J. Biochem. **143,** 813–820 (2008)
doi:10.1093/jb/mvn029

Affinity Modulation of Human Placental Insulin and Insulin-Like Growth Factor Receptors by Lectins

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Received February 14, 2008; accepted February 25, 2008; published online March 3, 2008

The ability of plant lectins to modify the interactions of the insulin receptor (IR) and insulin-like growth factor (IGF) receptors (IGFRs) with their ligands was investigated. The lectins profoundly affected the competition-binding curves for ^{125}I-labelled IGF-I and insulin, causing an increase in the affinity of placental IGF1R and IR towards their ligands. This increment was of such a magnitude that it could affect the receptors' specificity towards these ligands. The lower the ligand concentration, the greater was the lectin-induced affinity shift, which suggests potential physiological significance of the effect. The affinity modulation occurred in a lectin-specific and dose-dependent manner. In contrast to IGF1R and IR, the binding of ^{125}I-labelled IGF-II to its receptors resisted lectin modulation. Here we provide evidence of the possibility of external modulation of the affinity of placental IGF1R and IR *via* interactions of the receptors' carbohydrate moieties with lectins. The existence of modulators that would selectively inhibit or enhance the binding of IGFs or insulin to their corresponding receptors may have important implications for placental cell responses to these molecules.

Key words: IGF1R, IR, WGA, Con A, PHA.

Abbreviations: IGF, insulin-like growth factors; IGF1R, type 1 IGF receptor; IR, insulin receptor; IGF2R, type 2 IGF receptor; WGA, wheat germ agglutinin; Con A, concanavalin A; PHA, phytohaemagglutinin.

Insulin-like growth factors I and II (IGF-I and -II) are polypeptides that are essential for normal fetal and postnatal growth and development (*1*, *2*). Both IGF molecules act through the type 1 IGF receptor (IGF1R) (*2*), but its affinity for IGF-I has been reported to be several times higher than that for IGF-II (*3*). Moreover, IGF-I and IGF-II seem to utilize different molecular mechanisms to bind to the same ligand-binding site of the receptor (*4*). IGF1R is a transmembrane Tyr kinase, which is highly homologous to the insulin receptor (IR) (*5*). Insulin and IGF-I can bind to each other's receptors, although with a 100-fold lower affinity than that for the cognate receptor (*6*). IR exists in two isoforms (IR-A and IR-B) generated by alternative splicing of the IR gene (*7*). IR-A appears to be a high-affinity physiological receptor for IGF-II in fetal and some cancer cells (*8*). Individual αβ subunits from IR and IGF1R can combine to form hybrid receptors (*9*). Type 2 IGF receptor (IGF2R) is structurally dissimilar to IGF1R and IR and has no Tyr kinase activity. It binds IGF-II with a 100-fold higher affinity than that for IGF-I and, according to the available literature, it does not recognize insulin (*10*).

The hIR has 18 potential sites for *N*-glycosylation, of which 16 have been confirmed as glycosylated. The α-subunit, which binds insulin, contains 14 potential *N*-linked glycosylation sites (*11*). Both subunits of this receptor are heavily glycosylated such that 22% of its molecular mass is composed of carbohydrate (*12*). The roles of the saccharide chains in the functions and metabolism of the IR are not yet elucidated. Multiple potential sites for *N*-linked glycosylation (*16*) are also found in the hIGF1R molecule (*5*). IGF2R has 19 potential *N*-glycosylation sites of which at least two are utilized (*10*).

Using similar mechanisms, IGF1R and IR activate common intra-cellular pathways. However, it is an open question where the signalling specificity is or how highly homologous ligands contribute distinct signals to common downstream components (*13*). It has been proposed that other factors could affect signalling, including the time course of stimulation of a cell with different ligands, possible differences in the association or dissociation rates of the ligands and the internalization kinetics of the receptor (*14*). One question that has not been frequently addressed is the possibility that other molecules can influence the binding kinetics of the receptors (*15*, *16*).

During the partial purification of IGF1Rs, IGF2Rs and IRs from detergent extracts of human placental cell membranes using five different lectins immobilized on agarose, we observed that the specific binding of the ligand–receptor complexes depended both on the lectin and on the ^{125}I-labelled ligand used (*17*). In the present work, we examined the possibility that the interactions of oligosaccharide chains of IRs and IGFRs with plant lectins affect their reactivity towards homologous and heterologous ligands.

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MATERIALS AND METHODS

Materials—Porcine insulin was from Novo Nordisk (Bagsværd, Denmark). Human IGF-I and IGF-II were from GroPep Pty Ltd (Adelaide, Australia). HEPES, polyethylene glycol 8,000, bovine IgG (technical grade), BSA and Triton X-100 were purchased from Sigma–Aldrich Chemie (Steinheim, Germany). Na^{125}I for iodination was purchased from Isotope (Budapest, Hungary). Wheat germ agglutinin (WGA) was from Vector Laboratories (Burlingame, CA, USA). Concanavalin A (Con A) was from Amersham Biosciences (Little Chalfont, UK). Phytohaemagglutinin (PHA, a mixture of E-PHA and L-PHA) was isolated in our laboratory from *Phaseolus vulgaris* according to established protocols (*18*).

