THE EFFECTS OF MONOCLONAL ANTIBODIES AGAINST HUMAN GROWTH HORMONE ON HORMONE–RECEPTOR INTERACTIONS

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1. Introduction

Monoclonal antibodies, produced by somatic cell fusion [1] provide useful tools for (among other things) comparison of the structures of related molecules and for the study of structure–function relationships. Different monoclonal antibodies raised against a single protein antigen will often show specificity for different sites on that antigen; consequently some of these antibodies may interfere with biological activity (by blocking receptor-binding sites) whereas others may fail to do so (because they interact at some distance from the receptor-binding site).

A series of monoclonal antibodies to human growth hormone (hGH), only some of which cross-react with the structurally related hormone human placental lactogen, has been described [2,3]. Each of these monoclonal antibodies reacts with 1 of at least 4 different antigenic determinants on the hGH molecule. It was of interest therefore to determine whether these antibodies could interfere with the interaction between labelled hGH and receptors from rabbit liver or mammary gland. The mammary gland receptors are specific for lactogenic hormones such as prolactin and human (but not non-primate) growth hormone [4], whereas the liver receptors bind prolactins and growth hormones from several different species [5], but show a species specificity that does not accord well with the biological actions of the hormone [6,7]. These results show that 2 of the 4 monoclonal antibodies tested can block receptor binding almost completely, whereas the other 2 have a relatively small effect.

2. Methods

2.1. *Iodination of human growth hormone*

hGH was a gift from the National Institute of Biological Standards and Control (Hampstead) and was labelled with $Na^{125}I$ (obtained from the Radiochemical Centre, Amersham, Bucks) by the 'iodogen' method [8] as in [7]. ^{125}I-Labelled hormone and free $^{125}I^-$ were separated by chromatography on a column (0.78 cm^2 × 11–14 cm) of Sephadex G-50 (Fine grade). ^{125}I-Labelled hGH was rechromatographed before use on a column (0.78 cm^2 × 90–95 cm) of Sephadex G-100 (Superfine grade) equilibrated and eluted with Tris–HCl buffer (0.025 M Tris adjusted to pH 7.6 with HCl) containing 0.1% bovine serum albumin and 0.6 mM merthiolate, at 4°C.

2.2. *Preparation of membrane-bound receptors*

Membrane-bound receptors were prepared as a microsomal fraction from liver and from mammary gland of late-pregnant New Zealand White rabbits, by differential centrifugation of a crude homogenate, as in [7].

2.3. *Monoclonal antibodies to human growth hormone*

Antibodies from 4 hybridoma cell lines secreting mouse monoclonal IgG antibodies against hGH were used. These have been designated NA27, NA39, NA71 and QA68 and their preparation and characterization has been described [2,3].

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2.4. *Effect of antibodies on receptor binding*

When ^{125}I-labelled hGH was incubated with membrane-bound receptors from rabbit liver or mammary gland, a substantial proportion (20–40%, at the concentrations of receptors used) of the labelled hormone was bound specifically. The ability of antibodies to interfere with such binding was assessed by preincubating ^{125}I-labelled hormone with various concentrations of each of the 4 monoclonal antibodies.

All dilutions of tracer, antibody and receptors were carried out with 'Tris–HCl assay buffer' (0.025 M Tris–HCl (pH 7.6) containing 0.1% bovine serum albumin, 0.6 mM merthiolate and 10 mM $CaCl_2$). Antibody titration curves were set up in triplicate for each of the 4 monoclonal antibodies. 100 μl ^{125}I-Labelled hGH (~30 000 cpm) was added to 100 μl antibody (serially diluted over the appropriate range) and 200 μl 'Tris–HCl assay buffer'. Tubes were incubated at 20–25°C for 48 h or at 4°C for 72 h. 100 μl (200–300 μg protein) of receptor preparation (membrane-bound from rabbit liver or mammary gland) or buffer was then added and the tubes were reincubated overnight at 4°C. In those tubes containing antibody and receptor, receptor-bound tracer was precipitated by the addition of 3 ml ice-cold sodium acetate buffer (0.025 M sodium acetate (pH 5.4), containing 0.1% BSA). In those tubes containing antibody only, antibody-bound tracer was precipitated by the addition of 0.5 ml poly(ethylene glycol) mixture containing 25% (w/v) poly(ethylene glycol), 0.15% bovine γ-globulin and 0.05 M Tris–HCl (pH 8.5) [9]. In both cases free and bound hormone were separated by centrifugation (20 min, ~1000 × *g*, 4°C), supernatants were removed by aspiration and the precipitates were subjected to γ-counting in a Nuclear Chicago γ-radiation counter (model 1195); counting efficiency for ^{125}I was 50–55%.

