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ORIGINAL ARTICLE

INSULIN-LIKE GROWTH FACTOR BINDING PROTEINS FROM ADULT-HAMSTER PANCREATIC ISLETS : INFLUENCE OF GLUCOSE CONCENTRATION

L. MASSA (1), A.M. CORTIZO (2), J.J. GAGLIARDINO (1)

SUMMARY - This study investigated the effect of glucose on insulin-like growth factor binding proteins (IGFBPs) in islets isolated from pancreas of adult hamsters and compared the response pattern with that of their serum IGFBPs. Serum samples and islets were obtained from adult normal male hamsters, and IGF-binding capacity was measured in aliquots of serum, sonicated islets, or conditioned medium using either ^{125}I-hIGF-I or -II. IGFBPs were characterized in these samples by the ligand-blotting technique, and insulin was measured in conditioned medium by radioimmunoassay. Three IGFBP fractions were identified in serum, with relative molecular weights of 38, 30-33, and 24 kDa, while only two fractions of 30-33 and 24 kDa were identified in islets or in their conditioned medium. Islets cultured with 2 or 16 mM glucose for 48 h released more insulin in the presence of the higher glucose concentration. The binding capacity measured in the islet suspension or conditioned medium increased as a function of glucose concentration in the incubation medium. The IGFBPs present both in islets and conditioned medium had a 3- to 4-fold higher apparent affinity for IGF-II than IGF-I. The higher glucose concentration increased the intensity of the two IGFBP bands identified in the islet suspension by 2- to 3-fold. Our data show that two low-molecular-weight IGFBPs were released from adult hamster pancreatic islets, with a different distribution pattern from that of hamster serum, and that the amount of IGFBPs released by islets depended on the glucose concentration in the culture medium. Though not conclusive, these data suggest that IGFBPs may play a regulatory role in B-cell turnover in adult islets as they do in foetal islets. *Diabetes & Metabolism 1997, 23, 417-423.*

Key-words : hamster, isolated islets, islet culture, insulin-like growth factor binding proteins, islet function.

RÉSUMÉ - **Protéines de liaison pour les facteurs de croissance apparentés à l'insuline dans les îlots de pancréas de hamsters adultes : influence de la concentration en glucose.** Le but de ce travail est d'étudier les effets du glucose sur les protéines de liaison des facteurs de croissance apparentés à l'insuline (IGFBP) d'îlots isolés de pancréas d'hamsters adultes, et de comparer leur réponse à celle des IGFBP dans le sérum. Dans ce but, des échantillons de sérum et d'îlots ont été obtenus à partir de hamsters mâles adultes normaux. La capacité de liaison des IGF a été mesurée avec de l'^{125}I-hIGF-I ou -II dans des aliquots de sérum, d'îlots ou de milieu de culture conditionné. Les IGFBP ont été caractérisées dans ces échantillons par la technique de « ligand-blotting », tandis que l'insuline a été dosée dans le milieu de culture par méthode radioimmunologique. Trois fractions d'IGFBP, de poids moléculaires relatifs de 38, 30-33 et 24 kDa, ont été identifiées dans le sérum, alors que seulement deux fractions de 30-33 et 24 kDA ont été mises en évidence dans les îlots et le milieu de culture. Les îlots cultivés avec du glucose 2 ou 16 mM pendant 48 heures sécrètent plus d'insuline en présence de la plus haute concentration de glucose. La capacité de liaison des IGF, mesurée dans la suspension d'îlots ou dans le surnageant de culture, augmente en fonction de la concentration de glucose dans le milieu d'incubation. Les IGFBP présentes dans les îlots et le milieu de culture ont une affinité apparente 3 et 4 fois plus élevée pour l'IGF-II que pour l'IGF-I. La concentration supérieure de glucose accroît d'environ 2 à 3 fois l'intensité des deux bandes d'IGFBP identifiées dans la suspension d'îlots. Nos observations montrent que : (a) deux IGFBP de faible poids moléculaire sont secrétés par les îlots de pancréas d'hamsters adultes, avec un schéma de distribution différent de celui observé dans le sérum ; et (b) la quantité d'IGFBP libérée par les îlots dépend de la concentration de glucose dans le milieu de culture. Même si elles ne permettent pas de conclure de façon définitive, nos données suggèrent donc que les IGFBP pourraient jouer un rôle de régulation dans le turnover des cellules B dans les îlots adultes comme elles le font dans les îlots fœtaux. *Diabetes & Metabolism 1997, 23, 417-423.*

Mots-clés : hamster, îlots isolés, culture d'îlots, IGFBP, fonction insulaire.

