Immunization against GnRH in male species (comparative aspects)

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Abstract

Active immunization against gonadotropin-releasing hormone (GnRH) was recognized in the 1970s as a potential means by which the reproductive system of mammals might be shut down for various practical and clinical reasons. Numerous studies in males have been performed since that time to determine the applicability of the technique as an alternative to surgical removal of the testes. Reasons for such immunocastration include improvement of meat and carcass characteristics for cattle, sheep, goats, and swine; improvement in feed efficiency relative to castrates in those same species; reduction in male aggressive behavior; reduction in male-associated odors in goats and swine; and fertility neutralization in pet species. Although application as a fertility control agent in men is unlikely, there is renewed interest in active immunization against GnRH as a means of treating prostate cancers and related steroid-dependent pathologies. © 2000 Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

Active and passive immunization against the hypothalamic decapeptide gonadotropin-releasing hormone (GnRH; also referred to as luteinizing hormone-releasing hormone, LHRH) has proven to be a potentially useful tool for practical, on-farm applications as well as a very powerful tool for studying basic aspects of the hypothalamic-pituitary-gonadal axis. Like most hypothalamic releasing and inhibitory hormones, GnRH is produced in cell bodies of hypothalamic neurons and is transported by axonal flow to the terminal buttons, which synapse on the vessels of the primary capillary
plexus within the median eminence (Kainer, 1993; Guyton and Hall, 1996). Excitation of the GnRH neurons causes release of stored peptide from its secretory granules into the extracellular space, with eventual diffusion into the capillary blood. This blood then travels via the hypophyseal portal system to the sinusoidal (secondary) capillary plexus within the adenohypophysis, where a portion of the GnRH leaves the capillaries and thereby becomes available for binding to its pituitary target cells, the gonadotropes. The short distance (and time) that the GnRH travels in these vessels is where (when) it is vulnerable to attack by antibodies. If enough specific antibodies are present in the circulating blood entering the median eminence, then virtually all the GnRH secreted into the primary plexus is tightly bound by antibody. Binding to antibody ‘‘neutralizes’’ the GnRH either by preventing it from diffusing through the capillary walls (due to the size of the complex) or by masking the receptor binding site on the GnRH molecule itself. Either way, this immunoneutralization of endogenous GnRH results in profound effects on the gonadotropes, due to the absence of most or all of their normal trophic input.

Two major areas of interest in the technique have evolved since the first reports of successful immunization against GnRH. The first is the on-farm application of ‘‘immunocastration’’, in which males of various domestic species are potentially rendered reproductively inactive without actual removal of the testes and its inherent complications. Problems such as unwanted male aggressiveness, male associated odors, and fertility are targets for elimination. Also, in the arena of human medicine, immunization against GnRH has been considered as an alternative to treating various androgen-dependent pathologies, such as prostatic cancers and benign prostatic hypertrophy. In some of these areas, the reversibility of the testicular activity is a factor to be considered.

The second major area of interest in GnRH immunization has been the study of hypothalamic pituitary interactions. Immunization against GnRH allows the highly specific removal of hypothalamic input to the gonadotropes, which is not possible with the previously used paradigms of hypophysectomy or pituitary stalk–median eminence transection. Immunization alone, or immunization followed by controlled replacement of GnRH analogs, has provided a wealth of information on the requirements of LH and FSH for GnRH and the involvement of gonadal steroids. Moreover, immunization followed by selective replacement of LH, FSH and(or) testosterone has been used to study the relative roles of these hormones in spermatogenesis.

Several excellent reviews on the topic of immunization against GnRH have been published previously by Fraser (1980, 1986), Schanbacher (1984), Chaffaux et al. (1985), and D’Occhio (1993). Thus, in the interest of space, readers are referred to those reports for much of the pioneer research, particularly that in females and in laboratory species of both sexes.

2. Technical aspects of immunization against GnRH

Because GnRH itself is too small to be immunogenic, generating antibodies against it requires a unique approach to fool the animal’s immune system into recognizing it as
foreign. One of the first to achieve this was Arimura et al. (1973), who adsorbed GnRH to polyvinylpyrrolidone and immunized three male rabbits. Two of the males produced antibodies that specifically bound radioiodinated GnRH, and their testes showed marked atrophy after immunization. Also in 1973, Fraser and Gunn (1973) reported the use of the carbodiimide technique to covalently link GnRH to bovine serum albumin (BSA), a technique that has been used extensively since that time by Fraser as well as various other research groups. Immunization and subsequent booster of three male rabbits resulted in testicular atrophy and involution of the seminiferous tubules, and subsequent passive transfer of the GnRH antisera into female pro-estrous rats completely inhibited ovulation.

