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INVITED COMMENTARY

Testicular activin – too hot to handle?

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Activins are members of the transforming growth factor β (TGFβ) family of growth and differentiation factors (1). Like the other members of this family, they consist of dimers of glycosylated polypeptides, linked by a disulfide bond. To date, five different forms (A to E) of the activin subunit have been described on the basis of nucleotide sequences. However, biological activities have only been described for three of them: activins A, B and AB, the last being a heterodimer of the A and B form.

In common with TGFβ and its other family members, which include the bone morphogenetic proteins, the growth and differentiation factors and anti-Müllerian hormone, activins act by binding to members of a family of transmembrane receptors (1). These receptors consist of an extracellular domain, which can interact with the ligand, a transmembrane domain, and an intracellular domain, which can phosphorylate other proteins on serine or threonine residues. When activin is bound to the receptor, which is itself constitutively activated by phosphorylation, a second serine-threonine kinase can be recruited into this complex. Cross-linking experiments indicated the formation of a complex, in which interaction between ligand and both kinases occurs; for this reason the second kinase is also regarded as a receptor. On the basis of apparent molecular weight, the latter, smaller receptor is called the type I receptor, whereas the former, larger ligand-binding molecule is designated the type II receptor.

The type I receptor is activated by phosphorylation by the type II receptor, and itself phosphorylates a member of a family of intracellular signal transduction proteins, the so-called 'SMADs' (2), which can convey the signal to the nucleus. For activin, two subtypes of each of the receptors have been described: IIA and IIB and IA and IB.

The action of activin can be prohibited in two ways: the activin-binding protein, follistatin, can bind to the activin subunit and prevent the interaction of activin with its type II receptors (3) or, alternatively, the actions of activin can be counteracted by inhibin, a heterodimer of an activin subunit with a distantly related family member, the inhibin α-subunit. Because inhibin was characterized before activin, the activin subunit was earlier defined as the inhibin β-subunit; inhibin might act by binding to type II activin receptors, through this common β-subunit. Binding of inhibin to the IIB receptor appears to be more effective than binding to the IIA receptor (4), and this differential binding of inhibin might explain why some actions of activin can be blocked by inhibin, whereas other can not. Follistatin, in contrast, should block the actions of activin through type IIA and IIB receptors with similar effectiveness.

The Sertoli cells in the testicular seminiferous tubules produce inhibin, which is subsequently secreted and suppresses the production of follicle stimulating hormone (FSH) in the pituitary gland. It is likely that this action is caused by the above-described activin-antagonizing action of inhibin; activin stimulates FSH production and is locally produced by pituitary gonadotrophs (5). Because FSH can stimulate inhibin production by the testis, the activin–FSH–inhibin system is a closed feedback loop, which has a role in the regulation of the function of the seminiferous tubules, i.e. the production of spermatozoa. Recent evidence, however, indicates that spermatogenesis may proceed even in the absence of FSH (6).

A more direct level of involvement of the activin/inhibin system in the regulation of spermatogenesis may be at the level of the spermatogenic cells themselves. Activin is produced by Sertoli and peritubular cells (7, 8), and activin receptors are present in almost any testicular cell type: Leydig and Sertoli cells contain activin receptors (ActR) type IIA (9, 10), whereas the early spermatogenic cells – the spermatogonia – contain ActRIIB (11), and the further-developed cells – spermatocytes and spermatids – express ActRIIA (9, 10). Presence of type I receptors in spermatogenic cells has also been reported recently; from a study on the ontogeny of type I receptors, it appears that spermatids express the mRNA encoding activin receptor type IB (12).

Of course, the presence of the ligand and the receptors does not prove that there is an effect of activin on spermatogenesis. However, from the studies of Mather *et al.* (13), it appears that *in vitro* addition of activin to spermatogonia increases their mitotic activity. In contrast, inhibin can suppress the numbers of intermediate and late spermatogonia after intratesticular injection *in vivo*. This might be explained on basis of the presence of activin IIB receptors in spermatogonia (14). The role of inhibin in blocking the action of activin at the activin receptor type IIA in spermatocytes and early spermatids is less clear; as stated above, the potency to antagonize the action of activin at this receptor is less than that at the activin receptor type IIB. However, in a paper in this issue

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of the *European Journal of Endocrinology* (15), it is indicated that the immunoreactivity of follistatin increases in these spermatogenic cell types. For the pituitary gland and the ovary it is known that activin is able to stimulate the production of follistatin (16, 17), leading to a diminished activity of activin itself; in the spermatogenic cells, a similar mechanism might play a part.

Production of follistatin in cultures of Sertoli cells has been described (18). Follistatin mRNA is localized in Sertoli cells, but not in spermatogenic cells (9). Finally, follistatin was detected by immunocytochemistry in Sertoli cells, and mainly in Leydig cells (19). It is difficult to combine these conflicting data on localization of follistatin in one concept of the production and action of follistatin. The only reasonable explanation might be to postulate uptake of follistatin, together with activin, by the spermatogenic cell types that express activin receptor IIA. Such a mechanism can exist only if a follistatin–activin subunit–activin subunit–activin receptor type II complex could be imported into the cell; in this way interaction with the type I receptor can be prevented.

In this context, it is interesting to note that spermatogenesis itself can affect the expression of the subunits necessary for the production of activin or inhibin (20). By changing the ratio between α- and β-subunit expression, the ratio between the amounts of agonist and antagonist produced can be affected. If the hypothesis of activin-induced production of follistatin is true, the amount of activin that is not counteracted by inhibin would subsequently lead to production or import of an amount of follistatin, which would further fine-tune the activin signal in the complex process of spermatogenesis.

In summary, testicular cells are protected against activin by several mechanisms. Deletion of one of these mechanisms by deletion of the inhibin α-subunit gene leads to tumorigenesis of Sertoli cells (21). Follistatin may be another molecule that potects testicular cells against this 'hot' molecule. Unfortunately, it will be difficult to prove this hypothesis, as results of follistatin gene knock-out experiments indicate that this deletion leads to early postnatal death (22).

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