Preparation of ^{125}I-Labelled Ligands—^{125}I-labelled IGF-I, IGF-II and insulin (tracers) were prepared using the chloramine T method (*19*). Iodination was performed every 3 weeks and resulted in specific activities of $1–2 \times 10^8$ c.p.m./μg.

Binding Assays—Human placental tissue ($n = 5$) was obtained from uncomplicated pregnancies at term, according to protocols approved by the local ethical committee. Solubilized placental membranes were prepared essentially as described previously (*17*). The protein concentration in solubilized membranes was determined by the method of Bradford (*20*).

Lectins were dissolved according to the manufacturer's instructions to give stock solutions of final concentration 5 mg/ml. WGA was dissolved in 10 mM HEPES-buffered saline, pH 7.5; Con A in 50 mM phosphate-buffered saline, pH 6.5 and PHA in 50 mM phosphate-buffered saline, pH 7.5. The latter buffer containing 0.25% BSA was used to prepare serial lectin dilutions (ranging from 0 to 2,310 nmol/l for WGA, from 0 to 943 nmol/l for Con A and from 0 to 870 nmol/l for PHA) and serial ligand dilutions (ranging from 0 to 346 nmol/l IGF-I, IGF-II or insulin).

The binding assays were performed with solubilized membranes that were diluted in assay buffer (50 mM HEPES-buffered saline, pH 7.5) to give a membrane protein concentration of 1 mg/ml. The first set of assays employed solubilized membranes, ^{125}I-labelled ligands and increasing concentrations of plant lectins. Solubilized membranes (100 μg of membrane protein per tube) were incubated with 0.1 pmol of ^{125}I-labelled IGF-I, IGF-II or insulin (10^5 c.p.m/0.1 ml) and 0.1 ml of plant lectin solution in a fixed reaction volume of 0.5 ml containing assay buffer with BSA (final concentration 1.2%) at 4°C for 20 h.

After incubation, receptor–radioligand complexes were precipitated by the addition of bovine IgG (final concentration 0.05%) and 1.5 ml of polyethylene glycol solution (20% in 50 mM phosphate-buffered saline, pH 7.5) to each tube. The tubes were vortexed, centrifuged (4,500*g* for 45 min) and the supernatants were aspirated off. Precipitated ^{125}I-labelled ligand radioactivity was measured in an automatic gamma counter (1470 Wallac WIZARD, PerkinElmer, USA). Non-specific binding was measured in replicate reaction tubes that contained all reactants except the solubilized membranes. Maximal binding (B_0), the quantity of ^{125}I-labelled ligand bound to the receptors in the absence of lectin, was expressed as the percentage of total available ^{125}I-labelled ligand concentration (T). Specific binding in the presence of each lectin (B) was expressed as the percentage of that in its absence ($B/B_0 \times 100$).

In the competitive binding assays, the solubilized membranes (100 μg of membrane protein per tube) were incubated with ^{125}I-labelled ligands (10^5 c.p.m/0.1 ml) together with increasing concentrations of unlabelled ligands (competitors): IGF-I, IGF-II or insulin (in the range of 0 to 346 nmol/l). Parallel sets of tubes were run in which a fixed amount of different lectins was added to give a final lectin concentration of 20 μg/ml (92, 38 and 35 nmol/l for WGA, Con A and PHA, respectively). Reaction volumes in all tubes were adjusted to 0.5 ml using assay buffer with BSA (at a final concentration of 1.2%). The experiments were repeated three times in triplicate.

Maximal binding was the quantity of tracer bound in the absence of unlabelled ligand and lectin (B_0). In each experiment, specific binding was corrected for non-specific binding. Specific binding for each concentration of the unlabelled ligands (B) was expressed as the percentage of maximal binding ($B/B_0 \times 100$). These values were calculated separately for the tubes with and without lectins present. The data were plotted as a function of competitor concentration versus B/B_0.

Data Analysis—Sigmoidal (dose response, 4 parameter) regression analysis was performed on the data to determine IC_{50}, the concentration of unlabelled ligand required to inhibit the binding of ^{125}I-labelled ligand by 50%, both for the assays run in the absence and in the presence of the lectins, WGA, Con A and PHA.

All curves were created and fitted with the use of Origin Pro Version 7.5 (Origin Lab, Northampton, MA, USA). SPSS 10 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The B/B_0 data from the competitive binding assays were subjected to one-way ANOVA (with plant lectin as the independent variable) followed by the Least Significant Difference test. $P < 0.05$ was considered significant.

RESULTS

The ability of lectins to modify the specific binding of ^{125}I-labelled IGFs and insulin to their receptors was tested and the competition between ligand and lectin binding to the receptors on the solubilized membrane samples was also analyzed. The tracer quantities of the ^{125}I-labelled ligands corresponded to physiological concentrations of IGFs and insulin, thus ensuring that each ligand preferentially bound to its cognate receptor (*17*).

