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11β-Hydroxysteroid dehydrogenase in the testis of *Bufo arenarum*: Changes in its seasonal activity

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Abstract

In rat Leydig cells, glucocorticoids (GC) inhibit testosterone (T) synthesis via glucocorticoid receptor (GR). However, GC access to GR is regulated by the local expression of 11β-hydroxysteroid dehydrogenase (11β-HSD). Two isoforms were identified in mammals: type 1, a $NADP^+$-preferring enzyme with K_m in the μM range for GC and type 2, NAD^+-dependent, with K_m in the nM range for GC. In amphibians, a seasonal rhythm in baseline GC levels was described. However, a shift in the amount of deactivating 11β-HSD activity could alter GC effects. The purpose of this work is to describe seasonal changes in testicular activity of 11β-HSD in *Bufo arenarum* as well as the annual and seasonal patterns of plasma corticosterone (B) and T. The activity of 11β-HSD was assayed in homogenate and subcellular fractions in pre-reproductive (Pre-R), reproductive (R) and post-reproductive (Post-R) periods, using [^{3}H]B. Plasma B and T were determined by RIA. Testicular 11β-HSD is a microsomal NAD^+-dependent enzyme with a K_m in the nM order, its activity being strongly reduced by glycyrrhetinic acid. These results indicate that toad testes express an 11β-HSD similar to mammalian type 2. Although 11β-HSD activity is higher in the Post-R than in the R and Pre-R seasons (V_{max}: Pre-R: 0.26 ± 0.10, R: 0.14 ± 0.01, Post-R: 1.37 ± 0.45, pmol/min mg protein), K_m value remains constant throughout the year. A seasonal rhythm in baseline GC concentrations inversely correlated with plasma T was also described. T concentration is lower in the R season than in the other periods (Pre-R: 90 ± 6; R: 12 ± 1; Post-R: 56 ± 3, nM) while total B concentration is higher in the breeding than in the other seasons (Pre-R: 62 ± 10; R: 145 ± 18; Post-R: 96 ± 10, nM). Furthermore, free B (Pre-R: 51 ± 8; R: 94 ± 12; Post-R: 70 ± 7, nM) was always below K_m values. In conclusion, this work shows that the activity of 11β-HSD in toad testes could modulate GC action by transforming active hormones in the corresponding inactive steroid.

Keywords: 11β-Hydroxysteroid dehydrogenase; Corticosterone; Testis; Toad

1. Introduction

In mammals, glucocorticoids (GC) exert their biological action via the interaction with at least two different intracellular receptors named types I and II, or mineralocorticoid (MR) and glucocorticoid receptor (GR), respectively. However, the majority of GC effects occurs through GR (Funder, 1997). Several papers have also established that access of GC to their receptors within target cells is regulated by the local expression of the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD) (Monder and White, 1993; White et al., 1997). Two distinct gene products exhibiting 11β-HSD activity have been identified in mammals. The type 1 form (11β-HSD1) is a $NADP^+$-preferring enzyme with a K_m in the μM range for corticosterone (B) and cortisol (F). It was originally purified (Lakshmi and Monder, 1988) and cloned (Agarwal et al., 1989) from rat liver. Although 11β-HSD1 catalyses the interconversion of active B and F with the biologically inert 11-dehydrocorticosterone (A) and cortisone,

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respectively, this isoform appears to act essentially as an 11-oxoreductase (Duperrex et al., 1993; Jamieson et al., 1995; Low et al., 1994). In contrast to 11β-HSD1, the type 2 form (11β-HSD2) is NAD^+-dependent, has a K_m in the nM range for both B and F, and catalyses their conversion into A and cortisone, essentially in an unidirectional mode (Agarwal et al., 1994; Albiston et al., 1994; Naray-Fejes-Toth and Fejes-Toth, 1995; Zhou et al., 1995). Mammalian 11β-HSD2 cDNA was initially cloned from kidney of several species (Agarwal et al., 1994; Albiston et al., 1994; Cole, 1995; Naray-Fejes-Toth and Fejes-Toth, 1995; Zhou et al., 1995), and subsequently the protein was purified from human placenta (Brown et al., 1996). In mammals, 11β-HSD2 is expressed primarily in mineralocorticoid target tissues (Li et al., 1996; Naray-Fejes-Toth and Fejes-Toth, 1998; Smith et al., 1996), female reproductive tract (Burton et al., 1996; Roland and Funder, 1996), and corpus luteum (Waddell et al., 1996).

