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Original article

Characterisation and location of insulin-like-growth factor (IGF) receptors in the foetal bovine *Semitendinosus* muscle

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Abstract — We characterised IGFI and IGFII receptors and located them in bovine muscle during foetal growth. *Semitendinosus* muscle samples were taken from foetuses ranging from 80 to 270 days post-conception. The relative affinities of ^{125}I-IGFII and ^{125}I-IGFI mark the presence of typical type II and type I receptors in foetal muscle membranes. IGFII-specific binding is consistently five times greater than that of IGFI. The patterns of ^{125}I-IGFII- and ^{125}I-IGFI-specific binding are similar. They increase up to 110 and 170 days post-conception, respectively ($P < 0.05$); thereafter, they decrease ($P < 0.05$). This decrease was due to a fall in the number of receptors without any change in affinity. At the adult stage the specific binding of both the ^{125}I-IGF is very low. In foetal muscle, type II receptors are located both in the muscle bundles and in the connective tissue while type I receptors are only located in the muscle bundles. © Inra/Elsevier, Paris.

muscle / bovine / receptor / IGF / binding

Résumé — Caractérisation et localisation des récepteurs des IGF dans le muscle *Semitendineux* de fœtus bovins. Les récepteurs des IGF ont été caractérisés et localisés dans le muscle *Semitendinosus* au cours du développement fœtal. Les échantillons ont été prélevés entre 80 et 270 j post-conception. Les affinités relatives d'^{125}I-IGFII et d'^{125}I-IGFI mesurées sur membranes de muscle sont caractéristiques de la présence des récepteurs de type I et II. Au cours du développement la liaison spécifique d'^{125}I-IGFII et d'^{125}I-IGFI évolue parallèlement, celle d'^{125}I-IGFII étant cinq fois plus élevée que celle d'^{125}I-IGFI. Elle est maximale, respectivement, en début et en milieu de gestation, puis diminue progressivement jusqu'à 270 j post-conception ($p < 0,05$). Cette diminution est due à une chute du nombre de récepteurs, sans changement de leur affinité. Au stade adulte la liaison spé-

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cifique des deux d'^{125}I-IGF est très faible. Dans le muscle fœtal, les récepteurs de type II sont localisés dans les faisceaux musculaires et dans le tissu conjonctif, tandis que ceux de type I sont uniquement localisés dans les faisceaux musculaires. © Inra/Elsevier, Paris.

muscle / bovin / IGF / récepteurs / liaison

1. INTRODUCTION

The insulin-like growth factors (IGFI and IGFII) are mitogenic peptides that can stimulate cell division in vitro [44] and differentiation (for review, see Florini et al. [16]) of myoblasts, are anti-apoptose [43] and act on the metabolism (insulino-mimetic effects) (for a review, see Froech et al. [18]). In vivo, mRNAs and peptides for both IGFs have been detected in foetal muscle [4, 19]. Both these data show that foetal muscle is an important site of action for IGFs. This has been proved by knockouts of IGFI and -II (for a review, see Wood [45]). Disruption of the IGFII gene greatly diminishes prenatal growth in mice [9]. Knockout of IGFI leads to underdevelopment of muscle tissue [36], probably as a consequence of a decrease in tissue cell numbers [28]. During muscle cell regeneration in rodents, IGFI mRNA and the peptide appear first, followed by those of IGFII [26] which appear simultaneously with the formation of myotubes. This result suggests that both the IGFs act but that, as hypothesised by Florini (for a review, see Florini et al. [16]), their action is separated in time. Specific high affinity receptors for IGFI [6] and IGFII have been identified in rat muscle [1] and in several muscle cell lines, BC3H-1 [37], L6 [5] and C2 [44]. Most of the actions of IGFI and IGFII could be mediated by the type I receptor through auto/paracrine effects [14, 37]. The function of the type II receptor is not clearly established. The development of techniques based on autoradiography, especially on quantitative densitometric analysis, has enabled receptors to be located and, in particular, IGF receptors in the mammary gland [23] and in adult muscular tissue [33].

The aim of the present study was to characterise (specificity of binding, receptor number, affinity constant) IGFI and IGFII receptors and locate them in bovine muscle during foetal growth.

2. MATERIALS AND METHODS

2.1. Tissue samples

Animals were bred and slaughtered and samples collected at the Inra Research Centre (Theix, France) in compliance with ethical guidelines for animal care. Charolais foetuses were produced by artificial insemination of Charolais/Salers crossbreed heifers of mean age 2 years. *Semitendinosus* muscle samples were taken from foetuses and from two dams. For each gestational stage, several foetuses were obtained (number is indicated in brackets). They were 80 (n = 3), 110 (n = 3), 140 (n = 3), 170 (n = 3), 220 (n = 3), 270 (n = 2) days post-conception (dpc). Tissue samples were removed immediately after exsanguination of the dam, frozen in liquid nitrogen and stored at –80 °C.

2.2. IGFI and IGFII labelling

Human recombinant IGFI and IGFII were a generous gift from Eli Lilly Company (Indianapolis, USA). A modification of the method of Hunter and Greenwood, with a low concentration of chloramine T, was used to iodinate IGFI and IGFII in the presence of ^{125}I [18]. The specific activity, as calculated by isotope recovery, ranged from 1 000 to 1 800 Ci·mmole^{-1}.