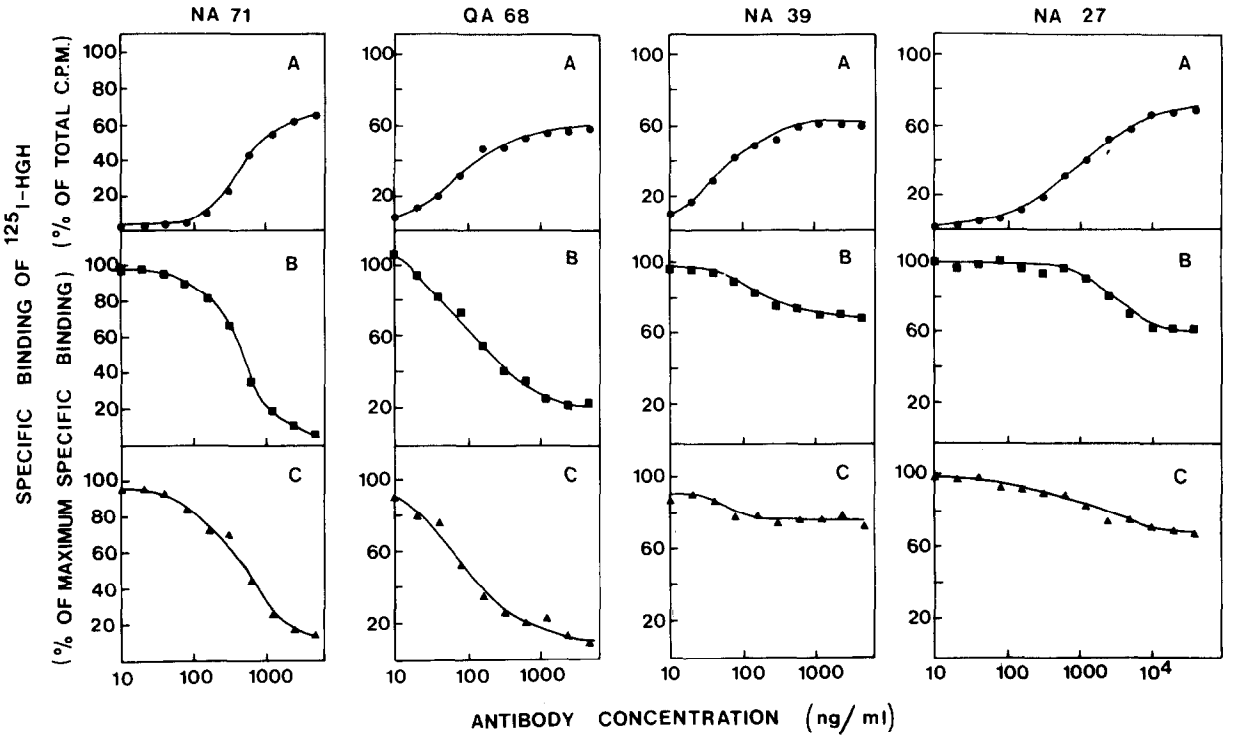

Fig.1. The effect of monoclonal antibodies on binding of ^{125}I-labelled human growth hormone to membrane-bound receptors from rabbit mammary gland and liver. ^{125}I-hGH (~30 000 cpm) was preincubated with various concentrations (based on freeze-dried material) of each antibody, for 48 h. Buffer (A) or liver receptors (B) or mammary gland receptors (C) were then added and incubation was continued for a further 24 h. Binding of ^{125}I-hGH to antibody (A) or receptors (B,C) was then determined as described in the text. Label 'bound' in the absence of receptor or antibody was considered 'non-specific'. Each value is the mean of triplicate determinations: (–●–) (A), specific binding to antibody (% of total cpm); (–■–) (B), specific binding to liver receptors (% of maximum specific binding); (–▲–) (C), specific binding to mammary gland receptors (% of maximum specific binding).

