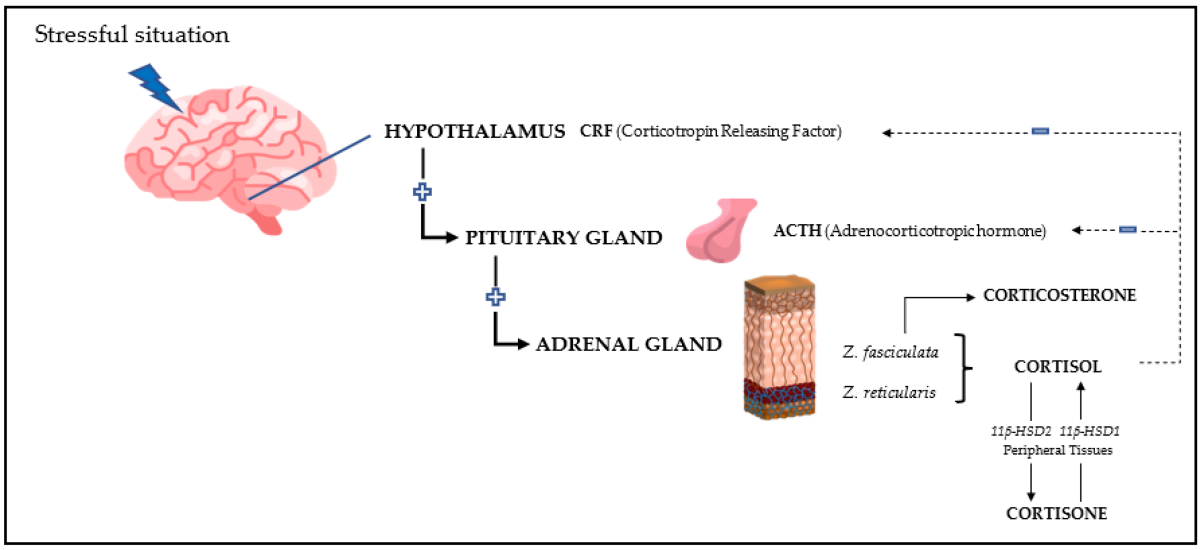


Figure 1. Schematic representation of glucocorticoid release following a stressful situation.

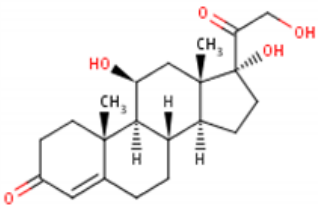
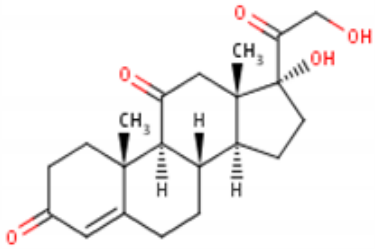
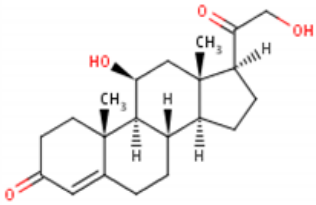
The most common glucocorticoid used to assess stress in humans and many animal species is cortisol [7]; although others such as cortisone [8] and corticosterone (this last one in species such as rats and mice, birds, and reptiles) [9][10] can also be measured. In addition, another way to assess the activity of the hypothalamic–pituitary–adrenal (HPA) axis in stressful situations is via the determination of "total glucocorticoids". The term "total glucocorticoids" refers to what is measured when immunoassays with non-specific antibodies showing cross-reactivity with different glucocorticoids or related metabolites are employed [11].

2. General Characteristics of the Main Glucocorticoids

Glucocorticoids are a group of endogenous adrenal hormones with a 21-carbon skeleton that are derived from cholesterol and that are released in a stressful situation. When released, they bind mainly to the corticosteroid-binding globulin (CBG), making them available for use at systemic or tissue level [12]. Their function is performed by intracellular binding to glucocorticoid receptors (GRs), which belong to the family of nuclear receptors [13]. Although the name "glucocorticoids" originates from their effects on plasma glucose, they are also involved in catabolic metabolism, inflammatory and immune response, and other physiological functions [14][15].