2.3. Muscle microsomal membranes preparation

Skeletal muscle membranes were isolated according to the procedure reported by Mickelson and Louis [30]. All steps were carried out at

4 °C. Briefly, muscle samples were homogenised in 250 mM sucrose, 50 mM Tris-HCl, pH 7.5, 1 mM EDTA (1 g·5 mL^{-1} of buffer) and centrifuged at 2 000 *g* for 10 min. The pellet was resuspended in 50 mL of 400 mM LiBr, 20 mM Tris-HCl, pH 8.5. The suspension was stirred slowly for 16 h then centrifuged at 1 000 *g* for 15 min. The supernatant was centrifuged at 100 000 *g* for 30 min. The pellet was resuspended in 5 mL of 600 mM KCl, 20 mM Tris-HCl, pH 8 and centrifuged at 10 000 *g* for 10 min. The supernatant was retained while the pellet was extracted twice with the KCl-Tris medium. The three resultant supernatants were combined and centrifuged at 100 000 *g* for 30 min. The pellet was resuspended in the homogenisation buffer and the samples stored at –80 °C. Protein concentration was determined by the method of Lowry et al. [29].

2.4. Biochemical IGFI and IGFII receptor assays

2.4.1. Preliminary assays

Time and temperature dependence (data not shown): these parameters were analysed by incubation of muscle membranes or muscle sections with a constant amount of ^{125}I-IGFI or ^{125}I-IGFII in the absence or presence of an excess of their unlabelled counterpart to assess non-specific binding. Specific ^{125}I-IGFII binding steady state on membranes was reached after 4 h of incubation and was greater at 20 °C than at 4 °C. On muscle sections, it was reached after 90 min and was greater at 20 °C than at 4 °C. Specific ^{125}I-IGFI binding steady state on membranes was reached after 16 h and was greater at 4 °C than at 20 °C. On muscle sections, it was reached after 120 min and was greater at 4 °C than at 20 °C. Consequently, for IGFII, all subsequent binding studies were conducted at 20 °C for 4 h on muscle microsomal membranes and at 20 °C during 90 min on muscle sections. For IGFI, all subsequent binding studies were conducted at 4 °C for 16 h on muscle microsomal membranes and at 4 °C for 120 min on muscle sections. The results obtained on muscle microsomal membranes were in accord with those of Disenhaus et al. [10].

2.4.2. Binding test

The binding test used was described by Disenhaus et al. [10]. Briefly, membrane proteins were incubated with iodinated IGFI or IGFII (80 nM; 50 000 cpm/0.5 mL) in the absence (total binding) or presence (non-specific binding) of an excess of unlabelled peptide (10^{-7} M). After incubation, membrane-bound iodinated ligand was recovered by centrifugation at 3 000 *g* for 30 min. The radioactivity of the pellet was determined by γ-counting. Binding specificity was determined by incubation of iodinated IGFI or IGFII (80 nM) and of increasing concentrations of unlabelled ligand (IGFI and IGFII, 10^{-11}–10^{-7} M; insulin (Sigma), 10^{-6}–10^{-4} M). The results were expressed as a percentage of the specific binding of ^{125}I-IGFII or ^{125}I-IGFI. The non-specific binding was subtracted. These measures were carried out only at 110, 170 and 220 dpc and in the adult. At these stages, the muscle samples obtained were pooled. The plots shown were representative of two individual determinations at each stage of foetal development.

2.5. Quantitative autoradiography of IGFI and IGFII receptors

Frozen sections (7 μm) were processed as previously described [20]. Briefly the slide-mounted sections were preincubated for 10 min at 20 °C in 50 mM Tris-HCl, pH 7.4 containing 2 mM $CaCl_2$ and 5 mM KCl. Preincubations of muscle sections in this buffer of high ionic strength dissociates the endogenous ligand from its binding sites and eliminates the soluble IGF binding proteins [31]. This treatment also increases specific binding (by 15 %) by decreasing non-specific binding. It was carried out before all assays. After preincubation, the sections were washed twice in 50 mM Tris-HCl, pH 7.4. Incubations were carried out in 120 mM Tris-HCl, pH 7.4 containing 0.1 % BSA and 0.45 nM ^{125}I-IGFI or ^{125}I-IGFII (106 cpm·mL^{-1}). Non-specific binding was determined in the presence of 10^{-7} M IGFI or IGFII and 10^{-5} M insulin. After incubation, the sections were washed three times for 10 min in ice-cold 50 mM Tris-HCl, pH 7.6, air dried, and exposed for 4–7 or 15 days at 4 °C to Amersham autoradiographic films (hyperfilm-3H). The films were processed and the relative density of IGFI and IGFII binding sites were quantified by computerised densitometry with an image analyser (RAG 200, BIOCOM, Les Ulis, France). The density was related to the concentration of radioactivity by a comparison with standard curves generated by processing sets of Amersham ^{125}I-microscale; the data were expressed as relative values to compare the dif-

ferent foetal stages. The results were expressed in fmol·mg^{-1} of protein. They are represented as means of triplicates.

2.6. Data analysis

The statistical significance of the differences between foetuses of different ages was analysed by variance tests using SAS software [39].

