Is the action of inhibin mediated via a unique receptor?

David M. Robertson, Ruth Hertan and Paul G. Farnworth

Prince Henry’s Institute of Medical Research, PO Box 5152, Clayton, Victoria, 3168, Australia

The receptor system and the molecular mechanisms by which inhibin acts on its target cells are poorly understood, in contrast to the situation for the structurally related molecule, activin. On the basis of evidence that the biological action of inhibin in a number of systems resembles that of an activin antagonist, it has been contended that inhibin operates by competition for the activin receptor rather than through a specific inhibin receptor. However, mounting evidence indicates that inhibin also interacts with high affinity and specificity with membrane-binding proteins that are likely to be the putative inhibin receptor.

Inhibin, like other members of the transforming growth factor β (TGF-β) superfamily, is a disulphide-linked dimer of homologous subunits that contain a characteristic set of seven cysteine residues that form a cystine knot within each subunit. Inhibin consists of α and either βA or βB subunits, while activins are dimers of the β subunits, which potentially include other β subunit forms (that is, βC, βD and βE) (Woodruff and Mather, 1995).

Receptors and binding proteins for members of the TGF-β family

A series of binding proteins has been associated with signalling by TGF-β, bone morphogenetic proteins (BMPs), Mullerian inhibitory substance and activins (Gaddy-Kurten et al., 1995). Some of these binding proteins are involved directly with the signal transduction process, but the way in which the proteins interact is proving to be highly complex. It is generally agreed that two groups of binding proteins, called type I and type II receptors, can bind weakly or strongly to their respective TGF-β superfamily member, but the combination is critical to transducing a signal. In the case of activin, the ligand first binds to an activin type II receptor that incorporates a constitutively active serine–threonine kinase domain. The complex then interacts with an activin type I receptor, which itself has low intrinsic affinity for activin. The union of type I and II receptors results in phosphorylation of the type I receptor which, thus activated, phosphorylates nuclear activating factors (Smads) specific to the pathway (Kawabata and Miyazono, 1999), leading to nuclear activation.

In addition to type I and II receptors, studies with members of the TGF-β superfamily have identified a number of associated binding proteins thought to modify the interaction of each family member with its putative receptors. These associated proteins are localized to the cell surface and apparently lack signal transducing activity, but exhibit both high affinity and specificity for the ligand. As an example, a large molecular weight protein called TGF-β receptor type III (or β-glycan; Lopez-Casillas et al., 1993) has been identified which is thought to concentrate or orientate TGF-β at the cell surface. Another accessory binding protein, endoglin (Barbara et al., 1999) binds the TGF-β, BMP-7 and activin type II receptor complexes, facilitating binding of each complex to its corresponding type I receptor. As a variation, endoglin also binds BMP-2 in association with its type I receptor.

A contrasting example is an activin–BMP type I-like receptor, which lacks the cytoplasmic tail and, hence, phosphokinase activity (BAMBI) (Onichtchouk et al., 1999). BAMBI is able to bind to an appropriate type II receptor–activin–BMP complex but is unable to transduce a signal and thus antagonises the actions of activin and BMP.

In addition to binding proteins involved in signal transduction, a number of soluble, secreted binding proteins have been identified; these proteins show high affinity and specificity for particular TGF-β superfamily members and neutralize their biological activities. Binding proteins identified include the latency-associated protein, which binds TGF-β; follistatin and cerberus, which bind activin; and chordin, gremlin, nogg, cerberus, follistatin and DAN, which bind BMP (Hsu et al., 1998). The action of each is attributable to competition for binding of the respective ligand to its receptors.

A separate signalling mechanism is used by a distant group of the TGF-β superfamily. Gliial cell line-derived neurotrophic factor (GDNF), persephin, artemin and neurturin bind to specific accessory proteins devoid of phosphokinase activity, termed GFRα1–3 (Soler et al., 1999). Each complex then binds to a common receptor, RET. GFRα1–3 proteins differ from the recognized TGF-β family type II receptors in their lack of structural homology and their glycolipid anchorage to the cell membrane. However, the most significant difference is that the ligand–GFRα–RET complex signals through the intrinsic tyrosine kinase activity of RET rather than through the serine–threonine kinase activity characteristic of type I receptors for most other TGF-β superfamily members. This finding establishes a precedent for alternative signalling within the superfamily, raising the possibility that inhibin receptors differ from other TGF-β receptor family members.

