Different inhibitory actions of IGFBP-1, -2 and -4 on IGF-I effects in vascular smooth muscle cells

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Abstract

IGF-I is involved in the regulation of metabolism, growth and migration of vascular smooth muscle cells (VSMCs). We have studied how IGFBP-1, -2 and -4 modulate IGF-I-induced DNA and protein synthesis in cultured rat VSMCs. DNA and protein synthesis were measured as incorporation of [³H]thymidine and [³H]leucine into DNA and protein respectively. Western immunoblot was used to detect IGFBPs in conditioned medium and solution hybridization was used to measure IGFBP gene expression. IGF-I stimulated DNA synthesis with an EC₅₀ of 44 pM, reaching a maximal effect at 1 nM. An IGF-I concentration of 1 nM was subsequently used in the experiments with IGFBPs. IGFBP-1 and IGFBP-4 acted in an inhibitory manner on IGF-I-induced DNA synthesis with calculated IC₅₀ values of 1.6 nM and 6.2 nM

respectively. IGFBP-2 (16 nM) also inhibited the growth response to IGF-I, but this effect could only be obtained if the two peptides were pre-incubated together for 2 h prior to addition to the cells. IGFBP-1, -2 and -4 inhibited IGF-I-induced protein synthesis in a similar way. Immunoblot of the incubation medium showed little degradation of IGFBP-2 and -4 for up to 24 h. mRNA for IGFBP-2 and -4, but not for IGFBP-1 was detected in the VSMCs. Endogenous IGFBP-2 and -4 could be detected by immunoblot in the conditioned medium but only if it was concentrated. In conclusion, IGFBP-1, -2 and -4, of which IGFBP-2 and -4 may be locally derived, act as inhibitors with different potencies on IGF-I effects in VSMCs.

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Introduction

In the circulation and throughout the extracellular space, the insulin-like growth factors (IGF-I and IGF-II) are complexed to a group of high affinity IGF-binding proteins (IGFBPs) (Rechler & Clemmons 1998). A minor part, less than 1% of the total IGF-I content, is unbound and only the free fraction is considered to be bioactive (Baxter 1994). So far, six different IGFBPs (1–6), which all share structural homology with each other, have been cloned and characterized (Shimasaki & Ling 1991). Although the 3-dimensional structure of the IGFBPs is not fully solved, it is accepted that IGFBPs consist of two globular N- and C-domains, with an intermediate, non-homologous domain connecting the other two (Spencer & Chan 1995).

IGFBP-1, -2 and -3 have been reported both to inhibit and enhance IGF-I effects, depending on cell type and other factors (Cornell *et al.* 1986, DeMellow & Baxter 1988, Bar *et al.* 1989, Camacho-Hubner *et al.* 1991, Gockerman *et al.* 1995). IGFBP-4 has been reported to inhibit IGF-I-induced effects (Kiefer *et al.* 1992, Cohick *et al.* 1993), while studies on IGFBP-5 have shown stimulatory effects (Andress

& Birnbaum 1992, Jones *et al.* 1993a). So far, little is known about IGFBP-6. IGF-independent effects have been described for IGFBP-1 and IGFBP-3 (Liu *et al.* 1992, Jones *et al.* 1993b, Oh *et al.* 1993a).

IGF-I is involved in the regulation of metabolism, growth and the migration of vascular smooth muscle cells (VSMCs) (Arnqvist *et al.* 1995, Delafontaine 1995). We have shown previously that gene expression of IGFBP-2 and IGFBP-4 is markedly increased in hypertrophied rat VSMCs *in vivo* (Chen *et al.* 1994, 1995). Fasting and diabetes also modulate the gene expression of IGFBP-2 and -4 in rat VSMCs *in vivo* (Chen & Arnqvist 1994) and have pronounced effects on circulating levels of IGFBP-1 (Brismar *et al.* 1994). Inhibitory effects of IGFBP-2 and -4 on IGF-I-induced DNA synthesis have been reported in porcine VSMCs under serum-free conditions (Bourner *et al.* 1992, Cohick *et al.* 1993). If pre-incubated with IGF-I, IGFBP-1 has been found to inhibit IGF-I-induced DNA synthesis in rat VSMCs (Motani *et al.* 1995). In the present study, we wanted to investigate how IGFBP-1, -2 and -4 modulate the effects of IGF-I on DNA and protein synthesis in rat VSMCs and to characterize possible differences between them.