In rat Leydig cells, GC can inhibit testosterone (T) production (Monder et al., 1994a) through its interaction with GR (Monder et al., 1994b). However, Leydig cells express a predominantly oxidative 11β-HSD activity, which could protect the testis from GC effects (Gao et al., 1996, 1997).

In *Bufo marinus* bladder, an 11β-HSD activity resembling mammalian type 2 isoform was characterised several years ago (Brem et al., 1991, 1993). In the bladder, 11β-HSD is a NAD^+-dependent enzyme with an affinity in the nM range for B and behaves as an unidirectional dehydrogenase. Moreover, its inhibition by carbenoxolone provokes an increase in GC-induced transepithelial Na^+ transport (Brem et al., 1989).

In amphibians, a seasonal rhythm in baseline GC concentrations has been extensively described (for review, see Romero, 2002). Those studies have also demonstrated that baseline GC levels were higher during the breeding than nearly all pre- and post-breeding concentrations (Romero, 2002). Since no suppression of reproductive behaviour or other reproductive processes were described, it is possible that elevated plasma levels of GC during the breeding facilitate several physiological aspects of reproduction (Moore and Jessop, 2003).

Of equal importance of GC delivery to target tissues is the quantity of active hormones that reaches the GR. Consequently, as in other hormonal systems, the physiological effects of GC depend not only on hormonal concentration and GR but also on the relative amounts of 11β-HSD that can activate or deactivate GC at the tissue level. As amphibian reproduction seems to be positively correlated with an increase in GC concentration, changes in the activity of testicular 11β-HSD could protect Leydig cells from a potentially negative effect of GC on steroidogenesis. However, data examining potential changes in 11β-HSD in amphibians do not exist, but are vital to understand any physiological consequence of annual GC rhythms on testicular physiology.

Bufo arenarum is a seasonal breeder, the breeding season being restricted to the period between September and December, i.e., springtime. Moreover, animals in reproductive condition show low levels of circulating androgens suggesting that *B. arenarum* is a species with a dissociated reproductive pattern (Canosa and Ceballos, 2002a). Besides, *B. arenarum* is a potentially continuous breeding species with high concentration of androgens in summer when spermatogenesis starts (Pozzi and Ceballos, 2000).

This paper describes seasonal changes in the activity of 11β-HSD in the testes of *B. arenarum* as well as its correlation with the annual pattern of plasma B and T.

2. Methods

2.1. Materials

Corticosterone, 11-dehydrocorticosterone, glycyrrhetinic acid, and cofactors were from Sigma Chemical (St. Louis, MO, USA) and [^{3}H]B (76.5 Ci/mmol) and [^{3}H]T (60 Ci/mmol) were from NEN (Boston, MA, USA). Silica gel plates 60GF 254 on aluminium were purchased from Merck (Darmstadt, Germany). All other chemicals were of reagent grade. Antiserum against T was from Colorado State University (Colorado, USA). Dr. Celso Gomez Sanchez from the University of Mississippi Medical Center (MS, USA) kindly provided the antiserum against B.

2.2. Tissue preparation

Testicular tissue was obtained from male toads *B. arenarum* collected in the neighbouring area of Buenos Aires throughout the year. Toads were kept at 25 °C for 2 days prior to use. Animals were anaesthetised with MS222, their death being the result of the ensuing surgical procedures. Testes were quickly removed and then carefully separated from Bidder's organ. Testes were homogenised in 50 mM Tris–HCl buffer, $MgCl_2$ 1 mM, and 20% glycerol, pH 7.4 or 8.0 (250 mg of tissue/ml).

2.3. Subcellular localisation of 11β-HSD

All subcellular fractions were prepared from the homogenate by differential centrifugation (Pozzi et al., 1997). Briefly, after sedimentation of the nuclear fraction at 800 g for 10 min, mitochondria were sedimented from the supernatant by centrifugation at 15,000*g* for 20 min. Mitochondrial fraction was purified using 0.88 M sucrose (Pozzi et al., 1997). For the separation of the microsomal fraction, the 15,000*g* supernatants were centrifuged at 105,000*g* for 90 min. All steps were carried out at 4 °C. All fractions were used immediately for enzyme assays. Protein concentrations were estimated by