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# The calcitonin receptor on T 47D breast cancer cells

## Evidence for glycosylation

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The glycosyl nature of the receptor for the peptide hormone calcitonin has been investigated in a human breast cancer cell line, T 47D. Studies have been carried out to assess the ability of various lectins and of the antibiotic tunicamycin to inhibit specific binding of calcitonin to the cells, to reduce cross-linking of photoactive calcitonin to a macromolecular receptor component and to influence calcitonin stimulation of cyclic AMP. Pre-incubation of cells with low concentrations of tunicamycin for 72 h resulted in a reduction of total specific binding by approx. 80% and a 40% reduction in calcitonin-stimulated adenylate cyclase; formation of the cross-linked receptor component was also inhibited. Wheat-germ lectin showed the most marked inhibition of total specific binding and cyclic AMP production. However, cross-linking of photoactive calcitonin to receptor component was totally inhibited by this lectin. Soya-bean lectin brought about very little reduction in total specific binding but had more profound effects on calcitonin-stimulated cyclic AMP production and cross-linking of photoactive calcitonin. Concanavalin A and lentil lectin showed some inhibition of all parameters. The data indicate that the calcitonin receptor in T 47D cells is associated with glycosyl moieties, the major contributors of which are *N*-acetyl-D-glucosamine residues, but *N*-acetyl-D-galactosamine and mannose residues are also associated.

The human breast cancer cell line, T 47D, possesses specific receptors for the peptide hormone calcitonin and a calcitonin-responsive adenylate cyclase (Findlay *et al.*, 1980*a*,*b*). The receptors and responses in these cells and in certain other cancer cell lines display many characteristics expected of a physiological calcitonin receptor, in that structure–activity relationships are strictly maintained (Hunt *et al.*, 1977; Findlay *et al.*, 1980*a*,*b*), and specific hormone-induced desensitization is observed (Hunt *et al.*, 1977; Lamp *et al.*, 1981). Since the major physiological target of calcitonin action, the osteoclast, has not been isolated in sufficient quantities to permit biochemical study, the abundance of calcitonin receptors in T 47D cells has allowed for the first time attempts to isolate the calcitonin receptor. Previous studies have shown that an iodinated photoactive derivative of salmon calcitonin that retains full biological activity will form a covalently cross-linked complex with a receptor component on T 47D cells after photolysis. The apparent molecular weight of the complex is approx. 85000, as assessed by polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate (Moseley *et al.*, 1982).

In the present work tunicamycin has been used to block post-translational glycosylation of T 47D cell proteins. The effect of this and of lectins of various sugar specificities on binding of calcitonin, formation of receptor complex with photoactive calcitonin and calcitonin-stimulated cyclic AMP production in T 47D cells have been investigated.

### Materials and methods

T 47D cells were grown in monolayer culture as described previously (Findlay *et al.*, 1980*a*) in Roswell Park Memorial Institute 1640 (RPMI) culture medium with 10% foetal-calf serum (Flow Laboratories Australia Ltd.), 10ng of insulin/ml (Novo Laboratories) and 1mg of cortisol hemisuccinate/l. Synthetic salmon calcitonin (lot no.

K715031; 4700 units/mg) was a gift from Armour Pharmaceutical Co., Kankakee, IL, U.S.A. Wheat-germ lectin, soya-bean lectin, lentil lectin and concanavalin A were purchased from Pharmacia (South Seas), Melbourne, Victoria, Australia, and *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine, methyl D-mannopyranoside, 3-isobutyl-1-methylxanthine and leupeptin were from Calbiochem. Tunicamycin was a gift from Professor G. Tamura, Department of Agricultural Chemistry, University of Tokyo, Tokyo, Japan. Adenylate cyclase assay procedures were described previously (Hunt *et al.*, 1976). Preparation of photoactive synthetic salmon calcitonin was carried out as previously described (Moseley *et al.*, 1982), by attachment of *N*-(β-aminoethyl)-4-azido-2-nitroaniline to the γ-carboxyamide side chains of glutamine-14 by using guinea-pig liver transglutaminase (Gorman & Folk, 1980). Iodination of calcitonin preparations was carried out to specific radioactivities of 160–200 Ci/g and competitive binding assays were performed as described previously (Findlay *et al.*, 1980*a*,*b*). Photoaffinity labelling of T 47D cells was carried out in monolayer cultures in 25 $cm^2$ flasks (Costar). $^{125}$I-labelled photoactive calcitonin was incubated with T 47D cells in serum-free RPMI medium containing 0.1% bovine serum albumin for 1 h at room temperature, followed by exposure to ultraviolet light as described previously. Cells were solubilized with 0.1% (v/v) Triton X-100/0.05 M-Tris / 0.15 M-NaCl / 10 mM-EDTA / 10 mM-phenylmethanesulphonyl fluoride/0.6% (w/v) *N*-ethylmaleimide (pH 8.0), and extracts were resolved on polyacrylamide gels in sodium dodecyl sulphate-containing buffers by the method of Laemmli (1970). All these methods have been described in detail previously (Moseley *et al.*, 1982).