Binding parameters (dissociation constants (Kd), number of binding sites) were calculated by fitting the experimental data with functions for either one or two binding sites with non-linear least square curve fitting programs from Multifit (Day Computing, Cambridge, UK), in accordance with the recommendations of Feldman [13].

3. RESULTS

3.1. ^{125}I-IGFII and ^{125}I-IGFI binding specificity

The binding specificity of ^{125}I-IGFII to the skeletal muscle membranes is shown in *figure 1*. For all foetal stages analysed, the binding of ^{125}I-IGFII was inhibited by unlabelled IGFII in a concentration-dependent manner and a 50 % ^{125}I-IGFII displacement was obtained at 5.5×10^{-9} M IGFII. IGFI (10^{-11}–10^{-7} M) was not very effective in the inhibition of ^{125}I-IGFII binding, 20 and 50 % of inhibition at 10^{-7} M for 110–170 days and 220 days dpc, respectively. In the adult, the competition curve for ^{125}I-IGFII obtained by increasing concentrations of IGFII was shifted to the left and 50 % inhibition was obtained at 10^{-10} M. IGFI (10^{-7} M) inhibited only 30 % of ^{125}I-IGFII binding. Insulin did not compete at all. The competitive binding curves and the ID_{50} of each ligand (IGFII >> IGFI) define a typical type II receptor (for a review, see Florini et al. [16]).

The binding specificity of ^{125}I-IGFI to the skeletal muscle membranes is shown in *figure 2*. At all stages of foetal development, the binding of ^{125}I-IGFI to the receptor was inhibited by unlabelled IGFI in a concentration-dependent manner (10^{-11}–10^{-7} M), with a 50 % inhibition of iodinated ligand at 5×10^{-10} M. IGFII (10^{-11}–10^{-7} M) also displaced ^{125}I-IGFI binding but was less effective than IGFI, with a 50 % inhibition of binding at 5×10^{-9} M. Insulin (10^{-6}–10^{-4} M) competed with ^{125}I-IGFI, but required about 10^{-4} M to achieve 80 % inhibition. In the adult, the 50 % inhibition of ^{125}I-IGFI binding was obtained at 10^{-10} M for IGFI and 10^{-8} M for IGFII. Insulin (10^{-4} M) inhibited only 60 % of the ^{125}I-IGFI. The competitive binding curves and the ID_{50} for each ligand (IGFI > IGFII > insulin) characterise a typical type I receptor for the binding of ^{125}I-IGFI (for a review, see Florini et al. [16]).

Specificity of the binding of ^{125}I-IGFII and ^{125}I-IGFI was also measured by autoradiographic analysis of muscle sections. For each time-point of gestation studied, ^{125}I-IGFII was displaced by 10^{-7} M IGFII (non-specific binding, 10 %), but not at all by 10^{-7} M of IGFI and 10^{-4} M of insulin (*figure 3A*). ^{125}I-IGFI was displaced totally by 10^{-7} M of unlabelled IGFI and IGFII (*figure 3B*).

3.2. Location of ^{125}I-IGFII and ^{125}I-IGFI binding on skeletal muscle sections

The autoradiographic analysis of ^{125}I-IGFII total binding showed a heterogeneous labelling from 110 dpc until the end of gestation. The labelling was located both in muscle and in the connective tissue, but it was higher in the connective tissue than in the muscle (*figure 4A*). The autoradiographic analysis of ^{125}I-IGFI total binding on skeletal muscle sections showed uniform staining at 110 and 270 dpc (*figure 4B*). From 140 to 220 dpc, the muscle tissue appeared to be labelled while no labelling was present on the connective tissue. This result was illustrated at 170 dpc (*figure 4B*). We revealed the contrast between the labelling in the connective tissue and the muscle tissue by increasing the exposure time (15 versus 7 days). This result is illustrated in the inset (*figure 4B*). *Figure 4C* shows muscle struc-