✍ : J.J Gagliardino, CENEXA (UNLP-CONICET), Facultad de Ciencias Médicas, UNLP, Calles 60 y 120, 1900 La Plata, Argentina. Tel : 54 21 83 63 03. Fax : 54 21 22 20 81.
E-mail : gagliard@isis.unlp.edu.ar
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(1) CENEXA - Centro de Endocrinología Experimental y Aplicada (UNLP-CONICET), Facultad de Ciencias Médicas, UNLP, 60 y 120, 1900 La Plata. (2) Cátedra de Bioquímica Patológica, Facultad de Ciencias Exactas, 47 y 115, UNLP, 1900 La Plata, Argentina.

The total mass of pancreatic B cells, which is a critical regulatory factor of glucose homeostasis in most species, depends on the rate of replication of differentiated B cells as well as on the genesis of new islets from proliferating ductules [1]. The rate of B-cell death might also be an ongoing and important factor in the regulation of B-cell mass [2].

Glucose stimulates the rate of B-cell genesis and replication [1] while decreasing that of B-cell death [3]. Although the mechanism by which glucose produces these effects is poorly understood, several growth factors, such as insulin-like growth factors (IGF)-I and -II, might participate in this process [4-6] in both paracrine and autocrine fashion [7-10]. In this regard, it has been shown that isolated foetal islets in culture release IGF-I in response to different metabolic and hormonal stimuli [7, 11].

IGFs circulate in serum and in other biological fluids relative to a family of six specific IGF binding proteins (IGFBPs) [5, 12, 13] that have been purified and structurally characterized. These IGFBPs not only transport IGFs but may also modulate their action by prolonging their half-life as well as by regulating both their clearance and their access to specific receptors [5, 12, 13].

The presence of mRNA specific for IGFBP-1 and -2, as well as the secretion of the encoded proteins, has been demonstrated in foetal rat islets [11]. Pure preparations of IGFBP-1 and -2 were also found to cause a synergistic increase in DNA synthesis in the presence of low doses of IGF-I and -II [11]. This evidence suggests that IGFBPs may play a role in foetal B-cell proliferation. Otherwise, the available information on the mitogenic action of the IGF/IGFBP axis on adult islets of Langerhans is both scanty and controversial [14, 15]. Moreover, no study reported to date has examined the possible control of the content and release of IGFBPs *in vitro* in adult islets. Accordingly, we studied the effect of glucose on the IGFBPs of islets of Langerhans isolated from adult hamsters and compared the response pattern with that of their serum IGFBPs. The Syrian golden hamster was selected as the experimental model because this strain responds to stimuli that can alter pancreatic growth and development but has a low incidence of spontaneous pancreatic tumors [4].

MATERIALS AND METHODS

Materials – Recombinant human IGFs (hIGF-I and hIGF-II) were purchased from Biochem Inc. (Torrance, CA), ^{125}I from Amersham (U.K.), RPMI 1640 from Gibco, and foetal-bovine serum from Gen (Argentina). Fatty-acid-free bovine-serum albumin (BSA), X-Omat Kodak film, prestained molecular-weight-marker proteins, and other reagents of the purest available grade were obtained from Sigma Chemical Co. (St. Louis, MO).

Animals and islet culture – Male Syrian golden hamsters (Unci : SYR) from the Eppley colony, maintained in a room at 21 ± 1 °C and 50 ± 5 % humidity with a 12-h light-dark cycle, were housed in plastic cages and fed with a commercial pelleted diet (Purina chow) and water *ad libitum*. Animals 8 weeks of age (body weight 100 ± 1.9 g) were sacrificed by cervical dislocation, and the entire pancreas was removed for islet isolation. Blood samples (2-3 ml), previously obtained from the retroorbital plexus under light aether anesthesia, were left to clot, then centrifuged at 4 °C and stored at – 20 °C for not longer than one week.

