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Review

Relative contribution of P450c17 towards the acute cortisol response: Lessons from sheep and goats

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ABSTRACT

The rapid release of cortisol from the adrenal cortex upon ACTH receptor activation plays an integral role in the stress response. It has been suggested that the quantitative control over adrenal steroidogenesis (quantity of total steroids produced) depends on the activities of cytochrome P450 side-chain cleavage and steroidogenic acute regulatory protein that supplies pregnenolone precursor to the pathway. The qualitative control (which steroids) then depends on the downstream steroidogenic enzymes, including cytochrome P450 17α-hydroxylase/17,20-lyase (P450c17). In this review we focus on the relative contribution of P450c17 in the qualitative control of cortisol production with data collected from studies on South African Angora and Boer goats, as well as Merino sheep. Unique P450c17 genotypes were identified in these breeds with isoforms differing only with a couple of single amino acid residue substitutions. This review demonstrates how molecular and cellular differences relating to P450c17 activity can affect physiological and behavioural responses.

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1. Introduction: the acute cortisol response via adrenal steroidogenesis

The rapid release of glucocorticoids (cortisol in sheep and goats) from the adrenal cortex on stimulation of the hypothalamic–pituitary–adrenal (HPA) axis plays a vital role in the stress response. Together cortisol release and the autonomic fight-or-flight responses constitute the acute stress response that functionally diverts physiological and behavioural processes to aid immediate survival, known as the

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"emergency life-history stage" during which other normal life-history functions are suspended (e.g., reproduction, growth, and immunity) (Wingfield et al., 1998). It is therefore reasonable to expect that a quick cortisol response from the adrenal cortex, of sufficient magnitude, would assist the animal to surmount the immediate danger that the stressor presents. With the negative feedback effect of cortisol on the HPA axis, together with the subsiding danger from the stressor, cortisol production reverts back to basal levels and normal life-history functions can be restored (Harbuz and Lightman, 1992; Wingfield et al., 1998). However, these normal life-history functions would remain suspended if the stressor persists; the production of cortisol from the adrenal cortex is impaired (slower response or lower production); or the animal becomes unresponsive to the produced cortisol (e.g. down-regulation of glucocorticoid receptors) (Harbuz and Lightman, 1992; Sarabdjitsingh et al., 2010).

Steroid hormones are not stored in secretory vesicles like polypeptide hormones, but synthesized *de novo* from intracellular cholesterol (Miller, 2007). A rapid cortisol response from the *zona fasciculata* of the adrenal cortex is therefore mediated via the synthesis of new steroid by the steroidogenic enzymes: P450scc (cytochrome P450 side-chain cleavage), P450c17 (cytochrome P450 17-α-hydroxylase/17,20-lyase), 3βHSD (3β-hydroxysteroid dehydrogenase/$\Delta^4 \rightarrow \Delta^5$ isomerase), P450c21 (cytochrome P450 21-hydroxylase) and P450c11 (cytochrome P450 11β-hydroxylase). Furthermore, the physiological demand for cortisol production is extremely dynamic as displayed by its pulsatile, diurnal and seasonal rhythms, in addition to its rapid release upon stimulation of the HPA axis in response to stress as an adaptive mechanism. Irrespective of the origin of demand, the increased cortisol supply has to be facilitated by the steroidogenic enzymes – together with the factors that regulate their activity – upon ACTH receptor activation (Miller, 2007; Spiga et al., 2011).

The conventional view is that the manner in which ACTH receptor activation stimulates cortisol production is via intracellular signalling pathways that rapidly increase substrate availability for steroidogenesis (Miller, 2007; Miller and Auchus, 2011). This is achieved by increasing the transport of cholesterol from the outer to inner mitochondrial membrane by steroidogenic acute regulatory protein (StAR) (Lin et al., 1995; Stocco and Clark, 1996), where it can be converted to pregnenolone by P450scc, which is located within the inner mitochondrial membrane (Churchill and Kimura, 1979). Pregnenolone is the first steroid intermediate that is committed to the steroidogenic pathway and together the actions of StAR and P450scc are considered to be the rate-limiting steps in adrenal steroidogenesis (Miller, 2007; Miller and Auchus, 2011). Miller (2007) describes that the "the net steroidogenic capacity of a cell is determined by the expression of P450scc". Recent studies have also demonstrated rhythmicity in expressed genes within the adrenocortical cell that coincides with rhythmic cortisol production, namely expression of StAR, P450scc and proteins related to ACTH-receptor signalling (i.e. melanocortin type-2 receptor and melanocortin 2 receptor accessory protein) (Oster et al., 2006; Park et al., 2013; Son et al., 2008; Spiga et al., 2011, 2014). Furthermore, these changes in circadian and ultradian rhythms relate to the behaviour and physiology of animals in health and disease (Sarabdjitsingh et al., 2010; Spiga et al., 2014; Windle et al., 1998). There has been no investigation to date to indicate whether the steroidogenic enzymes downstream from pregnenolone in the pathway also undergo these rhythmic alterations in expression, whereas it has been demonstrated that their overall expression increases after 24 hours of chronic ACTH receptor activation (Kempna et al., 2010; Miller and Auchus, 2011). Miller and Auchus (2011) summarize that 1) the acute upregulation of steroidogenesis is determined by the action of StAR (controlling the quantity of substrate available to the pathway); 2) the quantitative output of steroidogenesis is controlled by the expression of P450scc as the enzymatic rate-limiting step to the pathway; and 3) the qualitative output of steroidogenesis (type of steroid) is controlled by the expression of downstream steroidogenic enzymes, particularly P450c17.