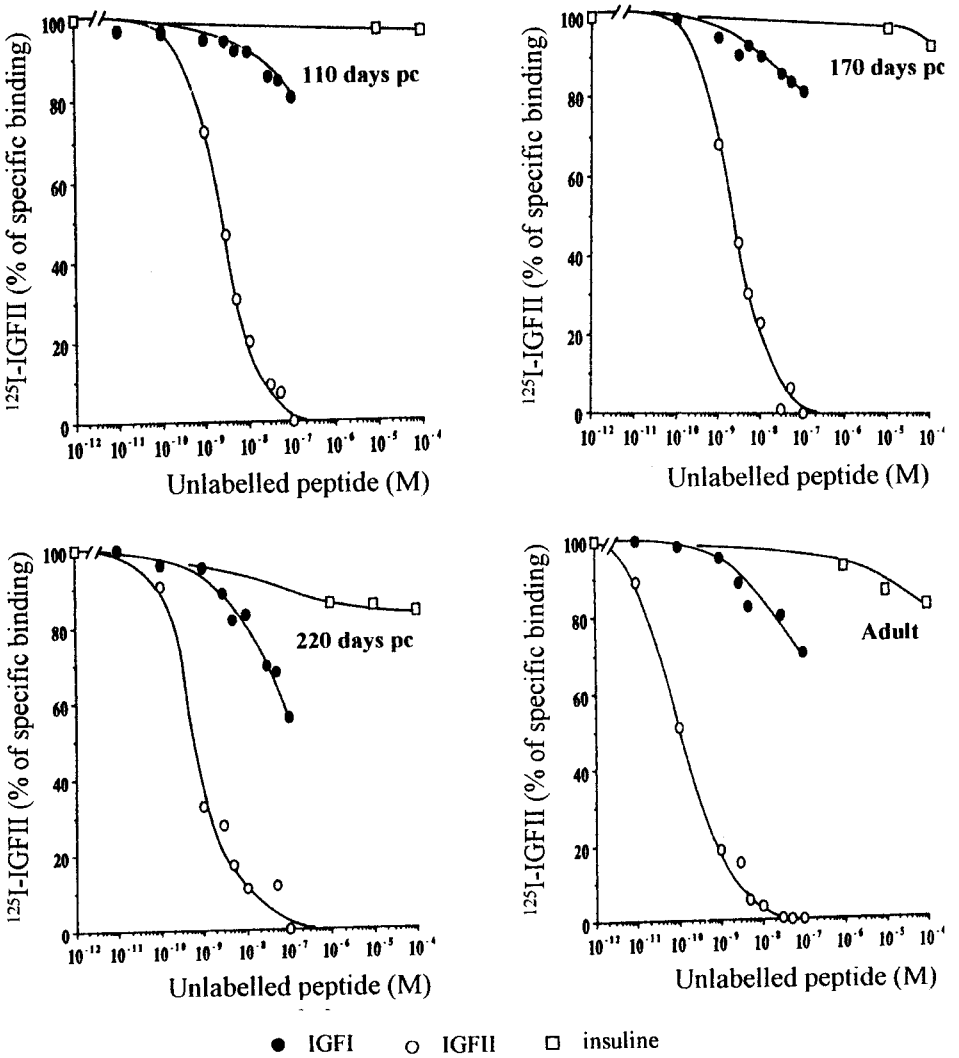

Figure 1. Displacement of ^{125}I-IGFII by unlabelled IGFI, IGFII and insulin. Binding of ^{125}I-IGFII was performed in muscle microsome preparations from foetal (110, 170 and 220 dpc) and adult skeletal muscle at 20 °C for 4 h, as described in the Materials and methods, in the absence or presence of unlabelled hormones (IGFI, IGFII and insulin) at the concentrations indicated. The results were expressed as a percentage of the specific binding of ^{125}I-IGFII. The non-specific binding has been subtracted.

ture at 110, 170, 270 dpc and in adult muscle. Muscle cells are darkly stained while connective tissue is stained white. At 110 dpc, muscle cells termed myotubes are grouped in bundles in a spacious extracellular matrix mainly composed of connective tissue. At 170 dpc, inside bundles, the space between the muscle cells (now muscle fibres) has decreased. At 260 dpc, the foetal muscle has the same structure as at 180 dpc, with major and minor networks surrounding muscle bundles.