The interactions of ^{125}I-labelled ligands with their receptors from the solubilized membranes were examined in the presence of various concentrations of lectins. The data obtained were grouped according to the ^{125}I-labelled ligand used and are presented in Fig. 1, A–C.

Maximal binding was $9.6 \pm 1.0\%$ (^{125}I-labelled IGF-I), $10.1 \pm 1.2\%$ (^{125}I-labelled IGF-II) and $15.4 \pm 0.9\%$ (^{125}I-labelled insulin). The experiments resulted in lectin dose-dependent binding curves for ^{125}I-labelled IGF-I (Fig. 1A) and ^{125}I-labelled insulin (Fig. 1C). The shape of the ^{125}I-labelled IGF-II-binding curves (Fig. 1B) differed from those obtained with the other two tracers. They were predominantly flat and did not reflect great

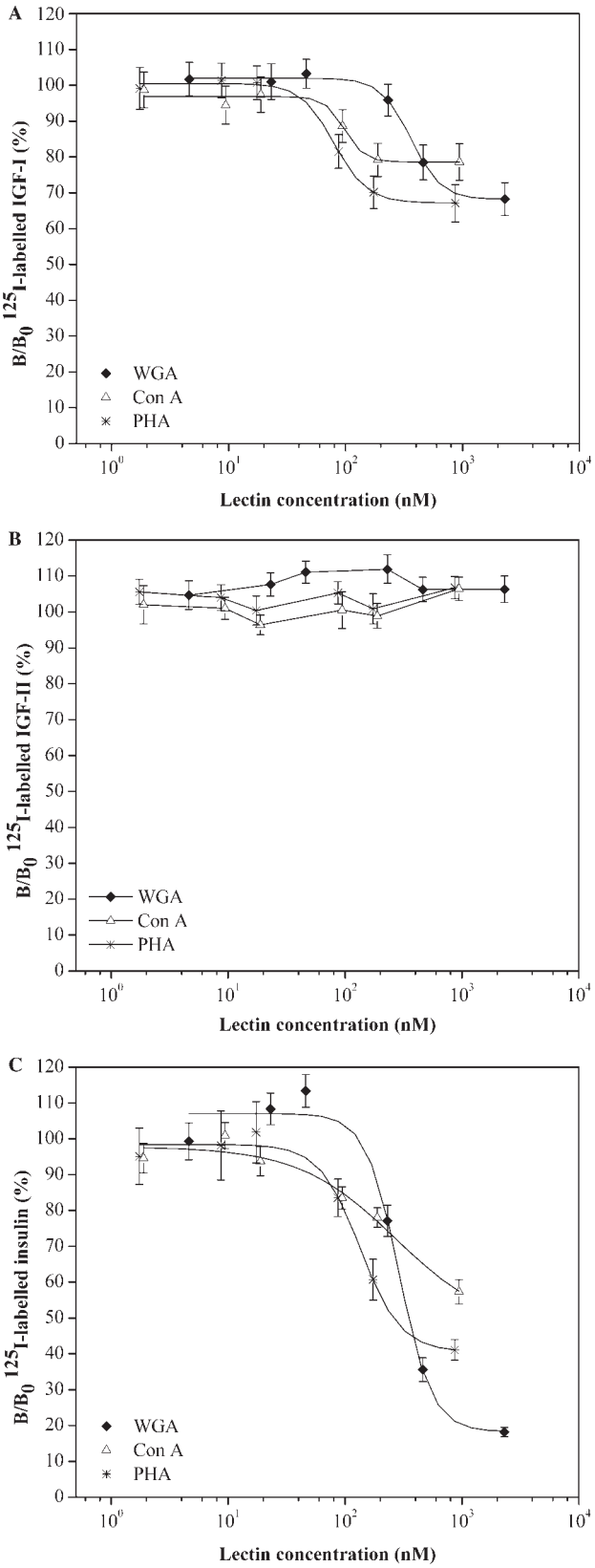

Fig. 1. **Effect of lectins on binding of ^{125}I-labelled IGFs and ^{125}I-labelled insulin to solubilized placental membranes.** Binding of ^{125}I-labelled IGF-I (A), ^{125}I-labelled IGF-II (B) and ^{125}I-labelled insulin (C) to solubilized placental membranes was measured in the absence or in the presence of increasing concentrations of lectins: WGA, Con A and PHA as described in the MATERIALS AND METHODS section. The means ± SD of three independent experiments, performed in triplicate, are shown. Specific binding in the presence of each lectin is expressed as a percentage of that in its absence. Maximal binding was 9.6 ± 1.0 (^{125}I-labelled IGF-I), 10.1 ± 1.2 (^{125}I-labelled IGF-II) and 15.4 ± 0.9 (^{125}I-labelled insulin) of the total radioactivity.

changes in tracer binding as a function of lectin concentration.