Competing lectins were added to incubations 10 min before the addition of calcitonin in all instances. Competing sugars were added to lectin solutions before addition to incubation medium. Freshly dissolved tunicamycin was added to culture medium and replaced at 24 h intervals during the incubation periods specified. Intact cell cyclic AMP was estimated as described previously (Lamp *et al.*, 1981) by using a protein binding assay (Brown *et al.*, 1971).

## Results

### *Effects of tunicamycin on calcitonin binding to T 47D cells*

T 47D cells were incubated with the antibiotic tunicamycin, which inhibits glycosylation of translated proteins (Olden *et al.*, 1978; Elbein, 1981). Fig. 1 shows the effects of tunicamycin treatment on the total specific binding of calcitonin. Incubation with tunicamycin (1 μg/ml) led to a reduction in binding over a 72 h period at which time binding was less than 20% that of untreated controls. The inhibition seen at 48 h was dose-dependent. During this period

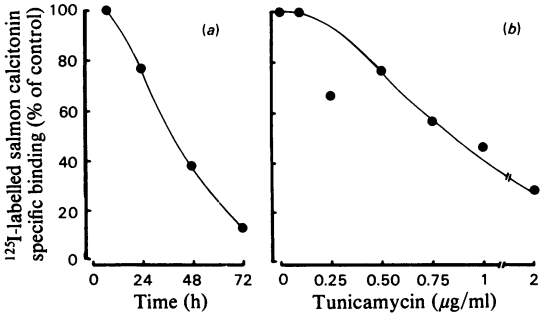


Fig. 1. *The effect of tunicamycin on binding of $^{125}$I-labelled salmon calcitonin to T 47D cells*

Confluent T 47D cells in 4.5 $cm^2$ multiwell tissue culture plates (approx. 500000 cells/well) were incubated in complete medium containing 1 μg of tunicamycin/ml for the times indicated (*a*) or with increasing concentrations of tunicamycin for 48 h (*b*). Specific binding of $^{125}$I-labelled salmon calcitonin (sp. radioactivity 180 μCi/g) was then assessed. Binding was carried out for 1 h at room temperature in 0.5 ml of RPMI 1640 medium supplemented with 0.1% (w/v) bovine serum albumin/well; 100000 c.p.m. of tracer were added to each well (Findlay *et al.*, 1980*a*,*b*). Non-specific binding was assessed in the presence of 2 μg of unlabelled salmon calcitonin/ml. Results are shown as specific binding to treated cells expressed as a percentage of that to untreated cells. Points are the means of triplicate estimations.

Table 1. *Effect of tunicamycin on protein synthesis and glycosylation in T 47D cells*

Confluent T 47D cells in 12-place multiwell plates were treated for 18 h with the indicated concentration of tunicamycin. In the continued presence of the drug cells were then assessed for incorporation of [$^3$H]glucosamine or $^{14}$C-labelled amino acids into trichloroacetic acid-insoluble material by the method of Housley *et al.* (1980). Trichloroacetic acid-precipitable material was collected on to Millipore filters (pore size 0.45 μm), which were then dissolved in 2 ml of tetrahydrofuran and then counted for radioactivity after the addition of 5 ml of Instagel.

| Tunicamycin (μg/ml) | Incorporation of [$^3$H]glucosamine (% of control) | Incorporation of $^{14}$C-labelled amino acids (% of control) |
|---|---|---|
| 0 | 100 | 100 |
| 0.1 | 87.3 | 97 |
| 0.25 | 77.6 | 82 |
| 0.5 | 34.5 | 79 |
| 0.75 | 36.6 | 81 |
| 1 | 39.6 | 74 |

the cells remained greater than 95% viable as assessed by Trypan Blue exclusion. Under conditions where incorporation of [$^3$H]glucosamine was decreased by greater than 60% of control, tunicamycin decreased protein synthesis by approx. 25% of control as assessed by incorporation of $^{14}$C-labelled amino acids into trichloroacetic acid-precipitable macromolecules (Table 1). Inclusion of leupeptin (at up to 150$\mu$g/ml) to inhibit non-specific effects that might result from the susceptibility of non-glycosylated protein to proteinases (Libby & Goldberg, 1978) had no effect on the observed inhibition of calcitonin binding. Scatchard analysis of binding in the absence and in the presence of tunicamycin revealed essentially parallel slopes with the tunicamycin line shifted to the left (Fig. 2).