These examples indicate many ways in which inhibin might act, each involving one or more novel accessory binding
proteins or specific classical type I and II receptors similar to those identified for other members of the TGF-β superfamily.

Models of the action of inhibin

There are two main hypotheses concerning the mechanism of action of inhibin (summarized in Fig. 1).

Model 1: inhibin competes with activin for the activin receptor

Since inhibin and activin share a common βA or βB subunit, the β subunit of inhibin should bind to the activin type II receptor. This hypothesis has been explored by several groups who have shown that the interaction of inhibin with subtypes of the activin type II receptor (in particular type IIB) results in up to 40% competition for activin-binding (Lebrun and Vale et al., 1997; Martens et al., 1997). However, the α subunit, although it is similar to the β subunits, is unable to recruit the signalling type I receptor (Xu et al., 1995). Thus, inhibin may be viewed as a strong or weak competitive antagonist of activin, depending on the activin type II receptor subtype involved (type IIB2 > type IIB1 > type II; Martens et al., 1997). Irrespective of the final biological response, it follows from this hypothesis that inhibin should antagonise the action of activin, and other superfamily members (for example BMP-7; Yamashita et al., 1995) when they use activin type II receptors, but that inhibin by itself should be inactive.

Model 2: inhibin binds specifically to its own receptor

Several research groups have attempted to identify the inhibin receptor by searching for proteins with sequence similarities in domains likely to be common to the TGF-β receptor family. Searches focused on either the serine-threonine kinase domain, in which high homology between TGF-β receptor family members is evident, or the transmembrane or...
extracellular ligand-binding domains have not been fruitful to date (Woodruff, 1999). The failure to find a candidate inhibin receptor by these approaches emphasizes the ability of inhibin to compete for the activin receptor.

An inhibin receptor would be characterized by interactions with inhibin with high affinity and high specificity in the picomolar concentration range with binding sites on target cells to generate a biological response.

Evidence of specific inhibin binding has been provided using autoradiography (Woodruff and Mather, 1995). Binding of iodinated inhibin to ovarian, testicular and pituitary sections is competed for by unlabelled inhibin but not activin. Studies of inhibin binding to cells from the ovine anterior pituitary, a known site of inhibin action, indicate the presence of two inhibin-binding sites \( K_d(1) = 0.3 \text{ mmol l}^{-1}; \ K_d(2) = 4 \text{ mmol l}^{-1} \), showing low crossreactivity with activin (\(< 2\%\)) and TGF-\(\beta\)1 (\(< 0.2\%\)) (Hertan et al., 1999). Other cell types not expected to respond to inhibin show low inhibin binding.

Further evidence for the existence of inhibin receptors has been obtained by Draper et al. (1998) who isolated a series of such proteins from ovarian cancer cells in mice in which the inhibin \(\alpha\) subunit gene had been deleted. The molecular sizes of the inhibin cross-linked binding proteins were reminiscent of those observed with other members of the TGF-\(\beta\) receptor family. These binding proteins, although purified to homogeneity, were N-terminally blocked and, thus, it was not possible to determine the sequences. Wang et al. (1999) identified an inhibin-binding protein with high specificity for inhibin in bovine pituitary membranes; this protein may be a component of the inhibin–receptor complex. Overall, these data support the presence of high affinity and specific binding proteins for inhibin, although the studies concerned did not address the mechanism of action of inhibin.

### Inhibin-binding proteins

Evidence of specific inhibin binding has been provided using autoradiography (Woodruff and Mather, 1995). Binding of iodinated inhibin to ovarian, testicular and pituitary sections is competed for by unlabelled inhibin but not activin. Studies of inhibin binding to cells from the ovine anterior pituitary, a known site of inhibin action, indicate the presence of two inhibin-binding sites \( K_d(1) = 0.3 \text{ nmol l}^{-1}; \ K_d(2) = 4 \text{ nmol l}^{-1} \), showing low crossreactivity with activin (\(< 2\%\)) and TGF-\(\beta\)1 (\(< 0.2\%\)) (Hertan et al., 1999). Other cell types not expected to respond to inhibin show low inhibin binding.