One hundred and fifty islets isolated by collagenase digestion [16] were cultured for 24 h at 37 °C in 250 µl of RPMI 1640 (islet/volume ratio 0.6), pH 7.4, containing 10 % (v/v) foetal bovine serum and 2 mM glucose in a humid atmosphere (5 % CO_2/95 % O_2 [v/v]).

After 24 h of culture, the islets were separated from the medium, rinsed twice in fresh serum-free RPMI culture medium, and cultured for a further 48 h in 250 µl RPMI 1640 plus 0.5 % (w/v) fatty acid-free BSA and the glucose concentrations indicated in the legends to the figures. An optimal ratio of the number of islets to the volume of culture medium (0.6) was selected for measurement of IGFBPs either by their binding capacity or Western blot analysis. Finally, islets from each well were separated from the conditioned medium, resuspended in 30 µl of 50 mM Tris-HCl, pH 7.4, and disrupted by ultrasound. The conditioned medium was concentrated by lyophilizing to a final volume of 30 µl. Both the islet-lysate suspension and the conditioned medium were maintained at – 20 °C until analysis.

Binding capacity – To measure IGF-binding capacity, different aliquots of hamster serum, islet lysates, or conditioned medium were incubated with ^{125}I-hIGF-I or ^{125}I-hIGF-II as previously described [17]. Briefly, samples were incubated for 3 h at 4 °C in the presence of ^{125}I-hIGF (10,000 cpm) with or without addition of an excess of unlabelled hIGF-I or -II, respectively, in a total volume of 500 µl of binding buffer (0.02 % [w/v] protamine sulphate, 0.3 mM NaH_2PO_4, 1.53 mM NaN_3, 0.01 M EDTA, 0.25 % [w/v] fatty-acid-free BSA, pH 7.5). One ml of 1 % (w/v) charcoal dextran in binding buffer was then added to each tube, and the mixture was incubated for another 10 min at 4 °C before the samples were centrifuged at 4,434 g for 10 min and ^{125}I-hIGF-IGFBPs complexes present in the supernatant were measured in a well-type gamma counter.

Western ligand-blotting – IGFBPs were characterized by means of the ligand-blotting technique described by Hossenlopp *et al.* [18]. Five microlitres of hamster serum, 30 µl of islet-lysate suspension, and 30 µl of a given conditioned medium sample were mixed with a fourfold-concentrated sample buffer (62.5 mM Tris-HCl, pH 6.8 ; 2 % [w/v] sodium dodecylsulfate [SDS] ; 10 % [v/v] glycerol ; and 0.001 % [w/v] bromophenol blue) [19] under nonreducing conditions and boiled for 3 min before being loaded onto a 12.5 % (w/w) SDS polyacrylamide gel. Samples were then electrophoresed at 200 V until the dye front reached the bottom of the gel. Prestained molecular-weight-marker proteins were run in a parallel lane. The proteins were next transferred to nitrocellulose membranes at room temperature in a 15 mM Tris base-120 mM glycine buffer, pH 8.3, containing 5 % (v/v) methanol. Electroblotting was performed under a constant current

of 100 mA for 16-18 h. After Western transfer, the gels were stained to detect any untransferred proteins. Nitrocellulose was blocked at 4° C and incubated with ^{125}I-hIGF-I or -II (350-400 µCi/µg) (3×10^5 cpm/ml) in 1 % (w/v) fatty-acid-free BSA and 0.1 % (v/v) Tween 20 in transfer buffer (150 mM NaCl ; 10 mM Tris-HCl, pH 7.4 ; 0.5 mg/ml NaN_3). To measure the amount of labelled IGF bound to each IGFBP fraction, the nitrocellulose membrane was cut into strips which were counted in a gamma counter.

The specificity of ^{125}I-hIGF-I binding to each IGFBP was verified through complete displacement of the tracer by addition of 2.5 µM unlabelled.hIGF-I to the incubation medium (data not shown). No IGFBPs were detected by Western ligand-blotting in nonconditioned media run in parallel as a blank. Following this incubation, the membranes were washed and autoradiographed using X-Omat Kodak film and a Dupont intensifying screen for approximately 3 days.