*Effect of tunicamycin on generation of calcitonin–receptor covalent complex*

Treatment of T 47D cells for 72h with 1$\mu$g of tunicamycin/ml almost completely eliminated the ability of photoactive calcitonin to bind and/or cross-link to its 85000-mol.wt. component (Fig. 3). Fig. 3(*a*) shows the 85000-mol.wt. cross-linked

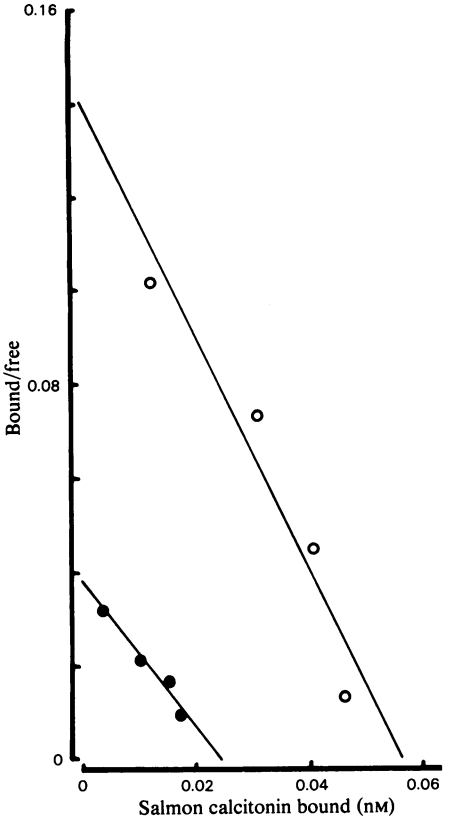


Fig. 2. *Scatchard plot of $^{125}$I-labelled salmon calcitonin binding to control and tunicamycin-treated cells*

Confluent T 47D cells in 4.5cm$^2$ multiwell tissue culture plates were incubated for 48h in RPMI 1640 medium containing 10% foetal-calf serum (○) or RPMI 1640 medium containing 10% foetal-calf serum and 1$\mu$g of tunicamycin/ml (●). Specific binding of $^{125}$I-labelled salmon calcitonin was then assessed as described in Fig. 1 in the presence of increasing concentrations of unlabelled salmon calcitonin. The standard errors of the data from which Scatchard plots were derived in no case exceeded 5% of the mean. In two separate experiments qualitatively similar results were obtained.

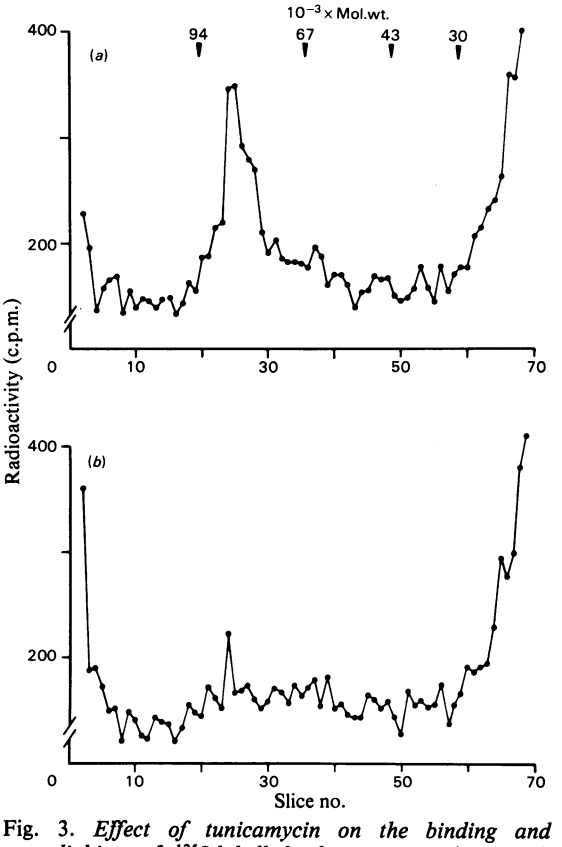


Fig. 3. *Effect of tunicamycin on the binding and cross-linking of $^{125}$I-labelled photoactive salmon calcitonin to receptor component in T 47D cells*

Profiles are shown of electrophoresis carried out on 10% (w/v) polyacrylamide gels in 0.1% (w/v) sodium dodecyl sulphate-containing buffers (Laemmli, 1970) of solubilized extracts of photoaffinity labelled T 47D cells. Photoaffinity labelling was carried out as described previously (Moseley *et al.*, 1982). Cells that had been grown for 72h in RPMI 1640 medium containing 10% (v/v) foetal-calf serum alone (*a*) or medium containing 2$\mu$g of tunicamycin/ml (*b*). Gels were sliced into 2 mm slices and counted for radioactivity in a spectrometer.

receptor component normally observed after photoaffinity labelling of T 47D cells with $^{125}$I-labelled photoactive salmon calcitonin. This cross-linked complex is calcitonin-specific and not observed when binding of photoactive salmon calcitonin is carried out in the presence of excess unlabelled calcitonin (Moseley *et al.*, 1982). In some experiments a small residual receptor peak remained after tunicamycin treatment for 72h as seen here (Fig. 3*b*) but in most experiments no such cross-linked component was recovered.