The binding curves obtained for ^{125}I-labelled IGF-I and insulin were similar in shape and mostly inhibitory throughout the lectin concentration range used. The most pronounced decrease of B/B_0 value occurred when ^{125}I-labelled insulin and WGA were incubated with the solubilized membranes. The decline of B/B_0 to 18.2 ± 1.3% (Fig. 1C) resembled the degree of displacement of ^{125}I-labelled insulin bound to placental IRs by unlabelled insulin (Fig. 4C). Con A and PHA demonstrated a weaker inhibitory effect on ^{125}I-labelled insulin–IR interaction, compared to that of WGA (B/B_0 decreasing to 57.3 ± 3.4% and 41.1 ± 2.9% for Con A and PHA, respectively) at the greatest lectin concentration used. The effect seemed to be receptor specific, as the same lectins exhibited a weaker inhibitory effect on ^{125}I-labelled IGF-I binding to its receptor (Fig. 1A). Thus, WGA and PHA lowered the specific binding of ^{125}I-labelled IGF-I to 68.2 ± 4.6% and 67.1 ± 5.2 of the initial value, respectively, whereas the specific binding of this tracer remained 78.6 ± 5.1% in the presence of the maximally effective Con A concentration. In contrast to the other labelled ligands, ^{125}I-labelled IGF-II binding to its receptors resisted the lectin modulation (Fig. 1B).

In the second set of binding assays, the competition binding curves for ^{125}I-labelled IGF-I, IGF-II and insulin were examined in the presence of a fixed lectin concentration. The final lectin concentrations of 92, 38 and 35 nmol/l for WGA, Con A and PHA, respectively, were chosen as they initiated a significant inhibition of the ^{125}I-labelled ligand-receptor binding, as shown in Fig. 1A and C. The aim of these experiments was to examine whether lectins influence the affinity of receptors for the ligands, *i.e.* whether they change IC_{50} values. The results obtained were grouped according to the ^{125}I-labelled ligand used and are presented in Figs 2–4. IC_{50} values, obtained from three independent experiments done in triplicate, were calculated and subjected to statistical analysis. The results are given in Table 1.

The presence of lectins affected almost all competition curves. Thus, the competition curves obtained with ^{125}I-labelled IGF-I and insulin were shifted towards lower B/B_0 values (Figs 2 and 4). In contrast, the ^{125}I-labelled IGF-II-binding curves were either unchanged or shifted towards greater B/B_0 values in the presence of lectins, the only exception being the combination of ^{125}I-labelled IGF-II/insulin and PHA (Fig. 3C).

The homologous competition curves obtained with ^{125}I-labelled IGF-I were altered in the presence of WGA and, to a lesser extent, PHA, whereas Con A had no significant effect (Fig. 2A). Compared with the control experiment (no lectins present), IC_{50} values obtained in

Table 1. **Effects of lectins on the displacement of ^{125}I-labelled ligand binding to solubilized placental cell membranes.**

^{125}I-labelled ligand	IC_{50} (pM)		
	IGF-I	IGF-II	Insulin
^{125}I-labelled -IGF-I			
No lectins	187 ± 9	573 ± 76	99,960 ± 11,451
+ WGA	65 ± 7[a]	209 ± 4[a]	55,130 ± 11,998[a]
+ Con A	200 ± 9[c]	327 ± 47[a]	46,273 ± 4,126[a]
+ PHA	140 ± 12[a]	300 ± 53[a]	9,120 ± 964[a]
^{125}I-labelled IGF-II			
No lectins	1,672 ± 88	2,163 ± 176	ND
+ WGA	3,683 ± 300[b]	1,969 ± 109[c]	ND
+ Con A	6,775 ± 1,428[a]	9,182 ± 93[a]	ND
+ PHA	1,898 ± 98[c]	1,813 ± 198[c]	ND
^{125}I-labelled insulin			
No lectins	ND	8,720 ± 673	175 ± 8
+ WGA	3,744 ± 1,002	492 ± 68[a]	21 ± 2[a]
+ Con A	ND	4,552 ± 545[a]	131 ± 5[a]
+ PHA	1,1091 ± 1,656	1,509 ± 36[a]	61 ± 2[a]

These data represent a composite of three independent experiments performed as described under MATERIALS AND METHODS section. Each value is the mean ± SD. [a]Significant at $P < 0.001$. [b]Significant at $P < 0.05$. [c]Not significant, as compared to control binding (no lectins). ND, not determined.

the presence of WGA and PHA showed a significant decrease (Table 1). All three lectins provoked a significant change in the IC_{50} values obtained for the heterologous pair ^{125}I-labelled IGF-I/IGF-II. WGA was the most effective (Fig. 2B) producing an IC_{50} value very similar to the control IC_{50} obtained for the homologous competition pair ^{125}I-labelled IGF-I/IGF-I (Table 1). As for the heterologous pair ^{125}I-labelled IGF-I/insulin, PHA most profoundly affected the interaction between the two (Fig. 2C), causing an 11-fold drop in the corresponding IC_{50} compared to the control value (Table 1).

The homologous competition curves obtained with ^{125}I-labelled IGF-II/IGF-II were noticeably changed only by Con A (Fig. 3B). This lectin caused a 4-fold decrease in the receptors' affinity. The heterologous competition curves for the pair ^{125}I-labelled IGF-II/IGF-I changed in the presence of WGA and Con A, but not with PHA (Fig. 3A, Table 1). The ^{125}I-labelled IGF-II/insulin competition curve shifted downwards in the presence of PHA, whereas those obtained in the presence of the other two lectins and the control curve overlapped (Fig. 3C). IC_{50} values for these interactions were never reached.