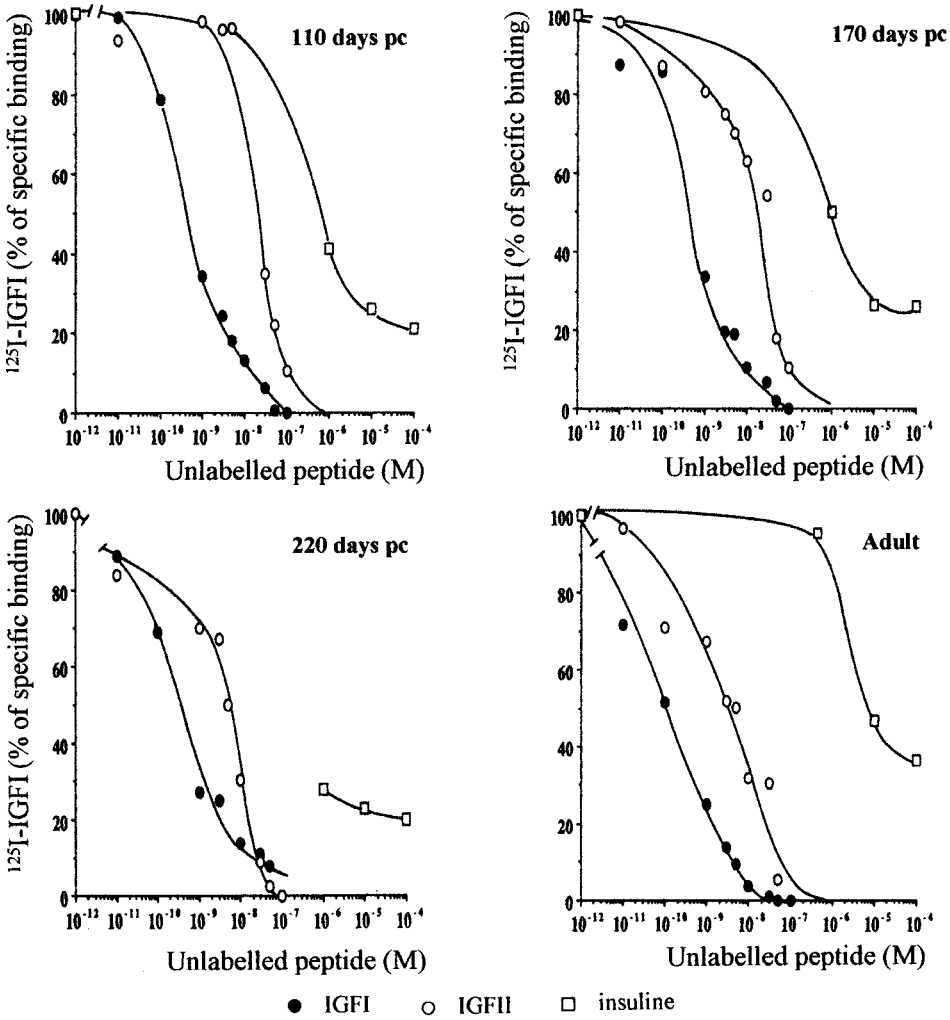

Figure 2. Displacement of ^{125}I-IGFI by unlabelled IGFI, IGFII and insulin. Binding of ^{125}I-IGFI was performed in muscle microsome preparations from foetal (105, 175 and 224 dpc) and adult skeletal muscle at 4 °C for 16 h, in the absence or presence of hormones (IGFI, IGFII and insulin) at the concentrations indicated. The results are expressed as a percentage of the specific binding of ^{125}I-IGFI. The non-specific binding has been subtracted.

3.3. Ontogeny of ^{125}I-IGF-II and ^{125}I-IGF-I specific binding in *Semitendinosus* muscle during foetal development

The binding assays were performed in skeletal muscle sections of adult and of 17 foetuses obtained between 80 and 270 dpc. *Figure 5A, B* shows the results of autoradiographic analysis by densitometry of ^{125}I-IGFII and ^{125}I-IGFI binding. At all stages analysed the ^{125}I-IGFII-specific binding was five times higher than the ^{125}I-IGFI-specific binding. ^{125}I-IGFII- and ^{125}I-IGFI-specific binding increased up to a maximum (110 and 140 dpc, respectively) ($P < 0.05$),