Insulin assay – Insulin was measured in aliquots of conditioned medium by radioimmunoassay [20] using an antibody against rat insulin, a rat-insulin standard obtained from Linco Research, Inc. (MI, U.S.A.), and highly-purified ^{125}I-labelled porcine insulin according to Linde *et al.* [21].

■ RESULTS

The capacity of IGF binding of hamster serum to both ^{125}I hIGF-I and -II is shown in *Figure 1A*. In both instances, specific binding increased as a function of the serum volume used, attaining maximal values at 20 µl and 5 µl with the two respective tracers. For each serum volume tested, higher binding values were obtained when ^{125}I-hIGF-II instead of -I was used as tracer.

Western blot analysis of the serum samples showed 3 IGFBP fractions with relative molecular weights of 38, 30-33, and 24 kDa (*Fig. 1B*). Eighty-five percent of the ^{125}I-hIGF associated with these bands was bound to the 38 kDa species, which possibly represents IGFBP-3.

Figure 2A shows that the binding of ^{125}I-hIGF to the islet suspension increased, though not linearly, as a function of the number of islets present in the sample, reaching a maximal value at 10 islet equivalents. The binding capacity of different aliquots of conditioned medium incubated with ^{125}I-hIGF also increased in relation to the volume employed, attaining a plateau at a volume of 50 µl (*Fig. 2B*). Taken together, these results suggest that hamster pancreatic islets contain and release a component with ligand activity for ^{125}I-hIGF.

When the nature of this ^{125}I-hIGF-binding activity was characterized by Western ligand-blotting, two bands of IGFBPs with apparent molecular weights of 30-33 and 24 kDa were detected in both the islet suspension and the conditioned medium (*Fig. 3*). Consistent with the observations on ligand-binding kinetics, the intensity of these bands increased as a function of either the number of islets present in the islet suspen-

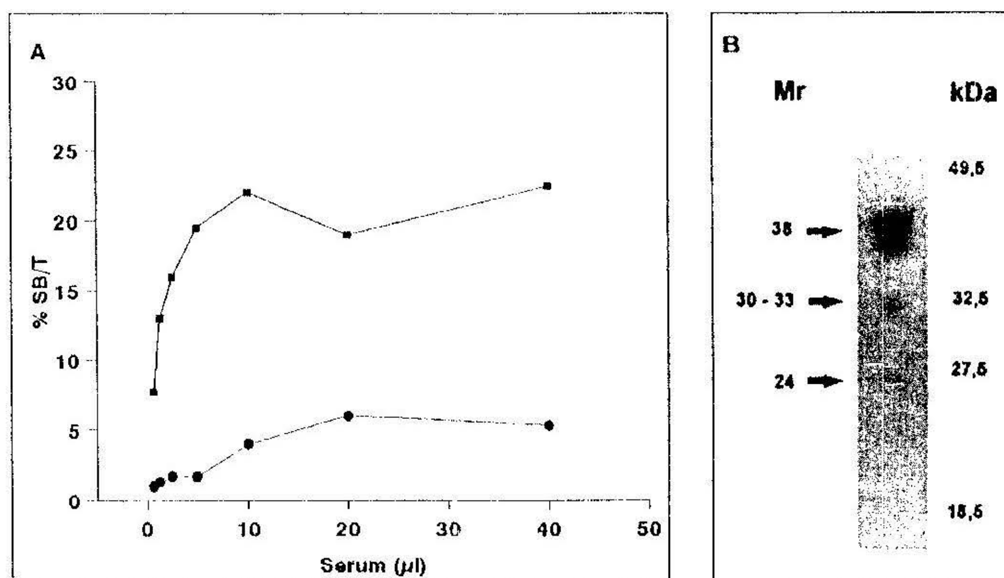

FIG. 1. *Binding capacity (A) and Western ligand-blotting (B) of serum from the adult Syrian hamster with ^{125}I-hIGF species as ligands. (Panel A) Different aliquots of serum were probed with ^{125}I-hIGF-I (circles) or ^{125}I-hIGF-II (squares). The percent specific binding relative to the total number of counts present is plotted as a function of the serum volume tested. The total radioactivity in each incubation was 10,000 cpm for each of the two labelled hIGFs. (Panel B) The IGFBPs in 5 µl serum were fractionated and characterized by Western ligand-blotting with ^{125}I-hIGF-I as a tracer. Positions of molecular-weight markers are shown on the right, while the locations of the IGFBP bands are indicated by arrows on the left.*