*Effect of tunicamycin on calcitonin activation of adenylate cyclase in T 47D cells*

Tunicamycin treatment of T 47D cells inhibited calcitonin activation of adenylate cyclase in a time- and dose-dependent fashion similar to the effects exerted on binding (Fig. 4). However, at 72h the effect of 1$\mu$g of tunicamycin/ml was to reduce activation by approx. 40% in contrast with the 80% reduction in binding capacity. Basal and guanosine 5'[$\alpha$,$\beta$-imido]diphosphate-stimulated levels of cyclase activity remained unchanged as did the response to prostaglandin $E_2$, an alternative agonist in these cells. These results suggest that it is unlikely that tunicamycin non-specifically interferes with membrane components.

*Inhibition of binding of calcitonin to T 47D cells by lectins*

Competitive binding assays were carried out after a 10min pre-incubation of T 47D cells with the different lectins before the addition of $^{125}$I-labelled salmon calcitonin (Fig. 5). Wheat-germ lectin, specific for *N*-acetyl-D-glucosamine, was most effective and brought about a dose-dependent inhibition of calcitonin binding at lectin concentrations between 0.5$\mu$g/ml and 100$\mu$g/ml. Maximal inhibition was never greater than approx. 45%, even at lectin concentrations as great as 1mg/ml (results not shown). Concanavalin A and lentil lectin, both specific for mannose residues, were less effective, but brought about some inhibition at concentrations from 1$\mu$g to 100$\mu$g of lectin/ml. Soya-bean lectin, specific for *N*-acetyl-D-galactosamine, showed virtually no inhibition of total specific binding. The effects of the lectins were only partially additive and total inhibition of binding was never observed. Scatchard analysis of $^{125}$I-labelled salmon calcitonin binding revealed a reduction in the slope in the presence of

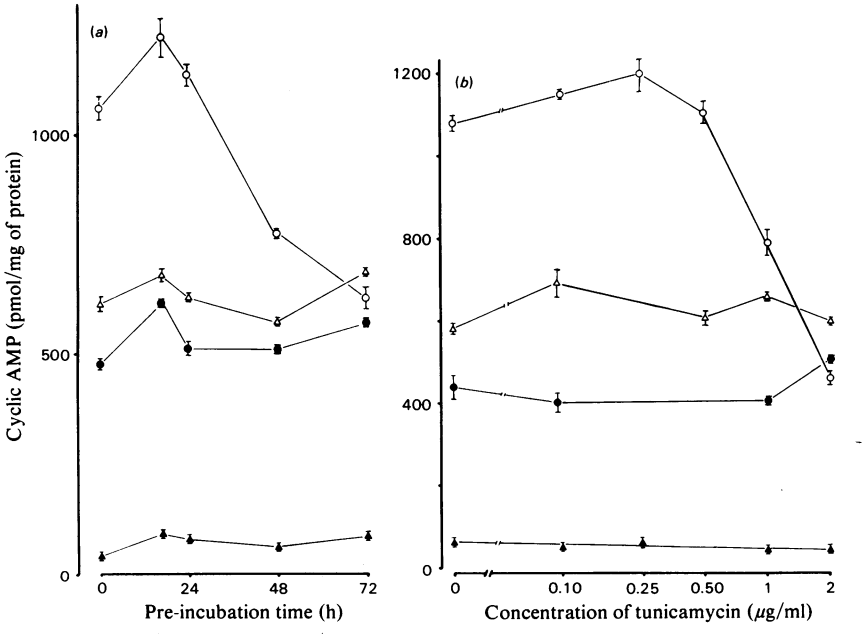


Fig. 4. *Adenylate cyclase response in tunicamycin-treated cells*

T 47D cells grown in 25cm$^2$ tissue culture bottles in complete medium were treated with 1$\mu$g of tunicamycin/ml for the times indicated (*a*) or with increasing concentrations of tunicamycin for 48h (*b*). Basal adenylate cyclase activity (▲) and that in response to 100ng of salmon calcitonin/ml (○), 0.1mM-guanosine 5'-[$\alpha$,$\beta$-imido]diphosphate (△) or 5$\mu$g of prostaglandin $E_2$/ml (●) were estimated as previously described (Hunt *et al.*, 1976) at the times and concentrations indicated. Activity is expressed as pmol of cyclic AMP produced/mg of protein during a 10 min incubation at 32°C. Points are means of triplicate estimations ± S.E.M. (indicated by the bars).