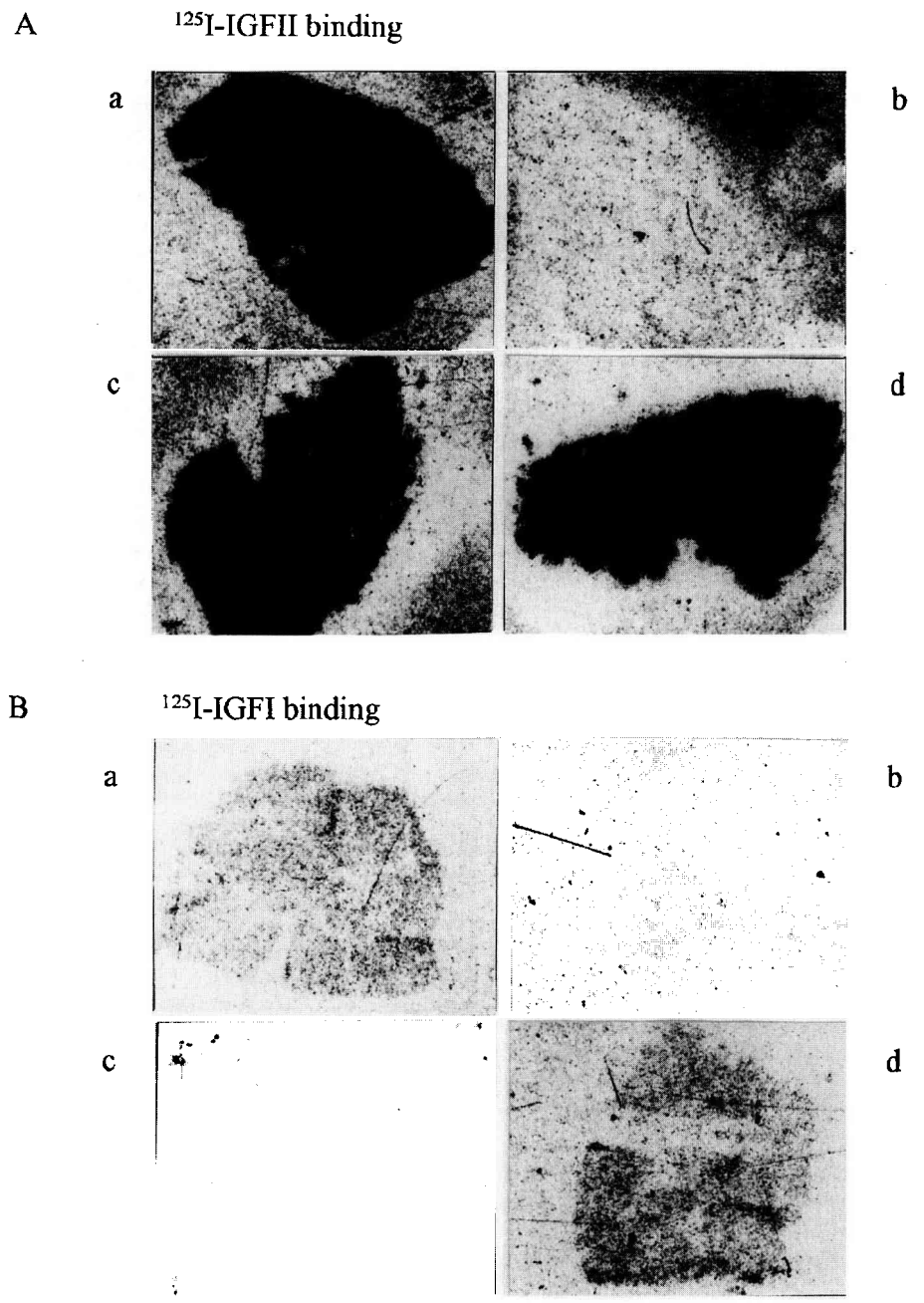

Figure 3. Autoradiographic analysis of ^{125}I-IGFII (**A**) and ^{125}I-IGFI (**B**) binding on cryostat foetal muscle sections at 170 dpc. Total binding was determined in the absence of unlabelled peptide (a), non-specific binding defined in presence of 10^{-7} M of unlabelled IGFII (b), 10^{-7} M of unlabelled IGFI (c), 10^{-4} M of unlabelled insulin (d) (non-serial sections) (original magnification × 10).

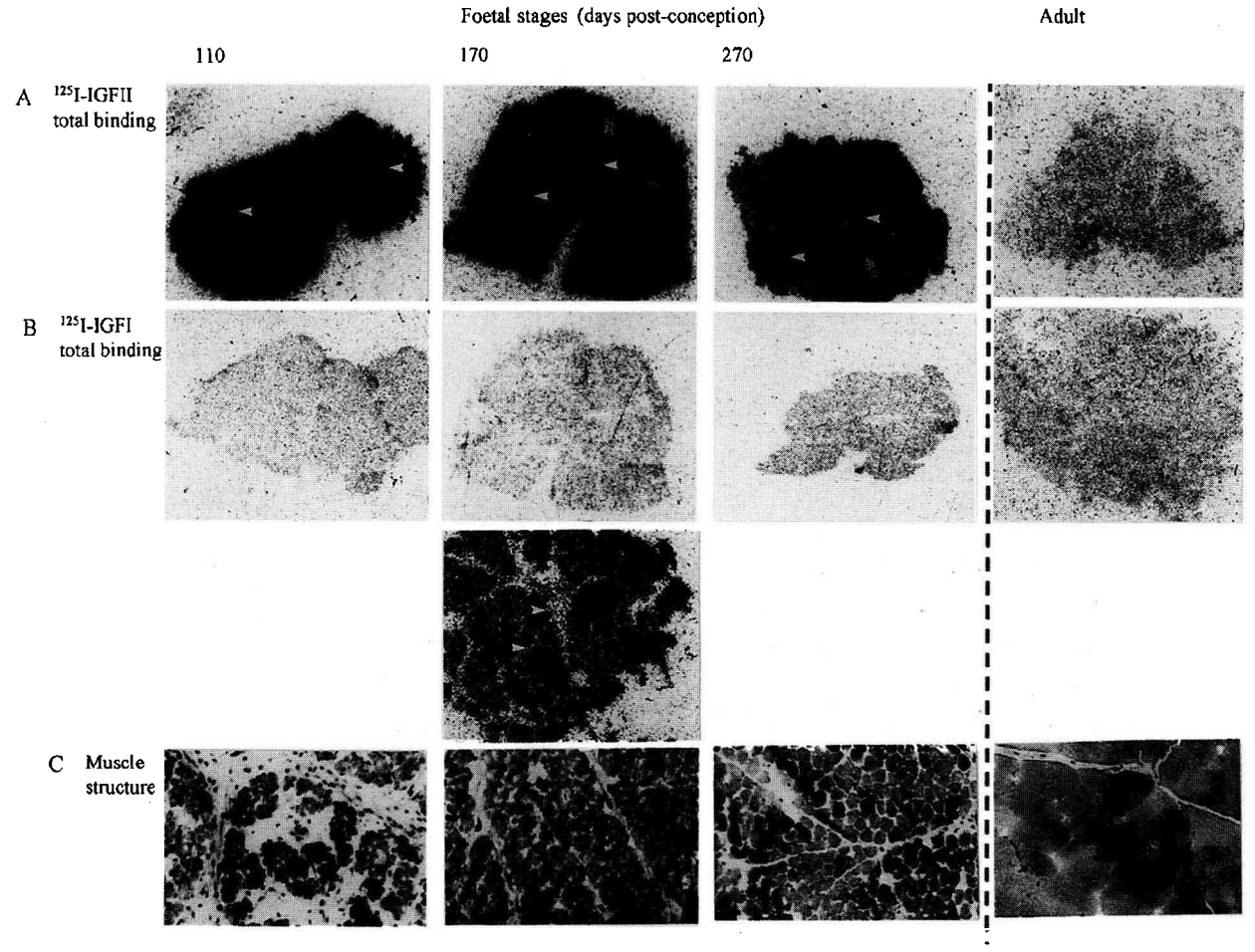

Figure 4. ^{125}I-IGF total binding at 110, 170 and 270 dpc and at the adult stage. **A)** ^{125}I-IGFII total binding (exposure time, 4 days). **B)** ^{125}I-IGFI total binding (exposure time, 7 days), inset at 170 dpc (exposure time, 15 days). Arrows indicate connective tissue (inside muscle bundles, endomysium; between muscle bundles, perimysium) (non-seriasl sections) (original magnification × 10). **C)** Histological structure of muscle tissue at 110, 170 and 270 dpc and at the adult stage. Sections were stained with haematoxylin (original magnification × 125).

