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Review

Diagnostic applications for inhibin and activins

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ABSTRACT

Inhibin and activins play major roles as paracrine and autocrine signaling molecules in reproduction and development where the main emphasis has been placed in developing potential diagnostic applications. While a role for activin assays in diagnostics has so far been unfounded, ELISAs specific for the biologically active inhibin A and B dimers, and for free inhibin alpha subunits, alone or in combination have found some specific diagnostic applications. Addition of inhibin A to the triple test for Down syndrome in the second trimester of pregnancy, measurement of total inhibin as a marker of certain forms of ovarian cancer in specific circumstances, and inhibin B for male fertility are useful diagnostics. A review of the evidence so far suggests that other applications for inhibin and activin assays have yet to be confirmed, or translated into reliable tools for clinical practice.

Contents

1. Introduction

The first demonstration of the major biological activity of "inhibin" in affecting gonadotropes in the pituitary (McCullagh, 1932) and subsequent purification of inhibin (Ling et al., 1985; Miyamoto et al., 1985; Robertson et al., 1985; Mason et al., 1986) prompted a surge in research into the biological functions of inhibin as exemplified in the contributions to this edition of MCE. The first indication that the biological activity of inhibin was measurable in plasma or serum came from the application of a sensitive bioassay. This bioassay was based on the rat bioassay used initially to purify inhibin (Eddie et al., 1979) and used sheep pituitary cells in culture which were more sensitive to the suppressive effects of inhibin on the secretion of FSH (Tsonis et al., 1986). Application to serum or plasma samples during the menstrual cycle showed that the bioassay gave a reliable estimate of inhibin bioactivity which could be neutralized by an antibody to inhibin. These early studies identified an increase in inhibin following FSH stimulation in both sheep and women suggesting a potential role in monitoring the success of ovarian stimulation programs in both women and domestic ruminants (Tsonis et al., 1988a,b) but future studies obviously re-

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quired development of either radioimmunoassays or ELISAs to be developed if inhibin measurements would prove to be of any diagnostic value. As the understanding of the structure and function of inhibin progressed and RIAs were developed so potential applications emerged. This short review will summarize the present state of diagnostic applications for inhibin assays, and mention possibilities for measurement of activins.

2. Development of assays

The first reliable and reproducible radioimmunoassays for inhibin were based on antibodies raised to intact dimeric inhibin but subsequently shown to recognize the alpha subunit of inhibin (McLachlan et al., 1986), or specifically the N-terminus of the alpha C subunit of inhibin (e.g. McNeilly et al., 1989). These showed that inhibin levels increased during the late follicular phase of the menstrual and estrous cycles of a number of species, but results in males were equivocal. While inhibin was recognized as being a dimer of an alpha subunit, coupled with a ß subunit, these assays were utilized with some effect to understand the biology of inhibin. However, there were inconsistencies between inhibin and FSH levels in women and men. Thus came the realization that there were two circulating forms of inhibin dimer, consisting of an alpha inhibin subunit associated with a ßA, or ßB subunit to form inhibin A or inhibin B and that RIAs relying on use of antibodies to the inhibin alpha subunit did not reflect both forms.

Furthermore it also became apparent that levels of free alpha inhibin subunit were present and secreted (Hamada et al., 1989; Knight et al., 1988; McNeilly et al., 1994; Schneyer et al., 1990) and these early RIAs could not distinguish between non-biologically active free alpha inhibin and biologically active inhibin A or inhibin B. In addition, in most female species where initial analyses were undertaken, it appeared that both inhibin A and B were produced by the ovary, the initial area of interest for potential diagnostic applications. With the biochemical analyses of inhibin alpha structure and the realization that the alpha subunit was initially produced as a pro-alphaN-alphaC precursor and that this could combine with the ß subunits to produce several different forms of inhibin (e.g. Robertson et al., 1997), it became clear that the RIAs were of limited use for many of the investigations into the biological function of inhibin which would lead to any diagnostic application.