Materials and Methods

Isolation and culturing of VSMCs

Rat vascular smooth muscle cells were isolated and cultured according to a modified method of Nilsson *et al.* (1983). Small pieces of the aortas were digested for 1 h in 0.1% collagenase in Ham's F12 medium, at 37 °C and then for 20 h in fresh collagenase/F12. The cell suspension was filtered through a nylon filter (pore size 48 µm) and the cells were then washed in F12 medium. The cells were transferred to a solution of the following composition: F12 medium, 10% new-born calf serum (pH=7.4), 50 µg/ml ascorbic acid, 2 µg/ml fungizone and 50 µg/ml gentamycin sulphate. Cells were plated in 75 cm² culturing flasks and kept at 37 °C in a humidified atmosphere of 5% CO₂ in air. The cells were harvested using a solution of trypsin (0.25%) and EDTA (0.02%) when passaged and the medium was changed twice a week. Cells from the second to the tenth generation were used throughout all experiments. The cells were characterized as smooth muscle cells by morphological criteria and with an antibody which recognizes a unique epitope of α -smooth muscle actin (Skalli *et al.* 1986). All experiments were performed on confluent cell cultures, which were kept for 24 h in serum-free F12 medium before the experiments.

DNA and protein synthesis

DNA synthesis was quantified by measuring [³H]thymidine incorporation into DNA. The cells were grown in 96-well plates and incubated for 24 h in serum-free F12 medium, with the addition of 1 µCi/ml [³H]thymidine and in the presence or absence of IGF-I and/or IGFBPs at the indicated concentrations in fresh serum-free F12 medium. The cells were washed with F12 medium and DNA was precipitated with 5% ice-cold trichloroacetic acid (TCA). DNA was solubilized in 0.1 M KOH. One hundred and seventy microliters of the solution (total 200 µl) in each well was added to scintillation liquid and the radioactivity was counted in a liquid scintillation counter. The data were presented as percentage change over control cells.

Protein synthesis was quantified by measuring [³H]leucine incorporation into protein. The cells were grown in 96-well plates and incubated for 20 h in serum-free F12 medium, in the presence or absence of IGF-I and/or IGFBPs at the indicated concentrations. [³H]Leucine was added in a final concentration of 1 µCi/ml for the last 90 min. The cells were rinsed with cold phosphate-buffered saline (PBS) (pH=7.4) and protein was precipitated in ice-cold 5% TCA. Protein was solubilized in a solution with the following composition: 5% sodium dodecyl sulphate (SDS), 20 mM Na₂CO₃ and 2 mM EDTA. Radioactivity was counted and data were presented as described in the [³H]thymidine section above.

mRNA levels of IGFBP-1, -2 and -4

VSMCs were grown to confluence in Petri dishes and were then kept in serum-free F12 medium for 24 h. Serum-free medium was changed and the cells were incubated for another 24 h. The cells were then harvested in SET buffer (1% SDS, 20 mM Tris, 10 mM EDTA, pH=7.5) and homogenized with a polytron. Proteinase K was added and the samples were extracted with phenol and chloroform according to the method of Durnam & Palmiter (1983). Total nucleic acids were precipitated in 95% ethanol. The DNA content was measured as described by Labarca & Paigen (1980). The mRNA levels for IGFBP-1, -2 and -4 were analyzed with a solution hybridization assay using [³⁵S]UTP-labeled RNA probes. The IGFBP-1 probe consisted of 407 bases of a rat cDNA clone. The IGFBP-2 probe consisted of 397 bases complementary to exons 2 and 3 and part of exon 4 of the IGFBP-2 gene. The probe for IGFBP-4 consisted of 444 bases of a rat cDNA clone. The probes were synthesized as described by Melton *et al.* (1984). The samples were hybridized with each probe for 18 h at 70 °C. The hybridization solution contained a total volume of 40 µl 0.6 M NaCl, 20 mM Tris, 4 mM EDTA, 0.75 mM dithiothreitol (DTT), 25% formamide and 0.1% SDS. At least 10 000 c.p.m. of the [³⁵S]UTP-labeled probe were used per sample. RNases were then added and the double stranded RNA was precipitated in 6 M TCA. The hybrids were collected on filter and the radioactivity was counted in a liquid scintillation counter. In each assay, a standard curve was included. The curve was created by hybridizing known amounts of *in vitro* synthesized sense RNA with antisense (probe).