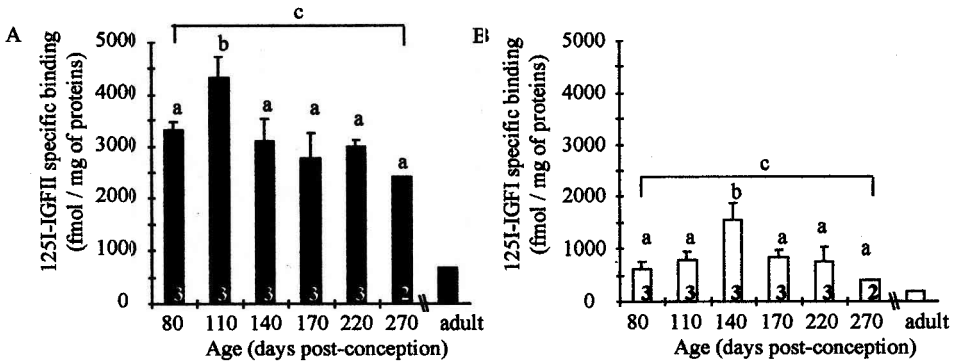

Figure 5. ^{125}I- IGF specific binding changes in foetal muscle between 80 and 270 dpc. **A)** Changes in ^{125}I- IGFII specific binding. **B)** Changes in ^{125}I-IGFI specific binding. Measurements are the result of triplicates on several foetuses (foetus number inside bars). The results are presented as the mean ± SEM of individual determinations and expressed in fmol·mg^{-1} of proteins. Specific binding at the adult stage is shown, but not compared with the foetal stages. Variance analysis was used. Values which have different index letters are significantly different: a, b, c ($P < 0.05$).

then decreased up to the end of the gestation ($P < 0.05$). At 270 dpc, they were lower than at 110 dpc ($P < 0.05$). In adult skeletal muscle sections, the specific binding of both ^{125}I-IGFII and ^{125}I-IGFI was lower than in the foetus.

As described in the preceding paragraph, the labelling obtained with ^{125}I-IGFI and ^{125}I-IGFII was heterogeneous. It was possible to quantify the specific binding in each compartment. In the connective tissue, ^{125}I-IGFII-specific binding was greater than in the muscle and remained stable in intensity throughout gestation (about 5 000 fmol per mg of protein) while ^{125}I-IGFI was not quantifiable (when exposure time was 15 days, it was near the background level). In muscle tissue, the patterns of ^{125}I-IGFII- and ^{125}I-IGFI-specific binding were similar.

3.4. Affinity constant and number of receptors

To investigate the basis for the changes in specific binding of ^{125}I-IGFII and ^{125}I-IGFI during foetal development, transformations of equilibrium binding data were performed. The binding parameters were calculated by fitting the experimental data with a non-linear least square curve. Analysis of ^{125}I-IGFII binding data performed at 110, 170 and 220 dpc in foetal muscle and in adult muscle produced linear plots at each stage, which is consistent with the presence of one affinity class of binding sites. Their number had a tendency to decrease from 110 to 220 dpc and was very low in the adult stage (*table I*). Analysis of ^{125}I-IGFI binding in foetal skeletal muscle, except at day 110 dpc, yielded a non-linear plot, which seemed to be due to the presence of two distinct binding components, the first of high affinity and low capacity, and the second of low affinity and high capacity. The number of high affinity binding sites had a tendency to decrease from 110 to 220 dpc and was low in the adult stage (*table I*). The slope of the straight line characteristic of the type II receptor and that characteristic of the high affinity component of the type I receptor remained constant during development, thereby indicating no changes in affinity for IGFII and IGFI (*table I*). Thus, the differences in binding measured in foetuses were entirely attributable to alterations in the number of type I and II receptors, with no changes in IGFI and -II binding affinity.

Table I. Presentation of binding parameters (dissociation constant (Kd), expressed in nM, and receptor number, expressed in fmol·mg^{-1} of proteins) for IGFII and IGFI, respectively, at the foetal timepoints, 110, 170, 220 dpc and in the adult state. Binding parameters were calculated by fitting the experimental data with functions for either one (IGFI) or two binding sites (IGFII) with non-linear least square curve fitting programs from Multifit. For IGFI, data correspond to the high affinity site.

Age (days post-conception)		Dissociation constant(Kd) (nM)	Receptor number (fmol·mg^{-1} of protein)
IGFII	110	1.44	2 000
	170	0.82	1 380
	220	0.83	950
	adult	0.30	165
IGFI	110	0.40	605
	170	0.35	378
	220	0.54	315
	adult	0.14	240

4. DISCUSSION

We report in this study the characterisation and location of IGFI and IGFII binding sites in the muscle during bovine foetal development. The binding specificity measured at all stages of foetal development and at the adult stage is characteristic of the presence of both IGF-type receptors in bovine skeletal muscle, type II and type I receptors. These results are in agreement with those obtained in other tissues by several authors (for a review, see Humbel [21]).