The breakthrough came from the development of ELISAs which were specific for inhibin A, inhibin B and the pro-alpha C forms of inhibin (Groome et al., 1994, 1995, 1996). The first data in the menstrual cycle using an RIA showed that inhibin levels were present throughout the menstrual cycle, increased only in the very late follicular and were high throughout the luteal phase of the cycle (Robertson et al., 1988). Application of the ELISA specific for inhibin A showed that levels were also very low early in the follicular phase, increased in the late follicular phase of the menstrual cycle, and were further increased in the luteal phase, correlating well with the RIA data (Groome et al., 1994). This showed for the first time that there was minimal production of inhibin A by early developing follicles at the start of the menstrual cycle and that inhibin A was secreted first in any quantity by the dominant preovulatory follicle(s) and then by the corpus luteum (Groome et al., 1994). Subsequent studies showed that in early pregnancy hCG stimulated a further increase in inhibin A secretion by the corpus luteum (Illingworth et al., 1996a,b) and that the placenta then maintained inhibin secretion throughout pregnancy (Lockwood et al., 1998a,b). These changes in inhibin A matched fairly closely those with the RIAs which detected dimerised or free alpha inhibin subunits. The development of the pro-alpha C ELISA showed a similar patter of secretion as inhibin A in early pregnancy (Illingworth et al., 1996a,b).

The major surprise came with the development of the specific ELSA for inhibin B which showed that levels increased as soon as follicle development was stimulated by FSH in the early follicular phase of the menstrual cycle and then declined towards the stages of preovulatory follicle selection when inhibin A secretion increased (Groome et al., 1996). Furthermore, while inhibin A had been undetectable in men, inhibin B was shown to be the inhibin secreted by men (Illingworth et al., 1996a,b), other primates (Ramaswamy et al., 1999), and rodents (Sharpe et al., 1999). Incidentally, in sheep, neither male nor female sheep produce inhibin B, both producing only inhibin A (McNeilly et al., 2002). The discovery of the different patterns of inhibin A and B secretion in the menstrual cycle and pregnancy in women, and that only inhibin B was produced in men began the search for potential diagnostic application of the specific inhibin assays. Careful analyses of plasma and tissue samples revealed the potential use in some instances for less inhibin-subtype specific assays and the usefulness of a global inhibin assay that would detect all forms of dimeric biologically active inhibin forms and non-biologically active alpha forms of inhibin. These potential diagnostic applications will now be discussed briefly.

3. Applications in female reproduction

Four areas of potential application have been assessed namely in ovarian reserve and function, polycystic ovary syndrome (PCOS), ovarian cancer, and in pregnancy, specifically pre-eclampsia and Downs screening.

3.1. Ovarian reserve

With the age of first attempts at conception increasing, the problem of ovarian reserve has become more acute for some women, as revealed in IVF where success declines with age (Templeton et al., 1996). Inhibin A levels increase from mid follicular phase onwards and only indicate if a preovulatory follicle(s) is present, but the preovulatory follicle can easily be detected by ultrasound. Some studies in animals have suggested that inhibin A in the late stages of ovarian stimulation gives some indication of health of oocytes and subsequent success in embryo transfer (Gonzalez-Bulnes et al., 2004; Veiga-Lopez et al., 2008) but there is no evidence that this is the case in women. Since inhibin B levels reflect the number of activated small follicles in the early follicular phase, the potential focus on early follicular phase inhibin B measurements to directly assess the ovarian follicle pool and predict ovarian response to FSH stimulation initially appeared to be of diagnostic value (Creus et al., 2000; Dumesic et al., 2001; Dzik et al., 2000; Lockwood et al., 1996), and certainly declining levels of inhibin B were related to increased FSH levels associated with older women approaching the menopause (Burger et al., 2008; Klein et al., 1996). However, subsequent studies using FSH-stimulated inhibin B levels found that levels of FSH were more reliable in determining ovarian function (Yong et al., 2003). Furthermore measurement of AMH appears to be more reliable indicator of overall ovarian reserve (Visser et al., 2006), and, together with more sensitive ultrasound to measure total antral follicle numbers (Muttukrishna et al., 2005; Hendriks et al., 2007) has superceded inhibin B for most routine diagnostic purposes.

3.2. Polycystic ovary syndrome

Given the increased numbers of small to medium sized follicles in women with PCOS it might have been expected that inhibin levels would be different from a normal women. Some small studies initially reported higher than normal plasma concentrations of