The main glucocorticoids involved in the stress response are cortisol, cortisone, and corticosterone (**Table 1**). Their concentrations allow the species to be classified as cortisol-dominant (most mammals) or corticosterone-dominant (such as rats, mice, birds or reptiles). Cortisone is produced mainly in the cortisol-dominant species, and its concentration depends on the activity of the 11β-hydroxysteroid dehydrogenase (11β-HSD) type 2 enzyme, which is expressed mainly in kidney, colon, and salivary glands [13].

Table 1. Main glucocorticoids and their main characteristics.

	Cortisol	Cortisone	Corticosterone
Formula	11β,17α,21-trihydroxypregn-4-ene-3,20-dione [16] [1] 	17-hydroxy-11-dehydrocorticosterone [17] [2] 	11β,21-dihydroxypregn-4-ene-3,20-dione [18] [3] 
Structural differences	An extra hydroxyl group attached to the 17th carbon [19].	A ketone group attached to the 17th carbon [20].	No extra hydroxyl group on the 17th carbon [19].
Metabolism	Synthesised from pregnenolone in adrenal gland. Inactivated mainly in the kidney by 11β-hydroxysteroid dehydrogenase (11β-HSD) type 2 into cortisone [21][22].	Transformation in the liver, lungs, ovaries, and central nervous system by 11β-HSD type 1 into cortisol [23].	Derived from pregnenolone in adrenal gland [18].
Activity	Active molecule [24]	Inactive molecule	Active molecule
Half-life	In plasma: 66 min In tissues: 12 h [25][26]	In plasma: 90 min [20]	In plasma: 60–90 min [27]
Predominant species	It is the main glucocorticoid in most mammals [28]	Same species as cortisol	It is the main glucocorticoid in rats, mice, birds, and reptiles, due to a lack of the enzyme 17-α hydroxylase [9]

ns of the pregnane-3β,11β,21-triol-20-one [29][30], can be measured. These are usually analysed in faeces [31][32][33] because of the variety of glucocorticoid-related metabolites present in them. In this line, the term “faecal corticoid metabolites” (fGCM) instead of “faecal total glucocorticoids” has been used since there are metabolites present in the faeces that can also potentially be measured [28].

1 Cortisol. (s.f.). ChEBI. https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:17650 (accessed on 15 November 2022); 2 Cortisone. (s.f.). ChEBI. https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:16962 (accessed on 15 November 2022); and 3 Corticosterone. (s.f.). ChEBI. https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:16827 (accessed on 15 November 2022).

3. Measurement

In general, there are two types of assays for the quantification of glucocorticoids:

(1) Those using techniques based on the reaction of an antibody with the analyte to be measured, such as radioimmunoassay (RIA), enzyme immunoassay (EIA), chemiluminescence, and, more recently, bead-based luminescent amplification assays (AlphaLISA). RIA assays are currently used with less frequency due to the need of special facilities and the radioactive nature of some components.

(2) Techniques based on the direct quantification of the analyte, including high-performance liquid chromatography (HPLC) [34][35] and liquid chromatography–mass spectrometry (LC-MS/MS) [36][37], with the latter being the most sensitive [8].

The main methods, with some selected references as examples of applications, used for the measurement of each type of glucocorticoid are listed in **Table 2**.

Table 2. Main methods used for glucocorticoid measurement.

Analyte	Analytical Method	Reference
Cortisol	EIA	[33][38][39]
	RIA	[40][41]
	Chemiluminescence	[42]
	AlphaLISA	[43]
	HPLC	[34][35]
	LC-MS/MS	[36][37]
Cortisone	AlphaLISA	[43]
	UHPLC-MS/MS	[44][45]
	LC-MS/MS	[46]
	LC-MS3	[47][48]
Corticosterone	EIA	[49][50]
	RIA	[51][52]
Total steroids	EIA	[53][54][55]
	RIA	[56][57]

Glucocorticoids can be measured in different sample types. Although blood has been traditionally frequently used, the stress that individuals suffer from blood collection [28] can interfere with the results. In this line, non-invasive alternatives, such as saliva, hair, faeces, or feathers, are becoming increasingly important [39][58][59][60].

There are three main glucocorticoids used to evaluate stress: cortisol, cortisone, and corticosterone, which vary in their amounts in different sample types and animal species. In addition, there is the concept of total steroids, which is used when immunoassays with antibodies showing cross-reactivity with different glucocorticoids or related metabolites are employed, being mostly used in faeces.

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