A high IGFII-specific binding was observed in foetal muscle. IGFII binding was greatest around 110 days of gestation, then significantly decreased up to 270 dpc. At the adult stage, the specific binding was very low. The analysis of the binding data showed only one type II receptor class with high affinity. The analysis of IGFI binding in foetal skeletal muscle yielded a linear plot at the beginning of gestation, whereas at 170 and 220 dpc, it yielded a non-linear plot, suggesting the presence of two binding site classes. Our results show that the first site was of high affinity and low capacity (type I) on which IGFI was ten-fold more potent to compete than IGFII and on which insulin was also able to compete, and that the second site was of low affinity and high capacity, displaced by IGFI and IGFII but not by insulin. For IGFI, the results obtained at 110 dpc are in agreement with those observed in cell cultures, C2 [44], L6 [5] and BC3H-1 [37]. It is possible, therefore, that the results obtained on cell cultures are only representative of the beginning of gestation. At 170 and 220 dpc, the characteristics measured may correspond to binding either on type II receptors or on binding proteins (IGFBP). As a matter of fact, several authors have shown the presence of IGFBP-4, -5, -6 in L6A1 muscle cell lines [12], of IGFBP-2 in C2C12 myoblasts [11], in vivo, of IGFBP-5 in muscle of mouse and chicks [22], and of IGFBP-4 mRNA in rat skeletal muscle [8]. Failure of the high concentration of insulin to compete for ^{125}I-IGFI binding suggests that the binding site of low affinity was that of binding proteins. In addition, on membrane preparation or on muscle sections, the use of an IGF analogue (long R^3 IGFI) (result not shown) does not change the results obtained. In the presence of this analogue, binding of ^{125}I-IGFI to the receptor was only inhibited at 85 or 90 % by 100 nM

of Long R^3 IGFI. The same result was obtained by Francis et al. [17].

The developmental changes in IGFII and IGFI binding were due to a decrease in the number of receptors, which, however showed no change in affinity. These results are in agreement with those of Alexandrides et al. [1] in rat skeletal muscle and of Beguinot et al. [5] on L6 cell line. In bovine muscles, Boge et al. [6] have also shown that the number of type I receptors decreased during foetal life, and then between the end of foetal life and adult age. In the rat, Sklar et al. [41, 42] have shown that the amounts of type II receptor mRNAs were proportional to those of receptors in the tissues and that their level, which was high during the foetal period in all tissues studied, decreased after birth. The changes in the number of receptors may therefore be the result of decreased transcription. As for IGFII, the developmental changes in IGFI binding were due to changes in the number of receptors, which, however, showed no change in affinity.

The location of type I and type II receptors during foetal muscle development was also determined. Type II receptors were located in the muscle tissue and in the surrounding connective tissue, IGFII-specific binding being greater in the connective tissue than in the muscle tissue. Type I receptors were located in the bundles of muscular tissue, both in the muscle cells and in the connective tissue surrounding them. For IGFI, Oldham et al. [33], in sheep muscle tissue, found a similar result, but for IGFII, unlike us, he did not find binding in the muscle fibres. These results obtained in different species lead us to suppose that the localisation of binding of both the IGF can vary during development but also between species. By in situ hybridisation, we observed that the expression of the IGFII gene during bovine foetal life was inside the muscle bundles, as are type I and II receptors [27]. So there was a co-location of IGFII mRNA and of type I and type II receptors on foetal muscle bundles. In addition, the IGFII mRNA [27] and the type I and II receptors are co-ordinately synthesised at the same foetal stages. Other studies on rats and pigs demonstrate the same evolution pattern for IGFII gene expression [4, 25] and for type I and type II receptors [1, 25]. At the adult stage, a simultaneous decrease in the three components was observed. If these transcripts are translated into protein similar to that shown by Brown et al. [7], a local production of IGFII suggests an auto/paracrine action via type I and type II receptors present in the same tissue. In the bovine, the presence of the IGFII mRNA and both the IGFs receptors coincides with muscle differentiation [35]. This coincidence is found in other species such as in the ovine [2, 34], in the porcine [3, 25], and in the murine species [4, 38]. Thus, in muscle cells, in vivo, IGF-II might act as an autocrine pathway on muscle cell differentiation, as has been shown in vitro by Florini et al. [15].

It is now admitted that the effects of the IGFs on proliferation and differentiation are mediated by the type I receptor (for a review, see Florini et al. [16]; Navarro et al. [32]). However, the physiological consequences of IGFII binding to the type II receptor (IGFII/Mannose-6-phosphate receptor) are not fully understood. During muscle differentiation, IGFII could modulate the lysosomal enzyme traffic by competing with these enzymes at the IGFII/mannose 6-P receptor level, suggesting that IGFII has a role in tissue remodelling [40–42]. Type II receptor could also act as 'a sink' to avoid elevated IGFII (for a review, see Florini et al. [16]).

The results obtained in this study show that type II and type I receptors are present in the same patterns during bovine muscle development, but that their respective location and their levels are different.

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