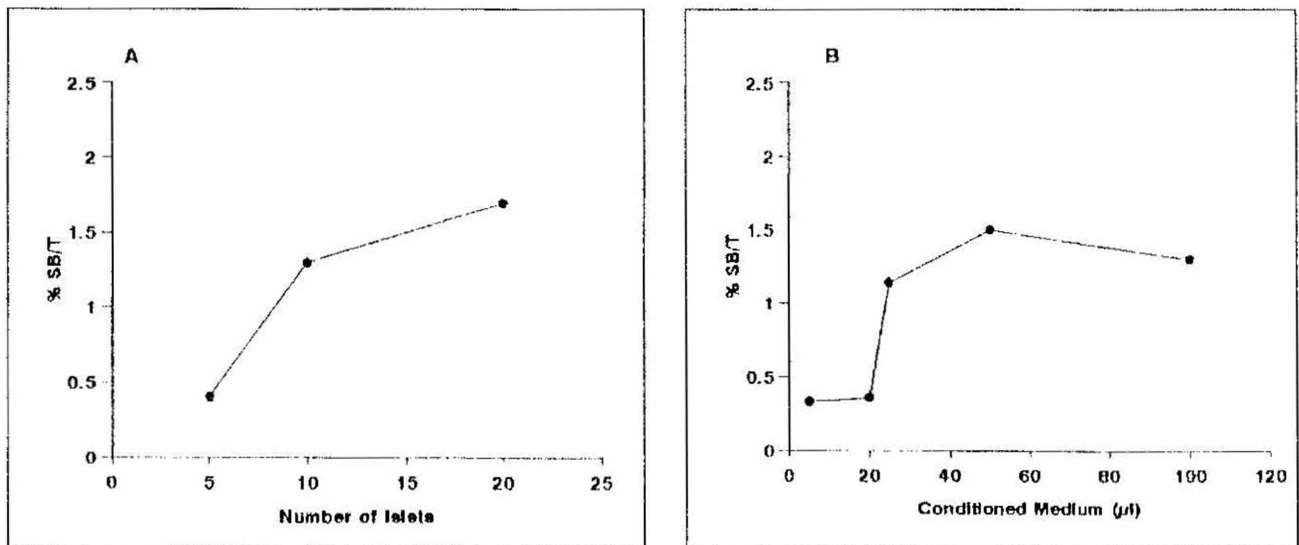

FIG. 2. *^{125}I-hIGF binding capacity of islet suspensions and conditioned medium. Isolated islets were cultured at 37 °C for 4 h in serum-free RPMI 1640 medium containing 2 mM glucose and assayed for ^{125}I-hIGF binding as described in Materials and Methods. Total counts bound minus nonspecific binding are plotted as a function of the number of islet equivalents in the suspension volume tested (Panel A) or the aliquot volume added from an 8-fold-concentrated preparation of CM (Panel B). The tracers were ^{125}I-hIGF-I and ^{125}I-hIGF-II, respectively. The total radioactivity in each incubation was 10,000 cpm for the labelled hIGF.*