As for the heterologous competition curves obtained with ^{125}I-labelled insulin, all three lectins potently influenced the affinity of tracer binding, with both IGF molecules following a similar pattern: WGA (most influential) > Con A > PHA (Fig. 4A and B). At a concentration of 100 times the concentration of ^{125}I-labelled insulin, IGF-I was not able to displace the tracer bound to IR to half-maximal binding (Table 1), except in the presence of WGA and PHA. IGF-II more effectively bound to IRs in the presence of lectins, especially WGA, which caused an 18-fold shift in the IC_{50} value.

Finally, of all lectins tested in the homologous competition between ^{125}I-labelled insulin and unlabelled

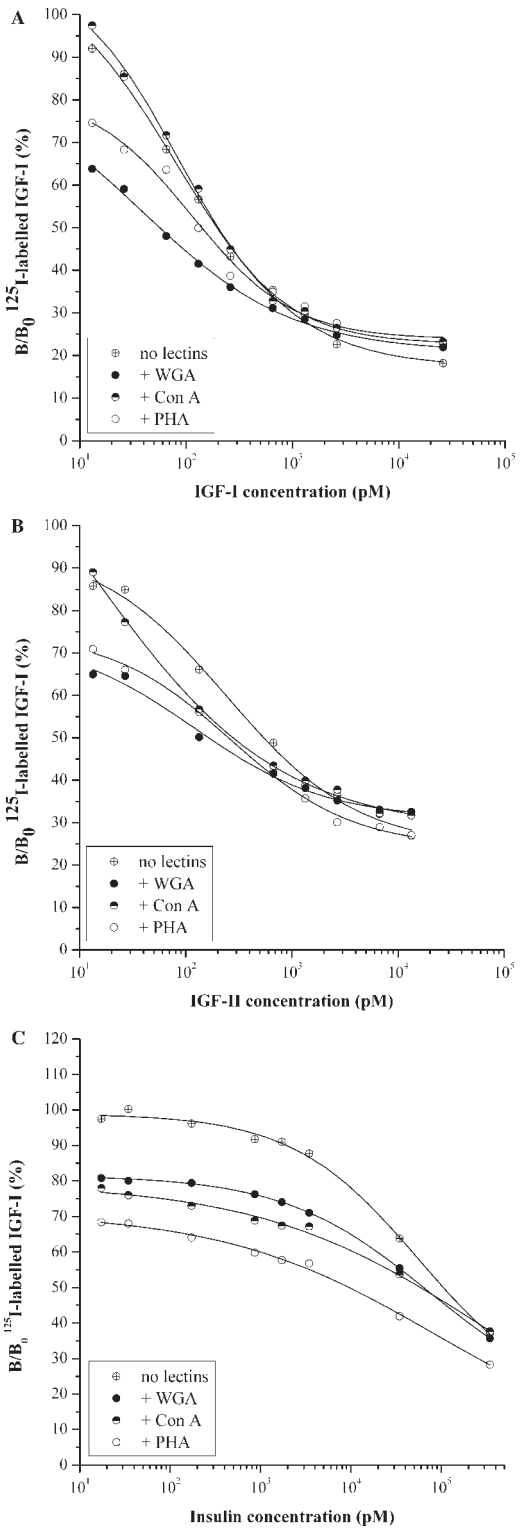

Fig. 2. **Effect of lectins on competitive ligand-binding curves for ^{125}I-labelled IGF-I.** Solubilized placental membranes were incubated with ^{125}I-labelled IGF-I alone or in the presence of increasing concentrations of unlabelled IGF-I (A), IGF-II (B) and insulin (C) as described in the MATERIALS AND METHODS section. All tests were run both with and without lectins added: WGA, Con A or PHA. The results are shown for one representative of three independently performed experiments.