The qualitative control of P450c17 over the steroidogenic pathway is unique as it is the only steroidogenic enzyme that shifts the steroidogenic output away from aldosterone biosynthesis towards cortisol biosynthesis, as well as towards adrenal androgen production. The 17α-hydroxylase activity of P450c17 mediates the 17α-hydroxylation of pregnenolone and progesterone to 17α-hydroxypregnenolone and 17α-hydroxyprogesterone respectively, whereas the 17,20-lyase activity of P450c17 converts these 17α-hydroxylated intermediates to dehydroepiandrosterone (DHEA) and androstenedione respectively. The 17,20-lyase activity is greatly augmented in the presence of cytochrome b_5 (Hough et al., 2013a; Kominami et al., 1992; Storbeck et al., 2007). Ovine and caprine P450c17 also mediates the 16α-hydroxylation of progesterone (Hough et al., 2013a; Storbeck et al., 2007). The Δ^5-steroids pregnenolone, 17α-hydroxypregnenolone and DHEA can be converted to their corresponding Δ^4-steroids by 3βHSD, namely progesterone, 17α-hydroxyprogesterone and androstenedione (Goosen et al., 2010). Progesterone and 17α-hydroxyprogesterone are subsequently converted to deoxycorticosterone and deoxycortisol, respectively, by P450c21. In sheep a single enzyme, P450c11, mediates the 11β-hydroxylation of 11-deoxycortisol to cortisol, as well as all three steps required for the synthesis of aldosterone from deoxycorticosterone, namely the 11β-hydroxylase, 18-hydroxylase and 18-methyl oxidase activities (Boon et al., 1997). These reactions may be mediated by more than one enzyme in other species (Miller and Auchus, 2011). One enzyme therefore mediates multiple reactions, whereas the expression of these steroidogenic enzymes is further restricted to specific subcellular compartments, as well as different zones within the adrenal cortex. P450scc and P45011β are expressed in the inner mitochondrial membrane, whereas P450c17, 3βHSD and P450c21 are expressed in the endoplasmic reticulum.

The *zona glomerulosa* of the adrenal cortex lacks the expression of P450c17 and therefore produces aldosterone. The *zona fasciculata* expresses P450c17, but lacks cytochrome b_5 expression (Miller and Auchus, 2011; Suzuki et al., 2000) and consequently this zone is the primary location for cortisol production. The *zona reticularis* expresses P450c17, cytochrome b_5 and very low levels of 3βHSD – expression of cytochrome b_5 changes with the onset of adrenarche in humans and changes with age – resulting in adrenal androgens (DHEA) being produced in this zone (Hui et al., 2009; Mapes et al., 1999). Given the facts presented, it is expected that an acute cortisol response, from the *zona fasciculata* upon ACTH receptor activation, will be attributed to the stimulation of StAR and increased P450scc activity to provide pregnenolone as the first committed steroid intermediate to the adrenal steroidogenesis pathway. However, the activity of P450c17 will determine the amount of pregnenolone to be converted to cortisol together with the reaction rates of 3βHSD and P450c21 not being restrictive to cortisol production. This review investigates the relative contribution of P450c17 in the qualitative control over adrenal steroidogenesis with data collected from studies on South African Angora goats, Boer goats and Merino sheep. These studies exploited P450c17 genotypes within ovine and caprine species that lead to differences in their cortisol responses upon stimulation of the HPA axis. Interesting variations within the P450c17 genotype of bovine species have also been described recently (Vanselow and Fürbass, 2011). We discuss the manner in which the different P450c17 genotypes affect the dynamic acute cortisol response at the molecular and cellular level, which can have considerable implications for behavioural and physiological responses of animals.

2. Hypocortisolism in South African Angora goats

The South African Angora goat has been subjected to intensive selection and inbreeding in an attempt to increase mohair