

**99. THE EFFECT OF TESTOSTERONE AND SEASON ON PREPROENKEPHALIN mRNA EXPRESSION IN THE PREOPTIC AREA–HYPOTHALAMUS OF THE RAM**

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Enkephalin appears to exert an inhibitory action on LH secretion (1), but whether testosterone regulates enkephalin gene expression is unknown. Testosterone regulates expression of proopiomelanocortin mRNA in the hypothalamus in a season-dependant manner (2). This study tested the hypothesis that testosterone and/or season modulate preproenkephalin mRNA expression in specific areas of the hypothalamus in the ram. Adult Romney Marsh rams were castrated (wethers) either during the 'breeding' season or 'non-breeding' season and 1 week later received intramuscular injections of either peanut oil (vehicle) or testosterone propionate (8 mg/12 h for 7 days) (5/group). Blood samples taken every 10 min for 12 h were assayed for plasma LH and testosterone. Preproenkephalin mRNA expression was quantified in hypothalamic sections by *in situ* hybridisation using an <sup>35</sup>S-labelled riboprobe and computer-aided image analysis. Plasma testosterone levels were higher in testosterone propionate-treated than oil-treated sheep. Mean plasma LH concentrations were reduced and the interpulse interval for LH pulses was greater in testosterone propionate-treated wethers compared to oil-treated wethers, with no change in LH pulse amplitude. Testosterone propionate treatment reduced proenkephalin mRNA expression in the diagonal band of Broca, the caudal preoptic area and the bed nucleus of the stria terminalis. Proenkephalin mRNA expression was higher in the 'breeding' season in the bed nucleus of the stria terminalis, lateral septum and periventricular nucleus than in the 'non-breeding' season but was higher in the 'non-breeding' season than 'breeding' season in the paraventricular nucleus. No differences were observed between treatments in seven other regions of the hypothalamus. We conclude that testosterone and season regulate preproenkephalin mRNA levels in the preoptic area/hypothalamus in the ram in a region-specific manner.

(1) Armstrong & Spears (1991) *J. Anim. Sci.* **69**: 774–781. (2) Hileman *et al.* (1998) *J. Neuroendocrinol.* **10**: 587–592.