STUDIES OF THE MAMMALIAN GONADOTROPHIN RELEASING HORMONE NETWORK AND THE RESPONSE OF MALE ANIMALS TO THE WITHDRAWAL OF THE GONADOTROPHINS FOLLOWING ACTIVE IMMUNISATION

By

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ABSTRACT

Recent evidence has shown the existence of several mammalian gonadotrophin releasing hormone (GnRH) isoforms, which have a high sequence homology. The objective of this study was to establish the distribution pattern and binding sites of GnRH-I, II and III in adult male rat tissues and assess the comparative efficacy of a GnRH-I vaccine in male animals. To establish the distribution pattern of GnRH-I, II and III, monoclonal antibodies (MAbs) were produced, using peptide conjugates. MAbs against GnRH-I and II were established using des2-GnRH-I and II peptide conjugates, coupled with 1-ethyl-3-3-dimethyl aminopropyl carbodiimide hydrochloride (EDC), but unfortunately all of the MAbs appeared 'sticky'. Therefore, the EDC method of coupling was found unsuitable for MAb production and the subsequent fusion protocol was switched to the hetero-bifunctional coupling agent sulpho-maleimidobenzoyl-N-hydroxy-succinimide (S-MBS). This protocol generated IgG1 MAbs (1D71C10, 4F22B6 and 7B101D10) to GnRH-I. MAbs against GnRH-II and III were also established using des1-cys-GnRH-II and III conjugates, made with S-MBS. Unexpectedly these MAbs showed IgM class specificity, displayed extensive non-specific reactivity and were unsuitable for the applications required.

Since 7B101D10 appeared to be a highly specific MAb, it was used to evaluate tissue distribution of GnRH-I, while a biotinylated des1-cys-GnRH-I was used to evaluate regions of GnRH-I binding. Co-expression of GnRH-I and GnRH-I binding activity was identified in the anterior pituitary, olfactory bulb, heart, liver, spleen, thymus and testes; this is evidence of the paracrine, autocrine or endocrine activities of GnRH-I.

Comparative efficacy trial of the TT-CHWSYGLRPG-NH_2 (GnRH-I) vaccine in adult male animals indicated that male Sprague-Dawley rats are a susceptible animal species to GnRH-I immunocastration, whereas, Scottish Suffolk-cross-bred rams and NZB x Balb/c male mice showed inconsistent response. Results indicated that GnRH-I and III peptides are actively involved in the regulation of testicular spermatogenesis, while the role of GnRH-II at the level of testicular spermatogenesis remain unidentified in this study.