Western blot analysis of IGFBPs

The cells were grown to confluence in 96-well plates and were then starved in serum-free medium for 24 h. Human recombinant IGFBP-2 and IGFBP-4 were added at the indicated concentrations and were incubated for 24 h. Some wells included cells with no exogenous addition of IGFBPs, while others included IGFBP addition but no cells. Four nanograms human recombinant IGFBP were used as a positive control. The conditioned medium (80 µl) was collected and 60 µl sample buffer were added. Fifty microliters of this solution were applied onto a 15% SDS-PAGE. Proteins were transferred onto polyvinylidene difluoride (PVDF) membranes for 1 h (200 mA) with transfer buffer (15 mM Tris, 120 mM glycine, 5% methanol, pH=8.3). The sheets were saturated with 0.2% polyvinyl alcohol dissolved in Tris-buffered saline (TBS)-Tween (TBS-T; 0.2%), pH=7.6 (overnight at 4 °C), and incubated for 1 h with polyclonal antibodies (1:3000) raised against bovine IGFBP-2 and human IGFBP-4. After washing 3 times (15 and 2 × 5 min each) with TBS-T, a donkey anti-rabbit immunoglobulin G horse-radish peroxidase-labeled antibody (1:3000) was added for

1 h. After washing three times as described above, the detection was visualized by enhanced chemiluminescence (ECL) analysis with the Amersham ECL system using ECL Hyperfilms for exposure. In order to concentrate conditioned medium, VSMCs were grown in Petri dishes (7 ml conditioned medium). Confluent VSMCs were starved for 24 h in serum-free F12 medium. After another 24 h incubation in fresh serum-free medium under similar conditions, the conditioned medium (5 ml/dish) was collected. EDTA in a final concentration of 5 mM was added to prevent protein degradation. The samples were immediately centrifuged to remove cells and other material in the medium. Fifty microliters 0.1% BSA and 5 ml 10% TCA were added to precipitate protein. The samples were incubated at 4 °C overnight and were then centrifuged at 11 000 r.p.m. at 4 °C for 30 min. The pellet was washed twice with 95% ethanol and was then dissolved in 250 µl electrode buffer. Forty microliters of the sample were mixed with 20 µl sample buffer and then applied on 15% SDS-PAGE. The further steps were performed according to the scheme described in the section above.

Chemicals

[³H]Thymidine and [³H]leucine were from Amersham International (Amersham, Bucks, UK). Human recombinant IGF-I was obtained from Pharmacia Upjohn (Stockholm, Sweden), human recombinant IGFBP-2 and IGFBP-4 (from yeast) from Austral Biologicals (San Ramon, CA, USA). IGFBP-1 was a gift from Dr Mats Lake, Pharmacia Upjohn, Stockholm, Sweden. Trypsin was from Difco Labs (Detroit, MI, USA) and collagenase type I was from Sigma (St Louis, MO, USA). The monoclonal antibody against α -smooth muscle actin was from Sigma Immuno Chemicals (La Jolla, CA, USA) and the polyclonal antibodies against IGFBP-2 and IGFBP-4 were from Upstate Biotechnology (Lake Placid, NY, USA) (see Bourner *et al.* 1992 and Camacho-Hubner *et al.* 1992). The source of both antibodies was rabbits immunized with bovine IGFBP-2 or human IGFBP-4 respectively. The antibodies detect human and rat IGFBP-2 and IGFBP-4. PVDF membranes were from Dupont (Boston, MA, USA) and scintillation liquid (Ultima Gold) was from Packard Instrument Company (Meriden, CT, USA). Chemicals and solutions for cell culture were received from GIBCO BRL Life Technologies (Täby, Sweden). Proteinase K was from Merck (Darmstadt, Germany) and the chemicals for antisense (probe) and sense synthesis were from Promega (Madison, WI, USA). RNase A, RNase T1 and herring sperm DNA were obtained from Boehringer (Mannheim, Germany). Phenol was from Fischer Scientific (Fair Lawn, NJ, USA).

Statistics

Values are given as means $\pm$ S.E.M. Statistical comparisons for more than two groups were made according to

Figure 1 The effect of different concentrations of IGF-I on [³H]thymidine incorporation in rat vascular smooth muscle cells. Confluent cell cultures were incubated in F12 medium for 24 h, in the presence of [³H]thymidine and without or with IGF-I at the indicated concentrations. Values are given as mean $\pm$ S.E.M., $n=4$ and the samples were measured in triplicate.