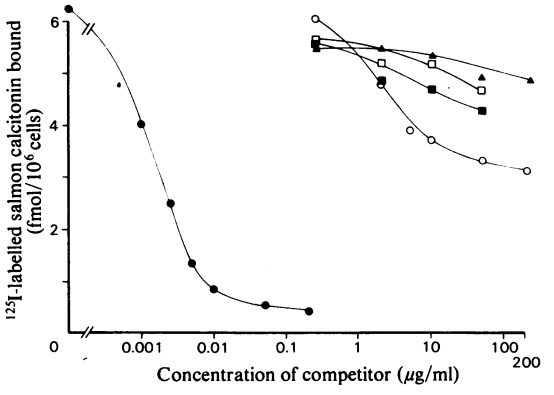


Fig. 5. *Competition by lectins for binding of $^{125}$I-labelled salmon calcitonin to T 47D cells*

T 47D cells in suspension (approx. $10^6$ cells/ml) in RPMI 1640 medium containing 0.1% bovine serum albumin were pre-incubated at 20°C for 10 min in the presence of increasing concentrations of unlabelled salmon calcitonin (●), wheat-germ lectin (○), concanavalin A (■), lentil lectin (□) or soya-bean lectin (▲). Incubation was continued for 1 h in the additional presence of $^{125}$I-labelled salmon calcitonin, after which total specific binding was assessed as previously described. Points are means of four determinations.

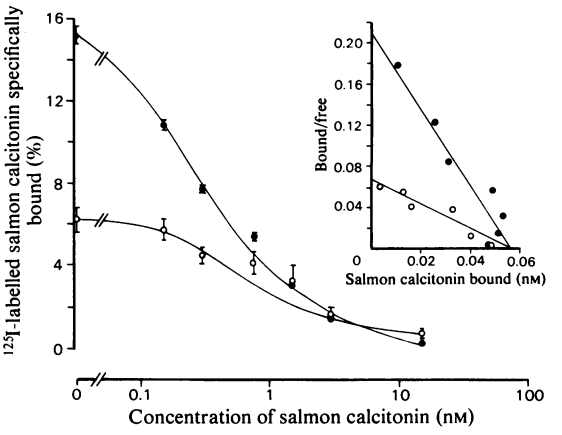


Fig. 6. *Binding of salmon calcitonin to T 47D cells in the absence (●) and in the presence (○) of wheat-germ lectin*

T 47D cells in suspension (approx. $10^6$ cells per ml) in RPMI 1640 medium containing 0.1% bovine serum albumin were pre-incubated at 20°C for 10 min in RPMI 1640 containing 0.1% bovine serum albumin alone (●) or with 100 μg of wheat-germ lectin/ml (○). Incubation was continued for 1 h in the additional presence of $^{125}$I-labelled salmon calcitonin and increasing concentrations of unlabelled salmon calcitonin, after which total specific binding was assessed. Results are shown as means of four determinations ± S.E.M. The insert shows a Scatchard analysis of the binding data illustrated in the main Figure.

wheat-germ lectin, indicating a reduction in the affinity of calcitonin for its receptor (Fig. 6). Calcitonin receptor number was unchanged in the presence of wheat-germ lectin. The effects of the other lectins on binding were too small to allow a similar comparison by Scatchard analysis.

When T 47D cells were incubated with wheat-germ lectin before binding and cross-linking of iodinated photoactive calcitonin, a roughly dose-dependent reduction in the peak height of the 85000-mol.wt. cross-linked component on polyacrylamide gels was seen. As we have noted previously (Moseley *et al.*, 1982), a non-specific peak of radioactivity corresponding to a mol.wt. of approx. 65000–68000 was seen in some experiments. A marked inhibition of the specific peak was seen at 2.5 μg of wheat-germ lectin/ml and at 50 μg/ml some component was still observed. However, as seen in Fig. 7, 100 μg of wheat-germ lectin/ml totally eliminated the ability to recover the cross-linked component. The effect of pre-incubation with 100 μg of soya-bean lectin/ml brought about an approx. 50% decrease in peak height, a consistent and noteworthy observation in the light of the relative lack of effect of this lectin on total binding (Fig. 5). The gel profiles showed no evidence that any of the lectins directly bound photoactive calcitonin. Specificity of the wheat-germ lectin binding is shown in Fig. 8, in which wheat-germ lectin (50 μg/ml) reduced the amount of radioactivity in the observed peak, but prior incubation of the lectin with its specific ligand *N*-acetyl-D-glucosamine eliminated the competition. *N*-Acetyl-D-galactosamine similarly eliminated the competition by soya-bean lectin, whereas mannose eliminated the effects of concanavalin A and lentil lectin (results not shown). Each of the latter two sugars showed very little competition for the cross-linked component. None of the sugars prevented competition except by its specific lectin.