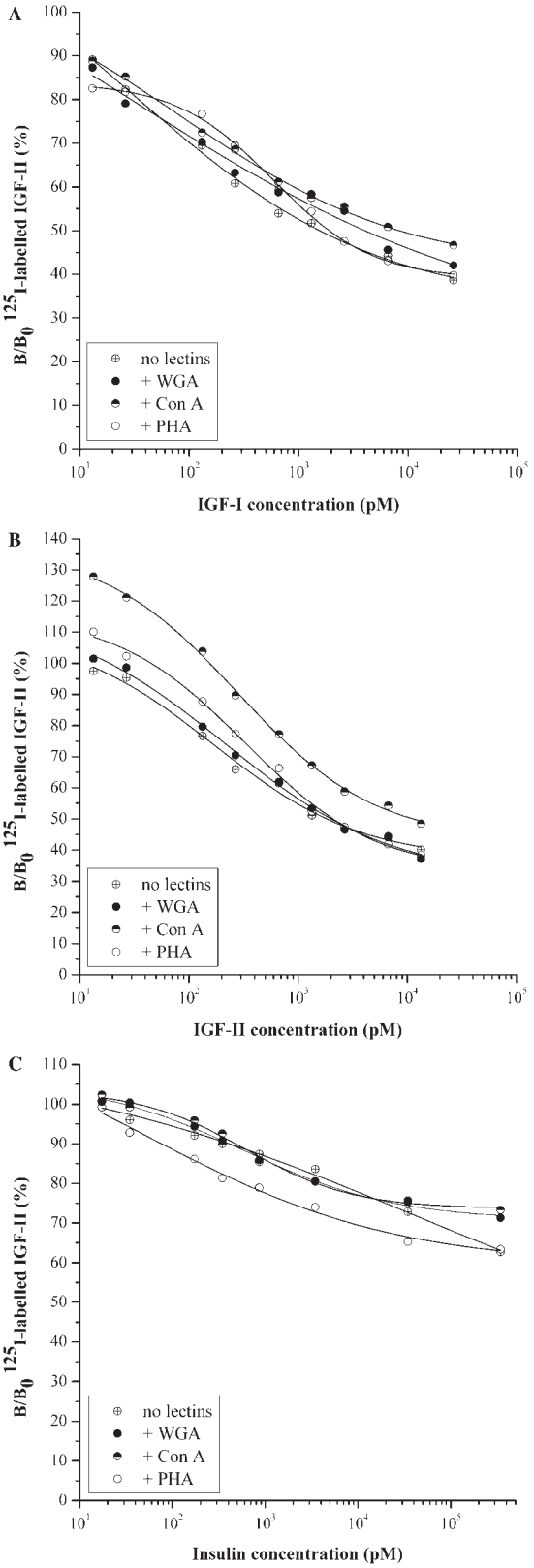

Fig. 3. **Effect of lectins on competitive ligand-binding curves for ^{125}I-labelled IGF-II.** Solubilized placental membranes were incubated with ^{125}I-labelled IGF-II alone or in the presence of increasing concentrations of unlabelled IGF-I (A), IGF-II (B) and insulin (C). See legend under Fig. 2.

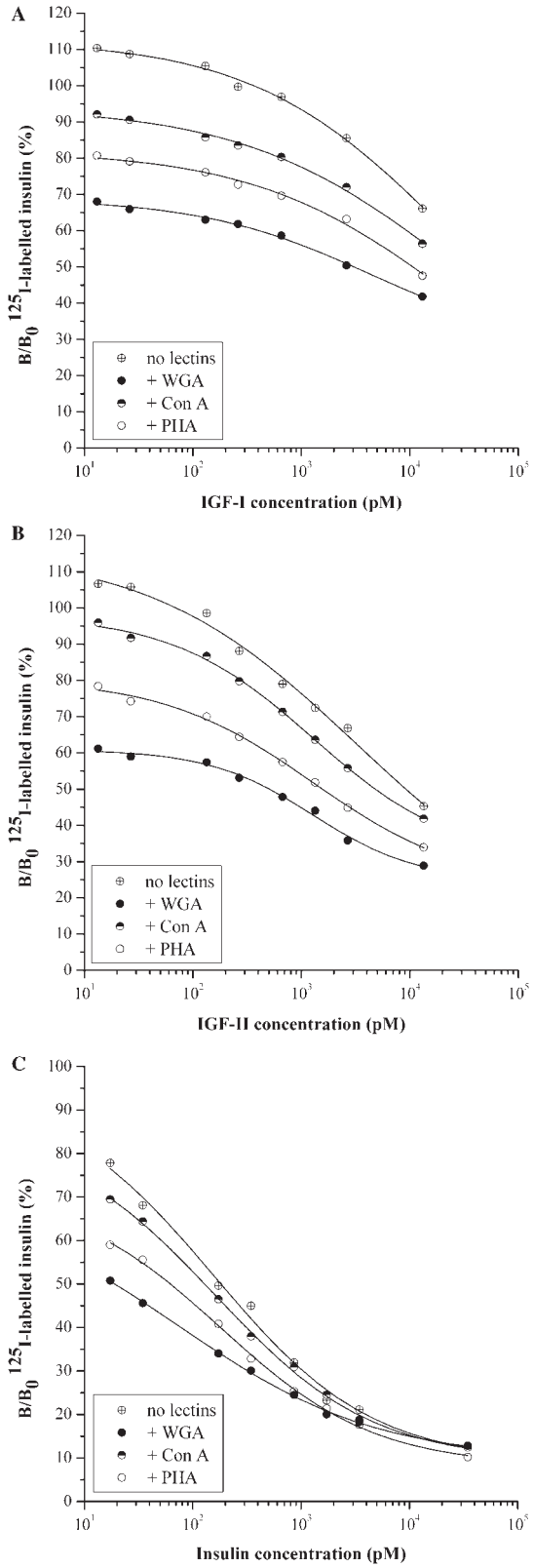

Fig. 4. **Effect of lectins on competitive ligand-binding curves for ^{125}I-labelled insulin.** Solubilized placental membranes were incubated with ^{125}I-labelled insulin alone or in the presence of increasing concentrations of unlabelled IGF-I (A), IGF-II (B) and insulin (C). See legend under Fig. 2.

insulin, WGA proved to be the most effective affinity modulator, causing an 8-fold shift in IC_{50} value when compared to the control (Fig. 4C), which is consistent with the data presented earlier (Fig. 1C). Con A and PHA also caused an affinity shift of placental IRs but less potently than WGA.