Other approaches to conjugating GnRH to large, non-self proteins include the use of glutaraldehyde (Kerdelhuet et al., 1976; Caraty and Bonneau, 1986; Hötzel et al., 1997) and substituted GnRH analogs, such as α-lyS-GnRH, which provides an extra amino group through which the molecule can be linked to proteins such as diphtheria or tetanus toxoid (Sad et al., 1991). Ladd et al. (1989, 1990) used GLY-OH-GnRH linked to tetanus toxoid to successfully immunize male rats. Goubau et al. (1989) compared the efficacy of GnRH analogs with a cysteine substituted at the 1, 6, and 10 position, conjugated to keyhole limpet hemocyanin (KLH) via the heterobifunctional reagent m-maleimidobenzoylsulfosuccinimide ester. Active immunization of ram lambs resulted in anti-GnRH antibody generation in all animals, with the titers being similar for all groups. More recently, Finnerty et al. (1994) used an analog of GnRH containing an extra CYS-GLY on the amino end of the GnRH molecule, and conjugated it to human serum albumin. Immunization of bull calves resulted in significant antibody titers and reduction in LH and testosterone concentrations in peripheral blood; the suggested protocol was 1 mg conjugate given in the adjuvant diethylaminoethyl dextran, with a lag of 56 days between the primary and secondary immunizations.

Morrison et al. (1987) tested an adjuvant-free GnRH conjugate preparation based on a carboxyl GLY-CYS analog of GnRH linked to a purified protein derivative of tuberculin. Immunization of male rats led to complete testicular regression in those previously immunized against a protein similar to the carrier protein. Hosmalin et al. (1987) were able to totally synthesize a linear monomeric GnRH-LYS-muramyl dipeptide (synthetic adjuvant peptide), and immunization of male mice resulted in testicular regression in all mice treated. Meloen et al. (1994) reported on the use of a 20 amino acid tandem repeat analog of GnRH, which was compared to the monomer (normal) GnRH, both conjugated to KLH and used to immunize 10-week-old boars. The tandem repeat conjugate was considerably more efficacious than the monomer, resulting in a much more consistent suppression of testicular size and testosterone concentrations in peripheral blood.

Based on the numerous and varied reports over the last 25 years, it appears that various sites and methods of conjugation of the GnRH molecule to carrier proteins have resulted in sufficient antibody titer to be biologically neutralizing. From the practical standpoint, the use of carbodiimide to link GnRH to a large non-self protein is easy to perform and very effective for research purposes. However, as will be discussed in the next section, when devising an immunogen for commercial use in domestic species or for use in human medicine, other factors become much more critical, and
some very sophisticated work has gone into creating acceptable conjugates for these purposes.

3. Development of commercial products

Two major factors to be considered in the development of vaccines against GnRH for commercial use in farm animal species are actually two aspects of the same problem: (1) the adjuvant used, and (2) the number of immunizations needed for effective immunocastration. Although Freund’s Complete Adjuvant (FCA), which contains Mycobacterium components, is an extremely effective adjuvant, it is not useful from a commercial standpoint due to its interference in tuberculin testing and due to the localized tissue damage it causes. Thus, non-FCA adjuvants are needed, or better yet, conjugates that are directly bound to adjuvant-like glycoproteins and need no further adjuvant action. Both of these approaches have been developed and appear to be potentially useful. The other aspect, that of frequency of immunizations needed, is a matter of how well the primary plus secondary immunizations stimulate the immune system. The best immunization schemes may neutralize GnRH for several months, however, for perpetual effectiveness, routine boosters have been required.

A commercially available GnRH vaccine (Vaxstrate®) designed for beef heifers based upon a carboxyl-containing GnRH analog conjugated to ovalbumin (described by Hoskinson et al., 1990) was tested on young rams by Brown et al. (1994) and on male goats by Godfrey et al. (1996) in Australia. In each case, a significant reduction in testicular size was noted, lasting up to 394 days after the first immunization in goats. Another proprietary GnRH immunogen preparation, based on Mycobacterium-free adjuvant derived from recombinant TraT-LHRH fusion proteins, has been described by McLachlan et al. (1995). Male rats immunized against this product had reduced testis weight and almost total elimination of sperm production.