### *Inhibition of total cyclic AMP production in intact T 47D cells*

The ability of the lectins to inhibit calcitonin-induced production of cyclic AMP in intact cells in the presence of 3-isobutyl-1-methylxanthine (150 nM) is shown in Fig. 9. Wheat-germ lectin (50 μg/ml) had the most marked effect, but at low calcitonin concentrations soya-bean lectin was almost as effective as wheat-germ lectin, again in contrast with the effects seen on total binding. Concanavalin A and lentil lectin both produced some inhibition of the response. Concentrations of

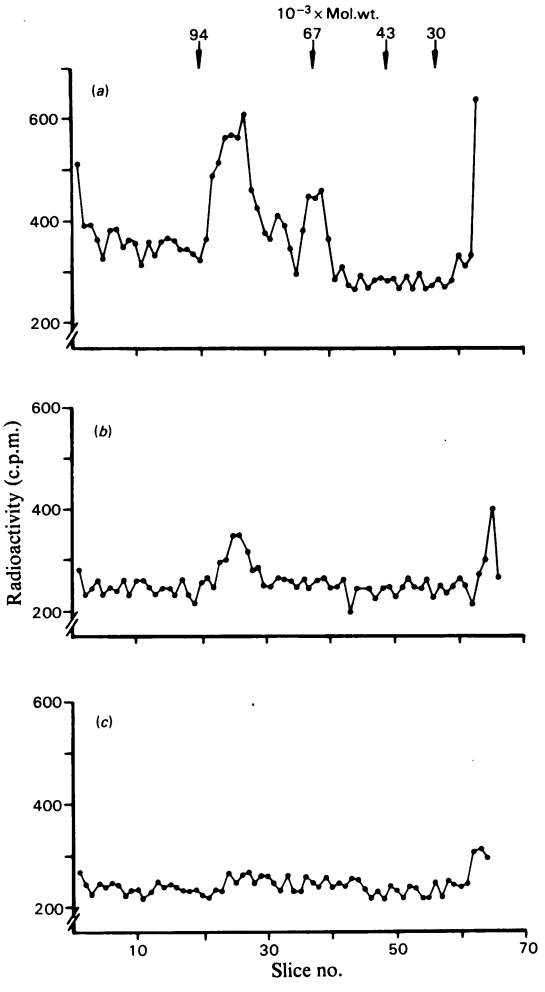


Fig. 7. *Comparison of the inhibition by wheat-germ lectin and soya-bean lectin of binding and cross-linking of $^{125}$I-labelled photoactive salmon calcitonin to receptor component*

Profiles of electrophoresis (carried out as described in the legend to Fig. 3) are shown of solubilized extracts from T 47D cells that had been pre-incubated for 10min at room temperature in the presence of RPMI 1640 medium containing 0.1% (w/v) bovine serum albumin alone (*a*), in the presence of 100$\mu$g of soya-bean lectin/ml (*b*), or in the presence of 100$\mu$g of wheat-germ lectin/ml (*c*). Photoaffinity labelling was then performed as described previously.

lectin above 50$\mu$g/ml showed no further inhibition of cyclic AMP production. None of the lectins showed independent effects on cyclic AMP stimulation and basal cyclic AMP levels were unaltered in the presence of lectin alone.

## Discussion

As techniques for isolation and characterization of cell-surface receptors improve, information is accumulating to support the contention that many cell-surface determinants are glycoprotein in nature. The use of lectins has demonstrated the glycoprotein nature of the insulin (Harrison & Itin, 1980; Saviolakis *et al.*, 1981), epidermal growth factor (Moriarty & Savage, 1980) and gonadotropin receptors (Azhar & Menon, 1981) and affinity procedures using lectins have been exploited for receptor purification (Saviolakis *et al.*, 1981). The use of tunicamycin has been largely directed at the study of generalized changes in patterns of cell-surface glycoproteins, but it is now becoming a useful tool in the study of more specific glycoproteins (Merlie *et al.*, 1982).

The data indicate that glycosyl moieties are associated with calcitonin receptors on T 47D cells. Tunicamycin treatment of T 47D cells resulted in a partial inhibition of calcitonin-induced activation of adenylate cyclase, though the effects on total specific binding and cross-linking of photoactive calcitonin were significantly greater. The discrepancies in the degree of inhibition of these parameters suggest that significant adenylate cyclase activation can be brought about by only a small proportion of the total receptors able to bind hormone as previously suggested (Lamp *et al.*, 1981). An alternative explanation is that non-glycosylated receptors might participate in the adenylate cyclase response.