ANOVA (Scheffé's test). Paired Student's *t*-test was used when comparing two dependent groups.

Results

IGFBP modulation of IGF-I-induced DNA and protein synthesis in VSMCs

IGF-I dose dependently stimulated DNA synthesis in VSMCs (Fig. 1). The EC₅₀ value was 44 pM ($n=4$). The maximum effect was reached at an IGF-I concentration of 1 nM and this concentration was subsequently used in the experiments with IGFBPs.

IGFBP-1 in a concentration of 18 nM (500 ng/ml) significantly inhibited the effect of IGF-I on DNA synthesis (Fig. 2a). The calculated IC₅₀ value for the inhibition was 1.6 nM ($n=5$). IGF-I-induced protein synthesis was also inhibited by 18 nM IGFBP-1 (Fig. 2b).

IGFBP-2 up to a concentration of 16 nM (500 ng/ml) did not inhibit significantly the IGF-I effect on DNA synthesis (Fig. 3a). When we performed experiments where IGF-I and IGFBP-2 were preincubated at 4 °C for 2 h prior to addition to the cells, IGFBP-2 at 16 nM (500 ng/ml) was capable of significantly inhibiting the effect of IGF-I on VSMC growth (Fig. 3b). IGFBP-2 (16 nM) also inhibited IGF-I-induced protein synthesis when the same pre-incubation procedure was used (Fig. 3c). The inhibitory effect of IGFBP-4 on IGF-I-induced DNA synthesis was more modest than that of IGFBP-1 (Fig. 4a). The inhibition was significant at

Figure 2 Modulation by IGFBP-1 of the response of rat vascular smooth muscle cells to IGF-I. (a) The effect of IGF-I added together with increasing concentrations of IGFBP-1 on [³H]thymidine incorporation in rat VSMCs ($n=5$). (b) The effect of IGFBP-1 on IGF-I-induced [³H]leucine incorporation ($n=4$). Values are given as means $\pm$ S.E.M. * $P<0.05$. Statistical comparison for more than two groups was made according to ANOVA Scheffé's test. Student's dependent t -test was used for two groups. The samples were measured in triplicate.

18 nM (500 ng/ml) IGFBP-4 ($IC_{50}=6.2$ nM, $n=4$). IGFBP-4 at 18 nM was also capable of significantly inhibiting the IGF-I effect on protein synthesis (Fig. 4b).

IGFBP-1, -2 and -4 mRNA levels in VSMCs

Confluent VSMCs were incubated for 48 h in serum-free F12 medium, with a change of medium after 24 h. IGFBP-2 and IGFBP-4 mRNAs could be detected with solution hybridization, whereas IGFBP-1 mRNA was below the detection limit. The level of IGFBP-2 mRNA was just above the limit of detection and could be determined in 2 out of 3 samples, with an average value of 0.87 amol/ μ g DNA. The mRNA levels of IGFBP-4 were considerably higher, at 9.6 ± 1.9 amol/ μ g DNA ($n=3$).

Endogenous and exogenous IGFBP concentrations in the conditioned medium

These experiments (see Fig. 5a and b) were carried out in order to investigate whether exogenous IGFBP was degraded by the VSMCs and whether there were endogenously produced IGFBPs which could interact with exogenously added IGFBPs in modulating the IGF-I effect. Both exogenous IGFBP-2 and IGFBP-4 (500 ng/ml) in the conditioned medium were detected with heavy bands (at 32 kDa and 26 kDa respectively) with Western immunoblot. The bands were obvious throughout the studied time course, i.e. for up to 24 h. The IGFBP bands in the conditioned medium were slightly weaker after 24 h in the presence of cells compared to control bands (exogenous IGFBPs in conditioned medium without cells). A faint immunoreactive band appeared for IGFBP-4 at 16 kDa, a possible degradation product. No endogenous IGFBP was detected and exogenous IGFBP added in a concentration of 5 ng/ml could not be detected.

To investigate whether IGFBP-2 and IGFBP-4 are secreted from the VSMCs at all, we collected and concentrated (1:20 times) conditioned medium. After 24 h incubation, we could detect an IGFBP-2 band at approximately 32 kDa with Western blot (Fig. 6a). IGFBP-4 was detected at approximately 26 kDa after 24 h incubation (Fig. 6b) and there was also a band at 16 kDa, probably an immunoreactive degradation product of IGFBP-4.

