Binding and signalling properties of a GH-enhancing monoclonal antibody

James Beattie*, James Mockridge*, Silvia Bramani*, Heather Davies*, Nicholas Marshall† and Philip E Buckle‡

*Hannah Research Institute, Ayr KA6 5HL, Scotland, †Clinical Pharmacology Unit, University of Cambridge, Addenbrookes Hospital, Cambridge CB2 2QQ, England, ‡Endocrine Unit, Department of Molecular Pathology, UCLMS, London W1N 8AA, Affinity Sensors, Cambridge CB3 8SL, England.

We have recently described in detail the structure of an epitope defined by a monoclonal antibody which enhances in vivo the biological activity of bovine GH (bGH) [1]. Although several theories have been advanced in relation to the mechanism associated with this enhancement phenomenon, there is little conclusive data supporting any hypothesis [2].

To examine the molecular and cellular aspects of this phenomenon more closely, we have undertaken studies using the well characterised GH-responsive 3T3-F442A preadipocyte cell line and the FDCP-1 cell line transfected with full length mouse growth hormone receptor (GHR). As a prelude to this, we examined the binding properties of OA15 in relation to the subsequent interaction between bGH and its receptor. Initial examination of the epitope defined on bGH by OA15 suggested that it was removed from binding sites 1 and 2 on GH required for interaction with the GH receptor [3]. Using the recombinant bGH binding protein (bGHBP), which represents the extracellular domain of the receptor, we have demonstrated, using optical evanescent wave biosensor technology, that engagement of bGH by OA15 still allows for the interaction between bGH and bGHBP. Although further experiments are underway to investigate the effects on the affinity of bGH-bGHBP interaction following bGH binding to OA15, at this stage we can tentatively conclude that these real time binding studies agree with our initial epitope mapping work that the epitope defined by OA15 is removed from receptor interaction sites on the hormone.

The results of these experiments suggested that in cell culture studies tripartite complexes of antibody-hormone and receptor would exist. In the context of mechanic studies, this in turn would suggest that OA15 would not inhibit GH signalling events through disruption of GH-GHR interactions. To examine this hypothesis in more detail we used two GH-responsive cell culture systems - the 3T3-F442A preadipocyte cell line and the FDCP-1 cell line which has been transfected with the full length mouse GHR [4]. In F442A cells, treatment with GH leads to the rapid tyrosine phosphorylation of proteins of the JAK-STAT-MAP pathway(s). Figure 1 is an antiphosphotyrosine (PY) blot of lysates from F442A cells treated with bGH alone or hormone pre-complexed with OA15. As can be clearly seen, GH treatment stimulates the level of a 91kDa tyrosine phosphorylated protein. This is most likely to be a member of the Stat (signal transducers and activators of transcription) family of transcription factors. The level of tyrosine phosphorylated 91kDa protein is not altered following treatment with antibody-hormone complexes. Again, this is consistent with no interference by OA15 in bGH signalling through its receptor. Another of the hypotheses which we were testing was that the enhancement of hormone activity seen in vivo would result in some amplification of bGH signalling events. The very limited evidence above of little effect of antibody complexation on hormone stimulated tyrosine phosphorylation of putative Stat protein(s) does not support this hypothesis. However more extensive time courses must be performed along with examination of the activation states of other GH-stimulated signalling events during stimulation with hormone-antibody complexes, before definitive conclusions can be drawn in this area.

Figure 2 presents data using the FDCP-1 cell line transfected with the full length mouse GHR. This tissue culture system provides a very sensitive MTS-formazan colourimetric system for bioassay of somatogenic hormones. As indicated, pre-complexation of bGH with OA15 did not enhance the biological activity of the hormone, but similarly to the results described for GH action in F442A cells, there was no evidence for any inhibition of activity. In summary epitope mapping studies indicate that the binding site for the epitope defined by OA15 is removed from receptor interaction sites on the hormone.

This work was partly funded by the Scottish Office Agricultural, Environment and Fisheries Department (SOAFD).


Fig. 1. The effect of bGH (50ng/ml) ± OA15 Mab (1:100 dilution of hybridoma supernatant containing 0.6ng/ml total protein) on tyrosine phosphorylation of 91kDa Stat protein. The location of 91kDa Stat is arrowed.