Inhibition of receptor glycosylation by tunicamycin could have two indirect effects. First, non-glycosylated receptor may not assemble correctly for insertion into the plasma membrane. Tunicamycin has been shown to prevent assembly of the subunits of the acetylcholine receptor in mouse muscle cells (Merlie *et al.*, 1982). Secondly, protein lacking its glycosyl complement may be rendered more susceptible to proteolytic degradation (Olden *et al.*, 1981). The first possibility cannot be excluded. However, the second possibility is unlikely since the proteolysis inhibitor leupeptin did not modify the effects of tunicamycin. Further, since tunicamycin treatment did not alter either basal adenylate cyclase levels or the response to an alternative agonist in these cells, non-specific effects of the drug due to general membrane perturbation are unlikely. Scatchard analysis of binding in the absence and in the presence of tunicamycin showed a parallel shift indicating a loss of receptors without alteration in the affinity of those remaining.

As with tunicamycin treatment, pre-incubation of cells with the lectins revealed differential effects on the parameters studied. The partial inhibition of total specific binding of calcitonin observed with each lectin suggested a lack of homogeneity in the distribution of glycosyl moieties associated with the

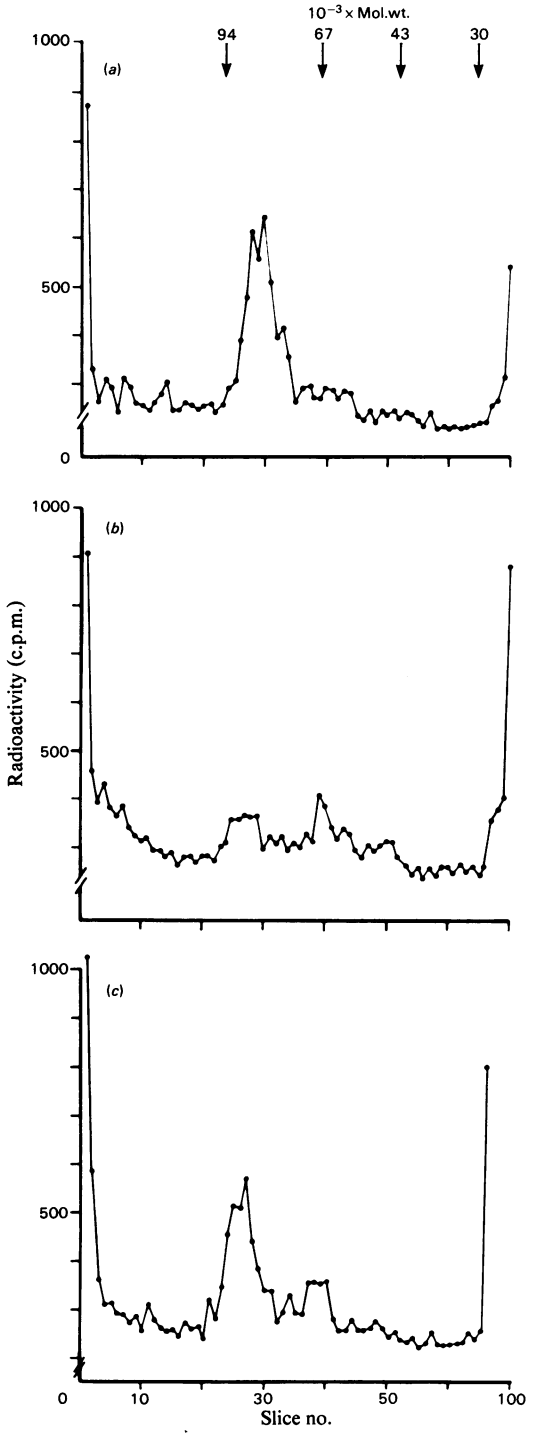


Fig. 8. *Specificity of inhibition by wheat-germ lectin of binding and cross-linking of $^{125}$I-labelled photoactive calcitonin to receptor component*

Profiles of electrophoresis, as described in the legends to Figs. 3 and 7, of solubilized extracts from T 47D cells pre-incubated for 10min at room temperature in the presence of RPMI 1640 medium containing 0.1% (w/v) bovine serum albumin alone (*a*), in the presence of 50$\mu$g of wheat-germ lectin/ml (*b*) or in the presence of 50$\mu$g of wheat-germ lectin/ml and 10mM-*N*-acetylglucosamine (*c*).