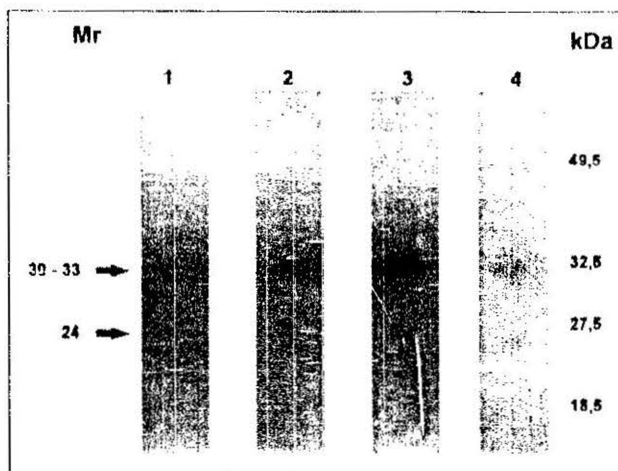

FIG. 3. *Western-ligand-blot analysis of islet suspensions and conditioned media cultured for 48 h in serum-free RPMI medium plus 16 mM glucose with ^{125}I-hIGF-I as a tracer. Positions of molecular-mass markers (in kDa) are shown on the right. The two IGFBP species migrating at 30-33 and 24 kDa are indicated by arrows on the left. The samples analysed were suspension volumes corresponding to 30 (Lane 1), 70 (Lane 2), or 140 islets (Lane 3), or 30 µl of conditioned medium (Lane 4).*

sion or the volume of conditioned medium tested. The major band of 30-33 kDa bound about 80 % of the ^{125}I-hIGF, while the less-pronounced band at 24 kDa accounted for the remaining 20 %. This pattern differed markedly from that found with hamster serum (*Fig. 1B*) in which a 38 kDa heavier band (not detected in islets or conditioned medium) was the main component.

Islets cultured for 48 h under basal (2 mM glucose) or stimulating (16 mM glucose) conditions released more insulin in the presence of the higher concentration of the sugar (1.2 ± 0.43 *vs.* 0.15 ± 0.03 ng/islet/h, $n = 9$, $p < 0.02$). Under these culture conditions, the values for the binding capacity of either islet suspension (*Fig. 4A*) or conditioned medium (*Fig. 4B*) with respect to ^{125}I-hIGF-I or -II was in each instance higher when the islets were cultured in the presence of 16 mM glucose than when the sugar concentration was 2 mM. However, these differences only became statistically significant with ^{125}I-hIGF-II as a tracer. We also observed that the IGFBPs present both in islets (*Fig. 4A*) and conditioned medium (*Fig. 4B*) had a higher (3- to 4-fold) apparent affinity for IGF-II than IGF-I, regardless of the glucose concentration to which the islets had been previously exposed.

Suspensions of islets cultured with 2 or 16 mM glucose, as well as their corresponding conditioned media, had the same pattern of 30-33 and 24 kDa IGFBPs (*Fig. 5*). High glucose concentrations increased the intensity of these two IGFBP bands in the islet suspension by 2- to 3-fold. Conversely, no IGFBPs were detected in the conditioned medium of islets cultured with 2 mM glucose, whereas these binding proteins were clearly observed in the presence of 16 mM glucose.

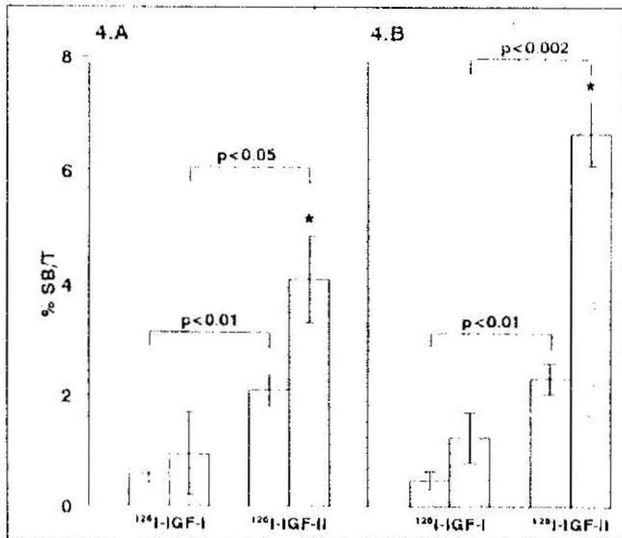

FIG. 4. *Effect of glucose on the binding capacity of islet suspensions and conditioned media. Isolated islets were cultured at 37 °C for 48 h in 2 (open bars) or 16 (stippled bars) mM glucose. Aliquots of 10 islet equivalents of the suspension (Panel A) or of 50 µl of the conditioned medium (Panel B) were incubated with either ^{125}I-hIGF-I (n = 3) or ^{125}I hIGF II (n = 5). Results are expressed as the percent of specific binding relative to the total number of counts. The values represent the mean ± SEM of 3 experiments performed in duplicate on different days and in different groups of islets obtained from 3 animals. Differences for 2 vs. 16 mM glucose are : Panel A, *p < 0.05 ; Panel B *p < 0.01. The total radioactivity in each incubation was 10,000 cpm for each of the two labelled hIGFs.*