In the area of human medicine, another factor that must be taken into consideration is the use of materials not approved for use in humans. Towards solving this problem, researchers have devised conjugates of GnRH linked to commonly used vaccine products, such as tetanus toxoid. Ladd et al. (1990) studied the effect of conjugation site and dose of antigen in male rats on the response to GnRH conjugated to tetanus toxoid via the 1, 6, or 10 position of GnRH. Rather than a dose–response curve in response, there was a threshold like response above which all doses responded similarly. Moreover, the conjugate linked at the 1 position gave the highest and most consistent response in antibody titers and testosterone suppression in both rats and rabbits. Although many studies are geared to studying the possibility of GnRH immunization as a means of fertility control, there is also interest in developing a product for treatment of humans with steroid-dependent pathologies, such as prostatic cancer in men and breast cancer in women. At this time, there are safety and dose trials ongoing in the United States and the United Kingdom with an anti-GnRH vaccine called Gonadimmune®, which is being developed through an alliance between Aphton and SmithKline Beecham (Talwar, 1997; Henderson, 1998).
4. Practical aspects of GnRH immunization in males

4.1. Cattle

Early reports on the immunization of bull calves against GnRH as a means of immunocastration by Robertson et al. (1979, 1981, 1982) and Jeffcoate et al. (1982) showed that if appropriate titers could be achieved, testis size and production of testosterone, and hence aggressive behavior, could be reduced. Moreover, relative to steers, bulls immunized against GnRH had approximately 32% less fat and 50% more rib eye area at the 10th rib, as well as a 12% reduction in feed:gain ratio (Robertson et al., 1982).

Adams and Adams (1992) used feedlot steers to show that a single immunization with GnRH conjugated to KLH was sufficient to raise anti-GnRH titers and reduce LH secretion for more than 28 weeks. Similar immunization of bulls retarded testis growth but did not affect performance characteristics, perhaps due to the late age at immunization (10.5–11 months). In a subsequent study (Adams et al., 1993), immunization of bulls at 3.6 months of age did improve feedlot gain, carcass weight, and loin eye area relative to steers. Lobley et al. (1992) reported that immunization of crossbred bulls against GnRH did not affect feed intake, but did alter carcass fat and protein content; bulls with the highest anti-GnRH responses tended to be similar in composition to steers, whereas those with lesser responses were closer to the control bulls.

In a study comparing ages at immunization, Adams et al. (1996) found that immunization at 7 months of age was most effective in reducing testicular size at slaughter at 18 months of age, although most carcass characteristics were unaffected. In a similar age study, Jago et al. (1997) reported that immunization of Friesian bulls at 2.5, 4, or 7.5 months of age resulted in a delay in development of sexual and social behaviors, and that there was no practical reason to immunize before 7.5 months. And finally, Huxsoll et al. (1998) immunized beef bulls at 1, 4, or 6 months of age, gave a single booster at 12 months of age, and slaughtered the animals at about 16.4 months of age. Feedlot gain in GnRH-immunized bulls was similar to control bulls, whereas aggressive behavior was reduced and carcass quality (marbling score and quality grade score) was improved regardless of age at first immunization.

4.2. Small ruminants

Jeffcoate et al. (1982) immunized ram lambs against GnRH at 16–20 weeks of age and gave secondary injections 6, 12, and 28 weeks later. Although no slaughter data were presented, immunized rams had essentially no LH or testosterone response when challenged with a GnRH analog at 27 weeks, and had no increase in LH concentrations in response to castration at 90 weeks; they also had an 81% reduction in testis size relative to control rams. Also, Schanbacher (1982) reported that active immunization against GnRH in crossbred lambs produced carcasses that were similar to castrate animals, except that backfat thickness was not different from intact control lambs. Brown et al. (1994) immunized Merino ram lambs either before puberty or around the
time of puberty and followed them through 2 years of age (secondary injections were administered 10 weeks after the first immunization in both groups). Growth rates of immunized and control rams did not differ throughout the study, although prepubertal immunization did delay testicular growth until about 27 weeks of age. Most of the rams had recovery of testicular function once anti-GnRH titers dropped, but a few immunized rams still had subnormal sized testes at 115 weeks of age.