receptors. Wheat-germ lectin was the most effective inhibitor of binding and cyclic AMP production. However, this lectin only partly inhibited total binding and cyclic AMP production even at high doses, at which formation of the cross-linked receptor component was totally abolished. Similarly soya-bean lectin, which had only a small effect on total binding, was a more effective inhibitor of both cyclic AMP production and the cross-linking of photoactive calcitonin to receptor component. This again is indicative that only a proportion of the receptors that bind calcitonin activate the cell. A correlation between binding to receptors rich in *N*-acetyl-D-glucosamine and *N*-acetyl-D-galactos-

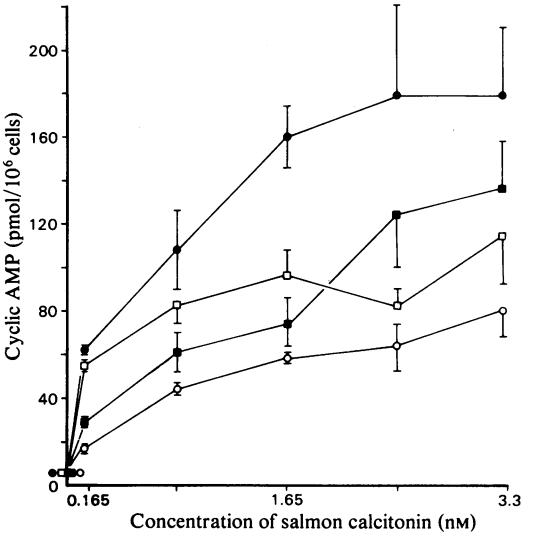


Fig. 9. *Inhibition by lectins of salmon calcitonin stimulation of cyclic AMP production in intact T 47D cells*

T 47D cells were suspended in RPMI 1640 medium containing 0.1% (w/v) bovine serum albumin and pre-incubated at 37°C for 10min in medium alone (●), 50$\mu$g of soya-bean lectin/ml (■), 50$\mu$g of concanavalin A/ml (□) or 50$\mu$g of wheat-germ lectin/ml (○). Cells from each pre-incubation group were then incubated for a further 10min with increasing concentrations of salmon calcitonin. Incubation was terminated by boiling and cell cyclic AMP was measured as described elsewhere (Brown *et al.*, 1971; Lamp *et al.*, 1981). Points are means of triplicate determinations ± S.E.M. (indicated by the bars).

amine and activation of cellular effectors could therefore be inferred.

Kinetic analyses of calcitonin binding to its receptor on T 47D cells indicate only a single class of receptor, although we have already drawn attention to the serious limitations of mathematical analysis of such binding data (Lamp *et al.*, 1981), as have others (Klotz, 1982). However, with these reservations, Scatchard analysis indicated that wheat-germ lectin reduced the affinity of the receptor for calcitonin and that receptor number was unaltered. The possibility thus remains that some of these observations could result from steric hindrance by lectin bound adjacent to the calcitonin-receptor binding site.

An alternative explanation to the presence of receptor subclasses is that the distribution of glycosyl moieties on each receptor is not homogeneous and some may exert their influence on receptor conformation. The effects of lectins may then manifest themselves according to the position of the appropriate sugar on the receptor. Wheat-germ lectin may produce its effects largely by sterically inhibiting calcitonin binding at the binding site. Soya-bean lectin may bind at a site distant to the calcitonin-binding site and thus be relatively ineffectual in its ability to block calcitonin binding as we have observed. However, it may change the conformation of the receptor in such a way as to alter the coupling between receptor and adenylate cyclase, which would explain the relatively greater inhibition of calcitonin-stimulated cyclic AMP production by this lectin. The region of the receptor to which photoactive calcitonin cross-links may also be influenced by adjacent sugar molecules but not necessarily those that influence binding and activation.

Lectins have been shown to mimic insulin action (Beachy *et al.*, 1981; Smith & Lui, 1981) by directly binding to the insulin receptor itself (Harrison & Itin, 1980; Saviolakis *et al.*, 1981) or indirectly by causing clustering of cell-surface glycoproteins, bringing about artificial clustering of receptor molecules (Neely *et al.*, 1976; Herzberg *et al.*, 1980). Microclustering of insulin receptors occurs normally when insulin binds to its receptor (Sandra *et al.*, 1979). However, lectin 'mimicking' of calcitonin action would tend to increase cyclic AMP levels and in these experiments basal cyclic AMP levels remained unaltered in the presence of lectins.

The evidence presented here indicates that the calcitonin receptor on T 47D cells has associated glycosyl moieties that can exert their influence on calcitonin binding and activation of the cell. Some subdivision of receptors may exist, the functional significance of which has yet to be clarified. However, the data are consistent with the existence of 'spare' receptors, as postulated previously (Lamp *et al.*, 1981), and these may possibly be defined through modifications in their associated sugars around the binding site.

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