3. Results

Fig.1 shows the effect of preincubation with each of the monoclonal antibodies on the binding of ^{125}I-hGH to rabbit liver and mammary gland receptors. In the case of NA71 an increase in the amount of tracer bound to antibody caused a proportional decrease in the amount bound to receptor, until at the highest concentration of antibody, receptor binding was negligible. The concentration of antibody required to cause 50% inhibition of specific binding to receptors was close to the concentration which gave 50% of maximum specific binding to antibody in the absence of receptors. The effects of NA71 on the binding of ^{125}I-hGH to both types of receptor, rabbit liver and mammary gland, were very similar.

A similar result was obtained with the antibody QA68, although in this case binding to the antibody could not entirely prevent binding of ^{125}I-hGH to liver receptors. Even at the highest concentration of antibody, specific binding to these receptors did not fall below 20%.

A very different result was obtained with the antibodies NA27 and NA39. Fig.1 (NA27) shows that an increase in the proportion of antibody-bound tracer caused only a small decrease in receptor binding, and this appeared to level off at 60–70% of total specific binding. The effect of preincubation with NA27 on the binding of ^{125}I-hGH to both types of receptor was similar. The results obtained with the antibody NA39 (fig.1) were almost identical to those obtained with NA27. Again specific binding could not be reduced below 70% and the effect of the antibody on binding of hGH to liver and to mammary gland receptors was similar.

4. Discussion

hGH binding sites prepared from rabbit mammary gland are specifically lactogenic and are very different from the bulk of those in rabbit liver, which bind both lactogenic and somatogenic hormones [6,7]. Distinct structural regions within the hGH molecule may be involved in binding to the different types of receptor, in which case a monoclonal antibody might be able to block binding to one type of receptor without affecting binding to the other type. In the event, however, the effect of antibody on receptor binding was almost identical for liver and for mammary gland receptors, in the case of each monoclonal antibody tested. This suggests either that one site within the hGH molecule is responsible for binding to both types of receptor, or that if 2 sites are involved they are so close together that they are identically affected by antibody binding. This accords with the finding [10] that the determinants for both hepatic and mammary gland receptor binding are within the first 134 residues of the amino-terminal region of hGH.

According to their effects on receptor binding, the 4 monoclonal antibodies could be divided into 2 categories, i.e., those which could almost completely inhibit (NA71 and QA68), and those which could only partially inhibit (NA27 and NA39) receptor-binding. There are 3 possible mechanisms by which antibody may effect receptor binding:

(i) The antibody could bind to the same site as the receptor, thus blocking receptor binding.
(ii) The antibody could bind to a site close to that for the receptor, and block receptor binding by steric hindrance.
(iii) A decrease in affinity of the hormone for the receptor could be caused by binding of the antibody to a site which, although distinct from the receptor site, affects the conformation of the molecule. (A conformational effect could also explain the cross-reactivity in an antibody–antibody competition assay between antibodies NA27 and NA39 which bind to hGH-specific and hGH-human placental lactogen-shared determinants, respectively [3].)

Any of these mechanisms could explain the complete inhibition of receptor binding obtained with antibodies NA71 and QA68. The partial inhibition seen with NA27 and NA39 could be explained by mechanisms (ii) or (iii), but could also be explained by heterogeneity of receptors; if more than one type of binding site was present in a receptor population it might be possible for the antibody to block binding to one type of receptor without affecting binding to a second type.

An assumption made in interpreting these data is that binding of hormone to receptors is reversible. In practice this is largely the case, although complete dissociation of hormone–receptor complexes is difficult [11,12]. If the hormone–antibody complex could dissociate easily whereas binding to receptors was irreversible, it might be possible for receptors to effectively 'strip' labelled hormone from the hormone–antibody complex. In this case, the incomplete

inhibition of binding seen with antibodies NA39 and NA27 might be a consequence of binding of free (dissociated), rather than antibody-bound, hormone to receptors. In fact, however, dissociation of ^{125}I-hGH from antibody NA39 appears to be very slow (like dissociation from NA71) whereas dissociation from QA68 and (especially) NA27 is more rapid [3]; so if receptors could 'strip' ^{125}I-hGH from antibody this would be most likely to happen in the cases of antibodies NA27 and (to a lesser extent) QA68, and not NA39 and NA71.

Whatever the explanation of the results obtained, it is clear that the different monoclonal antibodies have different effects on hormone–receptor interactions, and this may eventually help to determine the detailed nature of such interactions. It seems likely from these results that the receptor-binding site must be larger than an antigenic determinant (epitope) since at least 2 monoclonal antibodies (QA68 and NA71) of non-overlapping combining site specificities [3] almost completely blocked receptor binding.

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