Further evidence for the existence of inhibin receptors has been obtained by Draper et al. (1998) who isolated a series of such proteins from ovarian cancer cells in mice in which the inhibin \(\alpha\) subunit gene had been deleted. The molecular sizes of the inhibin cross-linked binding proteins were reminiscent of those observed with other members of the TGF-\(\beta\) receptor family. These binding proteins, although purified to homogeneity, were N-terminally blocked and, thus, it was not possible to determine the sequences. Wang et al. (1999) identified an inhibin-binding protein with high specificity for inhibin in bovine pituitary membranes; this protein may be a component of the inhibin–receptor complex. Overall, these data support the presence of high affinity and specific binding proteins for inhibin, although the studies concerned did not address the mechanism of action of inhibin.

### Proposed mechanisms of action of inhibin

On the basis of the above binding data, it appears that inhibin can act either as an activin receptor antagonist or by interaction with a specific inhibin receptor. The actions of inhibin in eight inhibin and activin responsive systems are presented (Table 1) to characterize which mechanism applies in biological systems. In four of the chosen cell systems, inhibin alone is inactive or of limited activity (EC\(_{50}\) value \(> 300 \text{ pmol l}^{-1}\)), activin is active (EC\(_{50}\) value \(= 20–120 \text{ pmol l}^{-1}\)) and inhibin is able to antagonise the action of activin. Presumably, this pattern reflects competition for the activin receptor (Xu et al., 1995; Lebrun and Vale, 1997; Martens et al., 1997). In four other systems, inhibin is active alone in the picomolar range (EC\(_{50}\) value \(= 30–300 \text{ pmol l}^{-1}\)), is at least as potent as activin (EC\(_{50}\) value \(= 20–300 \text{ pmol l}^{-1}\)), and also antagonises activin, which is consistent with the involvement of an inhibin receptor. It is important to note

<table>
<thead>
<tr>
<th>Proposed mechanism of inhibin action</th>
<th>Cell system</th>
<th>Action of inhibin alone</th>
<th>Inhibin EC(_{50}) (pmol l(^{-1}))</th>
<th>Action of activin alone</th>
<th>Activin EC(_{50}) (pmol l(^{-1}))</th>
<th>Activin + inhibin</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Via competition with activin receptor</td>
<td>DNA synthesis by liver (HepG2) cells</td>
<td>None</td>
<td>–</td>
<td>Suppression</td>
<td>100</td>
<td>Antagonism</td>
<td>Xu et al., 1995</td>
</tr>
<tr>
<td></td>
<td>DNA synthesis by ovarian (CHO-K1) cells</td>
<td>None</td>
<td>–</td>
<td>Suppression</td>
<td>120</td>
<td>Antagonism</td>
<td>Gonzalez-Manchon and Vale, 1989</td>
</tr>
<tr>
<td></td>
<td>ACTH secretion by pituitary (AtT20) cells</td>
<td>None</td>
<td>–</td>
<td>Suppression</td>
<td>20–30</td>
<td>No change</td>
<td>Bilezikjian et al., 1991</td>
</tr>
<tr>
<td></td>
<td>DNA synthesis by prostate (LNCAP) cells</td>
<td>Limited suppression</td>
<td>300 (EC(_{25}))</td>
<td>Suppression</td>
<td>9 (EC(_{25}))</td>
<td>Synergism</td>
<td>Wang et al., 1996</td>
</tr>
<tr>
<td>Via a specific inhibin receptor</td>
<td>FSH release by pituitary cells</td>
<td>Suppression</td>
<td>30</td>
<td>Stimulation</td>
<td>50</td>
<td>Antagonism</td>
<td>P. Farnworth, C. Cahir and P. Leembruggen, personal observations</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20–40</td>
<td>Stimulation</td>
<td>20–40</td>
<td>Antagonism</td>
<td>Bilezikjian et al., 1996</td>
</tr>
<tr>
<td></td>
<td>Folliculin synthesis by pituitary cells</td>
<td>Suppression</td>
<td>60</td>
<td>Suppression</td>
<td>50</td>
<td>Antagonism</td>
<td>Hseuh et al., 1987</td>
</tr>
<tr>
<td></td>
<td>LH-induced androgen production by rat testicular and ovarian thecal cells</td>
<td>Stimulation</td>
<td>30–300</td>
<td>Suppression</td>
<td>300</td>
<td>Antagonism</td>
<td>Hillier, 1991</td>
</tr>
</tbody>
</table>

EC\(_{50}\) value: concentration of ligand causing 50% of the maximum response.
that activin expression is widespread, implying that inhibin may inhibit the biological effects of endogenous activin as the basis of an apparent inhibin-specific action. As yet, there is little evidence of an inhibin action that is truly independent of activin.