Discussion

In the present study we show that IGFBP-1, -2 and -4 all act in an inhibitory manner on IGF-I (1 nM)-induced DNA or protein synthesis in rat aortic smooth muscle cells (SMCs). The inhibitory effects differed between the different IGFBPs with IGFBP-1 being the most potent.

In our study IGFBP-1 was the most potent inhibitor of IGF-I-induced DNA synthesis ($IC_{50}=1.6$ nM or

Figure 3 Modulation by IGFBP-2 of the response of rat vascular smooth muscle cells to IGF-I. (a) The effect of IGF-I added together with increasing concentrations of IGFBP-2 on [^3H]thymidine incorporation in rat VSMCs ($n=5$). (b) The effect of IGFBP-2 on IGF-I-induced [^3H]thymidine incorporation ($n=3$). In this case, IGFBP-2 and IGF-I were pre-incubated for 2 h at 4 °C prior to addition to the cells. (c) The effect of IGFBP-2 on IGF-I-induced [^3H]leucine incorporation ($n=3$). Values are means $\pm$ S.E.M. * $P<0.05$. Statistical comparison for more than two groups was made according to ANOVA Scheffé's test. Student's dependent t -test was used for two groups. The samples were measured in triplicate.

44 ng/ml). In addition, 18 nM (500 ng/ml) IGFBP-1 strongly inhibited IGF-I-induced protein synthesis. These results are in agreement with those of Motani *et al.* (1995).

In their experiments, however, IGF-I was pre-incubated with increasing concentrations of IGFBP-1 prior to addition to the rat aortic SMCs. An IGFBP-1 concentration

Figure 4 Modulation by IGFBP-4 of the response of rat vascular smooth muscle cells to IGF-I. (a) The effect of IGF-I added together with increasing concentrations of IGFBP-4 on [³H]thymidine incorporation in rat VSMCs ($n=4$). (b) The effect of IGFBP-4 on IGF-I-induced [³H]leucine incorporation ($n=4$). Values are means $\pm$ S.E.M. * $P<0.05$. Statistical comparison for more than two groups was made according to ANOVA Scheffé's test. Student's dependent t -test was used for two groups.

Figure 5 Levels of exogenous and endogenous IGFBP-2 and IGFBP-4 in conditioned medium (CM) of rat vascular smooth muscle cells. The cells were starved in 96-well plates for 24 h in F12 medium and were then incubated for another 24 h with or without the addition of exogenous IGFBP-2 (a) or IGFBP-4 (b). CM without cells served as control. The CM was collected and run onto a 15% SDS-PAGE. Western immunoblot was used to detect IGFBP-2 and IGFBP-4. The Figure shows representative blots ($n=3$).

of 100 ng/ml was capable of neutralizing the effect induced by 4 nM (30 ng/ml) IGF-I. Busby *et al.* (1988) have shown that IGFBP-1 purified from amniotic fluid inhibited IGF-I-stimulated DNA synthesis in porcine SMCs. In contrast to this, Elgin *et al.* (1987) reported that

(a)

Mw (kDa)

Endogenous IGFBP-2

hrIGFBP-2

32

(b)

Mw (kDa)

Endogenous IGFBP-4

hrIGFBP-4

26

16

IGFBP-1, isolated from human amniotic fluid, potentiated the effect of IGF-I on DNA synthesis in porcine aortic SMCs, but only if platelet-poor plasma was included in the culture medium. IGFBP-1 at concentrations of 1000 and 10 000 ng/ml has also been determined to inhibit IGF-I (100 ng/ml)-induced migration of porcine aortic SMCs (Gockerman *et al.* 1995). In summary, our results as well as those of other groups show that IGFBP-1 inhibits IGF-I effects on cultured VSMCs in serum-free medium. The inhibitory action appears when the concentration of IGFBP-1 is about equimolar or higher than the IGF-I concentration.

IGFBP-2 (16 nM or 500 ng/ml) pre-incubated with IGF-I for 2 h prior to addition to the rat VSMCs inhibited the effect of IGF-I on DNA and protein synthesis. IGFBP-2 was not capable of significantly inhibiting the effect of IGF-I without pre-incubation. Bourner *et al.* (1992) reported that bovine IGFBP-2 (5–20 ng/ml) potentiated IGF-I (10 ng/ml)-induced DNA synthesis in porcine SMCs in the presence of platelet-poor plasma, but was an inhibitor in serum-free medium. It could be speculated that IGFBP-2 binds to the SMCs or extracellular matrix (McCusker *et al.* 1990, Hodgkinson *et al.* 1994) and thereby is not able to bind IGF-I.