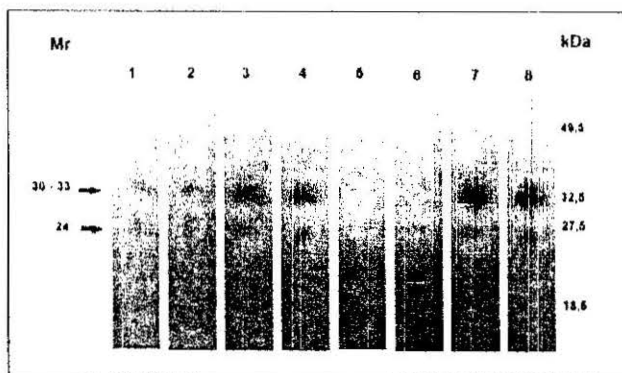

FIG. 5. *Effect of glucose concentration on the IGFBP pattern in islet suspensions and conditioned medium as detected by Western ligand-blotting with ^{125}I hIGF-I as a tracer. The samples analysed were islet extracts cultured with 2 (Lanes 1 and 2) or 16 (Lanes 3 and 4) mM glucose, or 30 µl conditioned medium from incubations with 2 (Lanes 5 and 6) or 16 (Lanes 7 and 8) mM glucose. Positions of molecular-weight markers are shown on the right, while the locations of the two IGFBPs migrating at 30-33 and 24 kDa are indicated by arrows on the left.*

DISCUSSION

Our results demonstrate for the first time that three IGFBP fractions with relative molecular weights of 38, 30-33, and 24 kDa are present in the serum of adult hamsters. Since the characterization of the various IGFBP species was not done by immunoblotting, we could only presume their identification as IGFBP-3 (38 kDa), IGFBP-1/-2 (30-33 kDa), and IGFBP-4 (24 kDa) on the basis of their apparent molecular weights [22] and their higher affinity for IGF-II than IGF-I [13]. Their relative proportion was similar to the IGFBP species observed in human serum, in which IGFBP-3 (38-40 kDa) represents some 80-90 % of the ^{125}I-IGF bound to the various peptide fractions. However, in rat serum, IGFBP-3 represents 60-70 %, while low-molecular-weight IGFBPs seem to be in greater abundance than in other mammalian systems [23].

Of the three IGFBP species present in hamster serum, only the two low-molecular-weight forms were detected in islet suspensions and conditioned media from adult hamster pancreas. This IGFBP pattern differed from that reported in foetal rat islets in which a 45 kDa fraction, probably identifiable as IGFBP-3, was observed [11]. However, mRNAs specific for only IGFBP-1 and -2 were detected in that study. Our failure to observe a high-molecular-weight IGFBP in both islet extracts and conditioned media cannot be attributed to an experimental artifact since the binding of ^{125}I-hIGF-I or -II clearly increased as a function of islet number and the 45-kDa species was detected without any difficulty in hamster serum.

It is noteworthy that the islets were cultured for one day in the presence of foetal-bovine serum in which there is only a small proportion of high-molecular-weight IGFBPs [23], and that they were then washed and incubated for 48 h in the absence of serum. Under such experimental conditions, even in the absence of direct measurement of IGFBP synthesis in islets, the presence of IGFBP species in the pancreas and not in serum suggests an endogenous origin.