The lower the concentration of unlabelled ligand in the competition with ^{125}I-labelled IGF-I or insulin, the greater was the lectin ability to lower the B/B_0 values. Compared to homologous curves, the heterologous competition-binding curves were changed to a greater extent in the presence of lectins. The affinity shift provoked by particular plant lectins was of such a magnitude that it could affect the receptors' specificity towards homologous and heterologous ligands.

DISCUSSION

The ligand-binding properties of the IGF1R and IR have been studied in considerable detail ever since Bhaumick *et al*. (*21*) established that, although remarkably similar, they represented distinct molecular species. Despite their extensive structural homology, the physiological functions of IR and IGF1R are also distinct. The molecular basis of this remains unclear, *i.e*. what determines signalling specificity *in vivo* (*13*). The importance of addressing these issues lies in the fact that the insulin/IGF family of ligands and receptors controls key aspects of mammalian life, including growth, metabolism and reproduction.

Ligand binding to IRs or IGF1Rs was frequently studied in an artificial milieu of cells that normally do not express high levels of these receptors and, therefore, display a limited range of responses to their ligands compared with physiologically relevant target tissues (*14*). Human placental membranes are rich in type 1 and 2 IGFRs as well as IRs (*22–24*) and, therefore, were chosen to characterize ligand receptor interactions in our study.

The IR has been extensively used as a model to examine the effect of glycosylation on receptor processing and targeting (*11*, *25*). It has been known for many years that some proteins undergo differential glycosylation in accordance with changes in cell phenotype (*26*). The potential role of variant *N*-glycosylation as a modulator of IGF1R and IR distribution and/or function has received limited attention. Recent results, however, support the hypothesis that there is a causal relationship between variant glycosylation and altered IR activity (*27*). We have recently demonstrated that human placental IGF1Rs, IGF2Rs and IRs differ with respect to their oligosaccharide moieties, which bear different sugars on their terminals, *i.e*. multiple populations of the receptors were detected. We also observed that the specific binding of the ligand–receptor complexes depended both on the lectin and on the ^{125}I-labelled ligand used (*17*).

The curves obtained using ^{125}I-labelled IGF-I in the absence of lectins are indicative of the binding of this tracer to the placental IGF1R. The affinity of IGF1R for IGF-I was in the same order of magnitude as that for IGF-II (IC_{50} values being 0.2 and 0.6 nM, respectively). Perdue and co-workers (*28*) showed that hIGF-II and rIGF-I bound to the purified placental IGF1R with the same affinity, with K_d values ranging from 0.03 to 0.07 nM. In our experimental system, K_d for binding of IGF-I to IGF1R partially purified from human placental membranes was 0.05 nM, whereas IGF-II bound to the same receptor with 2- to 3-fold smaller affinity (data not shown).

The curves obtained with IGF-II are not easy to explain as ^{125}I-labelled IGF-II binds to three different types of receptors on placental membranes: IGF2R, IGF1R and IR (*29*). The binding curves shown in Fig. 3, most probably, present the result of three different competition cases: (i) ^{125}I-labelled IGF-II bound to the IGF2R was most effectively competed by unlabelled IGF-II, much less by IGF-I, whereas insulin did not compete at all; (ii) ^{125}I-labelled IGF-II bound to the IGF1R was most effectively competed by unlabelled IGF-I, closely followed by IGF-II, whereas insulin was relatively effective only at high concentrations and (iii) finally, unlabelled insulin and IGF-II were equally potent in displacing ^{125}I-labelled IGF-II bound to IRs.

Frasca *et al*. (*8*) recognized IR isoform A (IR-A), lacking 12 amino acid residues at the C-terminus of the receptor α-chain, as a high-affinity IGF-II receptor. When we chromatographed the placental solubilizates on insulin–Sepharose column, the IR population eluted bound both ^{125}I-labelled insulin and ^{125}I-labelled IGF-II with the approximately same affinity (our unpublished data), which was the evidence of the abundant presence of IR-A in the solubilizates used in this work.

The competition-binding curves obtained with ^{125}I-labelled insulin reflected binding of the tracer to both IR-A and IR-B, as IC_{50} value obtained from the competition with unlabelled insulin was 0.2 nM, whereas IGF-II gave 9 nM (the ratio was 0.02). Frasca *et al*. (*8*) reported this ratio to be 0.36 in the cells designed to express only IR-A isoform. The same group reported values of IC_{50} to be 10 nM for unlabelled IGF-II, and 0.3 nM for unlabelled insulin, when displacing ^{125}I-labelled insulin bound to human fetal fibroblasts, in which the IR-A isoform made 80% of the IR population (*8*). These IC_{50} values, thus, closely fit those obtained in this work.