IGFBP-4 significantly inhibited the response of rat VSMCs to IGF-I-induced DNA synthesis. The overall inhibitory effect of IGFBP-4 was not as potent ($IC_{50}=6.2$ nM or 173 ng/ml) as that of IGFBP-1. In agreement with the effect on DNA synthesis, 18 nM (500 ng/ml) IGFBP-4 also inhibited IGF-I-induced protein synthesis. In a study by Cohick *et al.* (1993), IGFBP-4 (50–250 ng/ml) had no effect on IGF-I (20 ng/ml)-induced DNA synthesis in porcine SMCs, while 500 ng/ml IGFBP-4 gave a minimal decrease in the IGF-I response. It may thus be concluded that IGFBP-4 also has an inhibitory action on IGF-I effects in VSMCs.

The thermodynamic affinity of IGFBPs for IGF-I and the binding kinetics are the major determinants of how an IGFBP will modulate IGF activity. The affinity of the IGFBPs for IGF-I is about the same order of magnitude as the affinity of the type I IGF receptor for IGF-I (Arnqvist *et al.* 1995), which make them competitors for IGF-I. It should be mentioned that posttranslational modifications of IGFBPs, as occur following phosphorylation and glycosylation may influence their affinity for IGF-I (Jones *et al.* 1991, Rechler & Clemmons 1998). In a study by Oh *et al.* (1993b), the affinities of IGFBP-1 to -4 for IGF-I were

Figure 6 Levels of endogenous IGFBP-2 and IGFBP-4 in concentrated conditioned medium of rat vascular smooth muscle cells. The cells were starved in Petri dishes for 24 h in serum-free F12 medium and were then incubated for another 24 h in fresh serum-free medium. The medium was collected, concentrated (1:20) and run onto a 15% SDS-PAGE. Western immunoblot was used to detect IGFBP-2 (a) and IGFBP-4 (b). The Figure shows representative blots ($n=3$). hr, human recombinant.

measured with the ^{125}I displacement technique and according to that study the affinities for IGF-I were: IGFBP-1>IGFBP-4>IGFBP-2. The inhibitory actions of the IGFBPs found in the present study are in agreement with those results.

The physiological IGFBP-1 concentrations in the circulation of healthy individuals are in the range of 0–250 ng/ml (Brismar *et al.* 1994, Colett-Solberg & Cohen 1996, Nyström *et al.* 1997), depending on the nutritional state. Physiological concentrations of IGFBP-2 and IGFBP-4 are higher, about 250 ng/ml in serum (Colett-Solberg & Cohen 1996). This means that the circulating concentrations of IGFBP-1, -2 and -4 *in vivo* are in a similar range as used in the present study. The IC_{50} values determined for the IGFBPs on IGF-I action in our study suggest inhibitory actions of the IGFBPs at concentrations lower than 500 ng/ml. Since the modulatory effects of the IGFBPs are dependent on the concentration ratio between IGFBPs and that of free IGF-I, physiological IGFBP concentrations may, therefore, inhibit IGF-I effects on VSMCs *in vivo* at free IGF-I concentrations of about 1 nM or lower.

In the present study, we show that IGFBP-2 and -4 mRNAs, but not IGFBP-1 mRNA, are expressed in rat VSMCs. We found that endogenous IGFBP-2 and IGFBP-4 are secreted from rat VSMCs. IGFBP-4 production in rat VSMCs has previously been reported (Kamyar *et al.* 1994) and both IGFBP-2 and IGFBP-4 secretion has been shown for porcine VSMCs (Cohick *et al.* 1993). The fact that IGFBP-2 and -4 are expressed and secreted from VSMCs implies that the availability of IGFBP-2 and -4 in the vascular wall can be regulated by local synthesis. Both exogenous IGFBP-2 and IGFBP-4 were to a minor extent degraded by proteases in the presence of cells after 24 h incubation. This indicates that the difference in inhibitory action between IGFBP-2 and IGFBP-4 is not due to increased degradation of IGFBP-2.

In conclusion, IGFBP-1, -2 and -4 act in an inhibitory manner on IGF-I effects in vascular smooth muscle cells and have different potencies for inhibition of the IGF-I effects. The results suggest that these IGFBPs are capable of inhibiting IGF-I-induced effects at physiological concentrations.

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