The islets released insulin in culture as a function of the glucose concentration in the medium, thus demonstrating that the experimental conditions selected were appropriate for testing the protein-secretory capacity of the islets in response to stimulation by sugar. The two lower-molecular-weight IGFBPs (30-33 and 24 kDa) were also detected in samples of islets cultured with different concentrations of glucose (2 and 16 mM). The IGFBP content of islet suspensions and their corresponding conditioned media, as assessed by ligand-blotting or by IGF-binding capacity, increased significantly after islet exposure to high glucose, thus suggesting that the secretion, and presumably also the synthesis, of IGFBPs was stimulated by hexose metabolism within the islets. Since both the content and the release of IGFBPs increased at the same time in response to glucose stimulation, we considered that the islets secreted IGFBPs constitutively rather than through some regulatory mechanism involving membrane transport [11, 14]. Conversely, no IGFBPs of

any molecular-weight class were found in conditioned medium from islets cultured with 2 mM glucose by Western ligand-blotting. The failure to detect IGFBPs under such conditions was probably due to their low steady-state concentration rather than to a lack of release. The lower, though still detectable, binding capacity measured in the conditioned medium of islets cultured in 2 mM glucose offers support for this assumption.

Regardless of the glucose concentration to which the islets had previously been exposed, the apparent affinity of their IGFBPs was greater for ^{125}I-hIGF-II than ^{125}I-hIGF-I. The precise mechanism by which glucose stimulates the release of IGFBP, as well as the nature of the islet-cell component(s) responsible for this process or for IGFBP synthesis, is still unknown. In this regard, evidence obtained in other systems (human and rat osteosarcoma cell lines) suggests that levels of IGFBP-4 [24, 25] as well as its mRNA [26] are raised by cAMP; hence, a glucose-induced increase in islet cAMP content might account for the stimulatory effect of sugar on IGFBP-4 secretion.

Nonetheless, hypoinsulinaemia and fasting have been shown to increase the amount of hepatic IGFBP-1- and IGFBP-2-specific mRNAs, as well as the circulating levels of IGFBP-1 and, to a lesser degree, of IGFBP-2 [27, 28]. In other studies, the production of IGFBP-1 in human-foetal-liver explants was found to be inversely regulated by glucose [29], while the secretion of IGFBP-2 by a human-embryonic-kidney cell line increased in response to IGF-I and insulin as a result of overall stimulation of protein synthesis [30]. All these diverse data suggest that the action of glucose on IGFBP release may vary between tissues and at different stages in ontogenic development.

Hogg *et al.* [11] reported that a step-up in glucose concentration from 1.4 to 16.7 mM was accompanied by an increase in the release of insulin, IGF-II, and all four IGFBP species, as well as an elevation in the rate of DNA synthesis, in isolated foetal-rat islets. These authors also showed that exogenous IGFBP-1 and -2, synergized with subthreshold concentrations of IGF-I or -II, increased the rate of islet DNA synthesis. On the basis of those results, they suggested that glucose, IGFs, and IGFBPs interact to promote islet-cell hyperplasia during late gestation.

The mechanism by which IGFBP-1 and -2 potentiate IGF-stimulated DNA synthesis is not known, but it has been suggested that the binding of these species to the cell membrane may create a high concentration of IGF-I or -II at the cell surface for presentation to high-affinity receptors [31, 32]. Since IGFBP release was upregulated by glucose in islets from both foetal rats and adult hamsters *in vitro*, it is possible that a similar effect occurs *in vivo*, in which case nutrients may modulate islet-cell growth (at least partly) through IGFBP-mediated potentiation of IGF activity.

A recent report showed that the apoptosis rate of B cells is lower after exposure to high glucose, and that this protective effect disappears if protein synthesis is blocked [3]. Accordingly, within the array of proteins synthesized and released by islets in response to glucose, the IGF : IGFBP complex may constitute one of the components responsible for the antagonistic effect of sugar on B-cell apoptosis.

In conclusion, our data clearly show that two low-molecular-weight IGFBPs are released from adult hamster pancreatic islets, that the IGFBP distribution pattern is different from that of hamster serum or foetal-rat islets, and that islet IGFBP release depends on the glucose concentration in culture medium. These results suggest that IGFBPs may play a regulatory role in B-cell turnover within adult islets of Langerhans in the hamster, just as these proteins appear to do in islets from foetal animals.

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