In a similar study with goats, Godfrey et al. (1996) immunized feral adult bucks in the summer with Vaxtrate® and compared their reproductive characteristics, behavior and male odor with non-immunized bucks and castrates. Secondary immunizations at 2 or 4 weeks after the first produced equivalent responses, with a 78% and 63% reduction in testicular parenchymal weight, respectively, compared with control bucks 394 days after immunization. Odor score as well as concentrations of LH, FSH, and testosterone were all reduced in immunized bucks by 56 days after the first immunization. Body weights did not differ between immunized and control bucks, although control bucks had reduced feed intake during the same period of time.

Other recent reports on active immunization of rams against GnRH have been designed for hypothesis testing, and no slaughter information has been provided. Lincoln and Fraser (1979) used passive immunization against GnRH to show that high amplitude LH peaks in rams during the breeding season were GnRH-dependent, whereas normal FSH secretion in the short-term was unaffected by GnRH antibody administration. Chase et al. (1988) used GnRH immunization to show that continuous LH infusion was equivalent to high frequency, low amplitude replacement of LH in restoring normal Leydig cell function in yearling rams. And lastly, Hotzel et al. (1995, 1997) used GnRH immunization in conjunction with diet to show that there is a nutritional component of normal testicular growth that is independent of GnRH.

4.3. Swine

Like goats, male odor is a particular problem in male boars after puberty, when adult levels of androgens are produced by the testes. Falvo et al. (1986) immunized crossbred boars against GnRH coupled to human serum globulin with either FCA or mu-ramyldipeptide as adjuvant at 12 weeks of age, followed by secondary immunizations at 16 and 18 weeks of age. Adjuvant was not a significant factor for any characteristic throughout the study. Compared to adjuvant-immunized controls, immunized boars had undetectable LH and testosterone concentrations at 22 weeks of age, a 70% reduction in testicular weight, and accessory sex gland weights not different than castrates. Average daily gains through 24 weeks of age were not affected by immunization and carcass characteristics were generally similar among groups at slaughter at 24 weeks; however, boar taint was reduced in immunized boars to levels not different from castrates. Caraty and Bonneau (1986) reported similar results for boars immunized at 100 days of age with GnRH linked to BSA and injected in FCA; in contrast to FCA, boars immunized with alumina gel did not produce significant antibody titers. Awoniyi et al. (1988) reported no carcass data, but did find efficient immunoneutralization in crossbred boars immunized against GnRH linked to human serum globulin.
The problem of variation in response among boars was studied by Meloen et al. (1994), who hypothesized that a GnRH conjugate in which the GnRH moiety was less recognizable as ‘‘self’’ might produce better and more consistent antibody responses. Immunization of 9–10 week old boars with a 20 amino acid GnRH tandem repeat peptide linked to KLH followed by a secondary immunization 8 weeks later resulted in consistent suppression of testosterone concentrations in blood and an approximate 87% reduction in testis size at 26 weeks of age. Androstenedone concentrations in backfat at slaughter were undetectable (< 100 ng/g) in the boars immunized with the tandem conjugate. No carcass data were presented. Moreover, the authors pointed out that their use of FCA was not commercially viable due to the localized ulcerations it caused.

Bonneau et al. (1994) used highly purified mineral oil to emulsify a GnRH-globulin conjugate for immunization of crossbred boars at 29 kg body weight, and followed with secondary immunization at 89 kg with saponin as adjuvant. A transdermal, needle-less device was used to deliver multiple 0.2-ml aliquots behind the ears. Compared to castrates, immunized and control boars had less feed intake, greater feed efficiency rates, more muscle mass, and less fat content at slaughter (105 kg). Fat androstenone concentrations were reduced by immunization to approximately 210 ng/g, which was well below the reported threshold of 500 ng/g for human sensory detection (Bonneau et al., 1992).

4.4. Other species

Immunization against GnRH has been applied to other species either as a means of fertility control, as in the case of pet animals, or as a means of studying the hypothalamic–pituitary–gonadal axis. Ladd et al. (1994) used a GnRH-tetanus toxoid conjugate in conjunction with muramyl peptide adjuvant to immunize mongrel dogs and cats. The efficacy of the immunization, various doses of immunogen, and its reversibility were studied. In dogs, 0.5 mg of antigen administered at 0, 2, and 6 weeks resulted in responses better than 0.1 mg and equivalent to 2.5 mg. Serum testosterone concentrations were reduced to castrate levels in some, but not all, dogs. After a 3-month period of no immunization (9–10 months after primary immunization), the testis size and testosterone concentrations in blood had returned to normal, indicating the reversibility of the effect. No testicular or sperm data were collected at this time, thus fertility per se of these dogs was unknown. Re-immunization of these recovered dogs resulted in rapid antibody production and a return to castrate-like conditions. In contrast to dogs, male cats produced significant antibody titers to GnRH, but exhibited only marginal reductions in circulating testosterone concentrations.