The possibility that ^{125}I-labelled IGF-II and unlabelled IGF-II have somewhat different affinities for different types of IGF and insulin receptors cannot be excluded. Tyr27 residue in IGF-II was shown to be one of the key residues involved in the high-affinity binding of this molecule to IGF1R and IR, but not for its binding to IGF2R (*30*). The presence of significant quantities of ^{125}I(Tyr27)-labelled IGF-II in the tracer mixture might have interfered with its binding to IGF1R and IR-A from the placental solubilizate, leaving the tracer largely bound to the IGF2R. This fact may explain why the binding curves of ^{125}I-labelled IGF-II presented in Fig. 1B were not affected by the presence of lectins, as IGF2R (structurally and functionally dissimilar to the receptors of the Tyr kinase family) could be resistant to the plant lectin modulation.

The results presented in this article unequivocally show that the plant lectins: WGA, Con A and PHA modify the specific binding of IGF molecules and insulin

to their receptors from human placenta. The inhibition might have originated from overlapping of ligand-binding sites and lectin-binding sites on the receptors or as a result of a conformational change in the receptor molecule upon lectin binding. The possibility that one or more of the lectins tested did not bind to the IRs and IGFRs *via* their carbohydrate, but *via* their protein residues cannot be excluded. Kaur *et al*. (*31*) reported that a carbohydrate moiety (α-D-mannopyranoside) and a peptide molecule (DVFYPYPYASGS) bound to Con A at a common binding site (carbohydrate-binding site) as, although being chemically dissimilar, they shared surface topology.

Several lines of evidence indicate that the interactions of hormones with their glycoprotein receptors are affected by certain plant lectins. For example, the binding of ^{125}I-labelled NGF to its receptor in human melanoma cell membranes and detergent-soluble membrane extracts was shown to be significantly increased in the presence of WGA (*32*).

Moreover, Lou and co-workers (*33*) showed that the first three domains of the IR α-subunit (Leu-rich repeat domains and Cys-rich domain) differ from the IGF1R in the regions governing ligand specificity. The crystal structure of the IR fragment, consisting of the three domains, revealed *N*-linked carbohydrates attached at 8 of the 10 potential sites (Asn residues 16, 25, 111, 215, 255, 337, 397 and 418). Asn15 proved to be among the seven most important residues for high-affinity insulin binding (*33*). Interestingly, Asn16 is missing from the sequence of the IGF1R, as is Asn255 (*33*). Asn255 is positioned seven residues away from the sixth module of the Cys-rich domain of IGF1R, which is known to be involved in the high-affinity binding of IGF-I (*33*). Thus, the main differences in the glycosylation between IR and IGF1R seem to be positioned near amino acid sequences that take part in the high-affinity ligand binding to the receptors. These findings strongly suggest a possible role for the saccharides attached to IRs and IGFRs in interactions with other proteins.

The lectins WGA, Con A and PHA caused the specific binding of ^{125}I-labelled IGF-I and insulin to be altered in a lectin-specific and dose-dependent manner. The same lectins profoundly affected the competition-binding curves for ^{125}I-labelled IGF-I and insulin. As a rule, there was an increase in the affinity of placental IGF1Rs and IRs towards homologous and heterologous ligands. The lower the ligand concentration, the greater was the affinity shift induced by the lectin. This suggests potential physiological significance of the effect, as it occurred in the low ligand concentration range. In contrast, Con A caused a decrease in the affinity of placental ^{125}I-labelled IGF-II-binding receptors for IGF-I and IGF-II. This feature, again, distinguished the placental receptors that bound ^{125}I-labelled IGF-II from the IRs and IGF1Rs.

The results presented in this work reveal the susceptibility of the analyzed ligand–receptor interactions towards modulation *via* carbohydrate moieties of human placental IRs and IGF1Rs. To further elucidate the modulating effects of WGA, Con A and PHA on these interactions, the enzyme digestion (endo-β-*N*-acetylglucosaminidase, endo-ß-galactosidase and α2,3-specific sialidase) of affinity purified IRs and IGFRs, alone or ligand-complexed is under research.

We assumed the existence of placental lectins with the specificities and/or effects similar to those displayed by the examined plant lectins. We have recently isolated mannose- and *N*-acetylglucosamine-specific proteins from solubilized placental membranes using affinity chromatography on these immobilized sugars. The isolated placental lectins were then tested in the interactions of IRs and IGF1R with their ligands. The preliminary results suggest that they are potent modulators of the specific binding of ^{125}I-labelled IGFs and insulin to their placental receptors. The effects of the placental lectins were dose-dependent and differed among the ligands. The major proteins detected in the placental lectin isolates were the mannose receptor and mannose-binding lectin (monomeric and oligomeric), which are under investigation (our unpublished data).

The placental lectins could modulate the ligand activation of IGF1Rs and IRs in the unique milieu of the placental cell membrane in a dose- and time-dependent manner. These findings may have important implications for placental cell responses to IGFs and insulin.

This study was funded by Ministry of Science, Republic of Serbia (143019).

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