Thus, in almost all circumstances in which inhibin acts, it is counteracted by and can counteract activin, which is consistent, at least in part, with both proposed mechanisms of action. However, the potency of inhibin mostly exceeds that expected to arise from simple competition with activin for cell surface receptors. It has been proposed that ancillary inhibin-binding proteins can intercede and increase the interaction of inhibin with activin type II receptors, thus facilitating the competitive mechanism (Fig. 1, Model 1; for example, see Lebrun and Vale, 1997). An example of such a mechanism was described by Lewis et al. (2000) in which β-glycan functions as a co-receptor with activin type II receptor for inhibin binding. The affinity of the resulting complex for inhibin (0.2 nmol l⁻¹) exceeds that of either binding partner alone (0.6 and 6.3 nmol l⁻¹, respectively). Activin competes poorly with inhibin for binding to both β-glycan and the β-glycan–activin type II receptor complex. Expression of β-glycan by activin responsive cells, which were previously insensitive to inhibin, results in a potent inhibition of activin action.

Alternatively, with the inhibin receptor mechanism (Fig. 1, Model 2), mutual antagonism by inhibin and activin may reflect crosstalk between separate but converging signal transduction pathways. Since Smads identified to date can be stimulatory (Smads 1–3, 5 or 8 in combination with Smad 4) or inhibitory (Smads 6 and 7), inhibin may oppose the action of activin by using inhibitory Smads (Kawabata and Miyazono, 1999), or by dephosphorylating or differentially phosphorylating stimulatory Smads. A model in which inhibin binds an activin type II receptor and recruits an inhibin-specific type I receptor represents a hybrid of the two proposed mechanisms. It is also conceivable that binding of inhibin to a distinct type II receptor allows recruitment of the activin type I receptor to phosphorylate a unique inhibin signalling molecule. Finally, the case described by Wang et al. (1996) in which inhibin and activin share the same action (Table 1) provides a challenge to existing models of inhibin action.

Conclusion

Overall, the above data indicate that inhibin operates by two mechanisms: the first is based on competition of inhibin for activin receptors, which results in an antagonistic action; the second is by interaction with specific and high affinity receptors, which activate signal transducing pathways that oppose activin action. Additional inhibin-binding proteins may be involved in either process. The strongest evidence for inhibin receptors is the coincidence in the pituitary and gonads of potent inhibin actions and tissue-specific and inhibin-specific high-affinity binding proteins. Progress in this field is likely to depend on the isolation and characterization of the key proteins involved in the interaction of inhibin with the target cell, and the discrimination of receptors from ancillary binding proteins. These advances will clarify the signalling pathway of inhibin and whether its action is dependent on activin or is unique.

Work in the authors’ laboratories was funded by a Program Grant (number 983212) of the National Health and Medical Research Council of Australia.

References

Key references are identified by asterisks

Barbara NP, Wrana JL and Letarte M (1999) Endoglin is an accessory protein that interacts with the signalling receptor complex of multiple members of the transforming growth factor-β superfamily Journal of Biological Chemistry 274 584–594


Bilezikjian LM, Corrigan AZ, Blount AL and Vale WW (1996) Pituitary folistatin and inhibin subunit messenger ribonucleic acid levels are differentially regulated by local and hormonal factors Endocrinology 137 4277–4284


Lebrun JJ and Vale WW (1997) Activin and inhibin have antagonistic effects on ligand-dependent heteromerization of the type I and type II activin receptors and human erythroid differentiation Molecular and Cellular Biology 17 1682–1691


Lopez-Casillas F, Wrana JL and Massague J (1993) Betaglycan presents ligand to the TGF-β signalling receptor Cell 73 1435–1444


Soler RM, Dolcet X, Encinas M, Egea J, Bayascas JR and Comella JX (1999) Receptors of the glial cell line-derived neurotrophic factor family of neurotrophic factors signal cell survival through the phosphatidylinositol 3-ketase pathway in spinal cord motoneurons Journal of Neuroscience 19 9160–9169


membrane-bound protein which interacts with inhibin Proceedings of the 81st Annual Meeting the Endocrine Society Abstract P3–489