Lincoln et al. (1982) used active immunization against GnRH to study antler growth in sexually mature red deer stags. Three of the four immunized stags shed their antlers prematurely in autumn (instead of spring) and subsequently grew new antlers. The stag with the highest anti-GnRH titer did not experience antler hardening, and remained ‘in velvet’ for more than 6 months. No immunized stag showed normal sexual behavior in autumn, and all had reduced testes size relative to control stags. In a subsequent report, Lincoln et al. (1984) used previously immunized stags in comparison with non-im-
munized stags and stags with superior cervical gangliectomy to study the role of melatonin in the seasonal reproductive cycle. Melatonin treatment in May (the nadir of the seasonal cycle) induced premature hardening of the antlers and stimulated testosterone concentrations and male sexual behavior. These responses to melatonin were dependent upon GnRH, given that re-immunization of the previously immunized stags blocked the effects.

The practical application of GnRH immunization in the horse was first reported by Schanbacher and Pratt (1985), who actively immunized a cryptorchid stallion as an alternative to surgical removal of an abdominal testis. Serum LH and testosterone concentrations remained low while anti-GnRH titers were high, but returned to normal over a 7-month period between secondary immunizations. Re-immunization resulted in rapid restoration of titers and a drop in testosterone concentrations to castrate levels.

Garza et al. (1986) used active immunization against GnRH to study the differential dependency of LH and FSH on GnRH input to the pituitary of horses. Long-term concentrations of LH in plasma of mares were totally suppressed by immunization, whereas FSH secretion was reduced by only 50%. Further study of these mares (Garza et al., 1988) showed that the testosterone-induced stimulation of FSH production in the pituitary (which had been previously reported for castrate males as well as females; Thompson et al., 1979, 1983, 1984; Reville-Moroz et al., 1984) was GnRH-dependent, given that it was not inducible in GnRH-immunized mares. To further study this phenomenon, and to determine the effects of immunization on hypothalamic GnRH characteristics themselves, we immunized castrate male ponies against GnRH linked to BSA for 6 months and determined tissue contents of gonadotropins and GnRH at slaughter (Rabb et al., 1990). Immunization against GnRH reduced pituitary weight by 31%, reduced pituitary LH content by 91%, but only reduced pituitary FSH content by 55%. In fact, challenge with a GnRH analog that did not bind to the anti-GnRH antibodies prior to slaughter resulted in a similar FSH response in immunized and control ponies, even though the LH response was reduced by 90%. There was no effect of immunization on prolactin response to thyrotropin releasing hormone injection or on pituitary prolactin content. Moreover, GnRH concentration in the median eminence, preoptic area, and body of the hypothalamus was unaffected by immunization, indicating that there was likely no short-loop feedback of GnRH on its own production and storage.

### 4.5. Primates and humans

Hodges and Hearn (1977) first immunized marmoset monkeys against GnRH and reported significant reductions in testis size and plasma testosterone concentrations. They suggested that similar immunizations, after improvements upon adjuvants, might be useful for fertility control in humans. Current potential applications in human medicine go beyond fertility control, and now include the possibility of GnRH immunization for control of prostate and breast cancers, which are dependent upon steroid secretion from the gonads. Towards this end, Giri et al. (1991) showed that immunization of male bonnet monkeys with GnRH linked to diphtheria toxoid produced variable anti-GnRH titers, which were generally associated with reduced testosterone concentra-
tions in blood; prostate weights were reduced by about 67% at 20 weeks after onset of immunizations. Most recently, Gual et al. (1997) immunized three post-menopausal women against LYS\(^4\)-GnRH linked to diphtheria toxoid and showed that both LH and FSH were reduced within 60 days. The effects of immunization were reversed within 180 days in the absence of further injections. And, as mentioned earlier, clinical safety trials have already begun on the use of GnRH immunization for treatment of prostate carcinomas (Talwar, 1997).

References


