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Glucose does not influence the insulin-like growth factor (IGF) binding to carrier proteins (IGFBPs): Analysis of rat and human serum by western ligand blotting

S. Cianfarani[a,b], V. J. Frost[b], M. O. Savage[c] and J. M. P. Holly[b]

[a]Clinica Pediatrica, 'Tor Vergata' University, via O. Raimondo 8, I-00173 Rome (Italy), [b]Department of Chemical Endocrinology, and [c]Division of Pediatric Endocrinology, Department of Endocrinology, St. Bartholomew's Hospital, London EC1A 7BE (England)

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Abstract. The insulin-like growth factors (IGFs) circulate bound to specific proteins (termed IGFBP-1 through IGFBP-6) that modulate IGF bioactivity in tissues. The aim of this study was to analyse the effects of glucose on IGF binding to IGFBPs in rat and human serum by means of western ligand blotting. Serum samples were incubated with increasing concentrations of glucose (0 to 50 mmol/l), and EDTA (25 mmol/1) was added to inhibit protease activity. To analyse the effect of glucose on protection of IGFBPs from protease activity, serum from pregnant women (reported to be very rich in proteolytic activity against IGFBPs) was added to rat serum previously incubated with glucose. Glucose did not affect the ^{125}I-IGF binding to rat and human serum IGFBPs. The intensity of IGFBP-3 bands decreased considerably during the incubation. This appeared to be due to endogenous protease activity, since the decrease was blocked by addition of EDTA. The incubation of rat serum with pregnant human serum produced a marked attenuation of IGFBP-3 and disappearance of IGFBP-4 bands. In conclusion, our study shows that glucose does not influence the IGF binding to IGFBP-3 either in rat or in human serum, confirms the presence of endogenous proteolytic activity in normal non-pregnant serum, and demonstrates that glucose has no protective action against protease activity.

Key words. IGF; IGFBP; diabetes; glucose; proteases.

The insulin-like growth factors, IGF-I and IGF-II, circulate bound to specific proteins (IGFBPs) that prolong the IGF half-life, help to transport IGF to the various tissues, and modulate IGF bioactivity[1]. To date six structurally distinct human IGFBPs (termed IGFBP-1 through IGFBP-6) have been identified[2].

In insulin-dependent diabetes mellitus, IGF bioactivity is reduced[3] and IGFBP-1 levels are increased, being inversely related to insulin[4]. In addition, IGF bioactivity is inversely related to the levels of IGFBP-1, which therefore has been suggested to be one of the IGF inhibitors[5].

Cortizo and Gagliardino[6] have recently reported a glucose-dependent increase in IGF binding to IGFBP-3 in rat serum. They have suggested that the protein glycation might enhance the IGFBP-3 affinity for IGFs, thus reducing the amount of bioactive free IGF, and eventually leading to the decreased IGF bioactivity observed in diabetes.

In view of this observation we set out to analyse the effects of glucose on IGF binding to IGFBPs in rat and human serum using the technique of western ligand blotting.

Materials and methods

Pools of normal adult rat (male Wistar, 140–160 gm) and human sera were studied. Recombinant human IGF-I and IGF-II were purchased from Kabi, Stockholm, Sweden. Samples were incubated for 48 h at 37 °C in the presence of different concentrations of glucose (0, 5, 25, 50 mmol/l). EDTA (25 mmol/l) was added to inhibit protease activity. To analyse the effect of glucose on protection of IGFBPs from protease activity, serum from women in the third trimester of pregnancy was incubated at 37 °C for 4 h with rat serum (50% v/v) previously incubated with glucose. To rule out any effect of the incubation time on glucose stability in serum samples, we also tested different incubation times: 2 h, 6 h and 24 h. Fructosamine concentrations of the samples were assessed as an index of protein glycation (Test-combination Fructosamine, Boehringer, Mannheim, Germany)[7].

The binding of IGFs to IGFBPs was examined by western ligand blotting using a method based on that described by Hossenlopp et al.[8] with minor modifications as recently published[9]. Samples were diluted with buffer (0.15 mol Tris-HCl/l, pH 6.8, 6% (w/v) SDS, 22% (v/v) glycerol and 0.02% (w/v) bromophenol blue), and electrophoresed through a 12.5% SDS polyacrylamide gel. Electrophoresed proteins were then electroblotted onto Hybond-C Extra (Amersham International plc, Amersham, Berks, U.K.) at 4 °C for 4 h at constant current (0.8 A). After blotting, the Hybond-C was probed with ^{125}I-labelled recombinant IGF-I or IGF-II (1×10^4 c.p.m./100 µl/10 sec) for 2 h at room temperature. Radiolabelled IGFBPs were visualized by autoradiography using X-Omat Kodak film, after 48 h exposure.

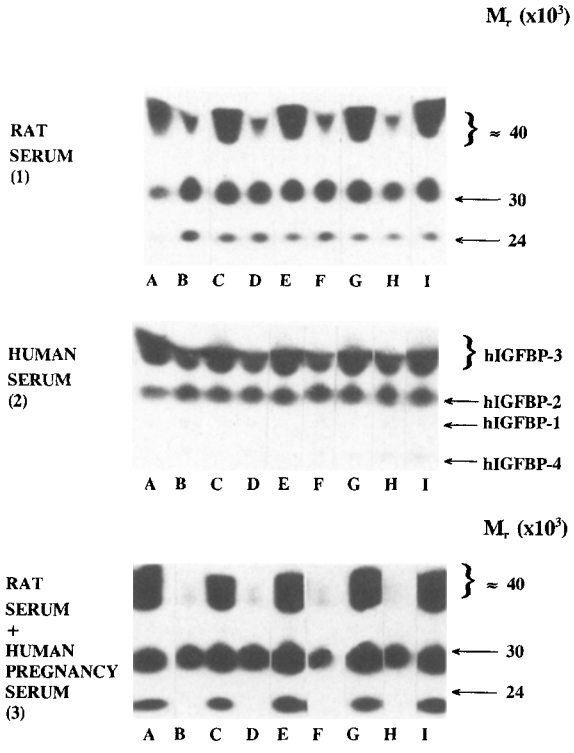

Figure. Western ligand blot analysis of 1, rat serum; 2, human serum; and 3, rat serum incubated with human pregnancy serum. Samples and molecular mass markers were incubated with SDS loading buffer, electrophoresed, electrotransferred, and probed with ^{125}I-IGF-I. The nitrocellulose was exposed to x-ray film for 48 h. Molecular mass of rat serum bands is reported in panels 1 and 3. The 40 Kd band corresponds to IGFBP-3, the 30 Kd band to a mixture of IGFBP-2, IGFBP-1 and N-terminal fragment of IGFBP-3; the 24 Kd band corresponds to IGFBP-4. Human serum bands are directly identified by names (panel 2). Lane A, unincubated serum; lanes B-D-F-H, samples incubated (at 37 °C for 48 h) with increasing concentrations of glucose (0, 5, 25, 50 mmol/l respectively); lanes C-E-G-I, corresponding samples incubated with increasing concentrations of glucose and added EDTA (25 mmol/l).

Results and discussion

Increasing concentrations of glucose did not affect the ^{125}I-IGF binding to rat and human serum IGFBPs (fig., panels 1 and 2), despite a dose-dependent increase in fructosamine levels (from 173 to 439 µmol/l in rat serum; from 393 to 967 µmol/l in human serum).

In rat serum the intensity of IGFBP-3 bands decreased considerably over the 48 h of incubation. This appeared to be due to endogeneous protease activity since the decrease could be blocked by addition of 25 mmol/l EDTA but not by 1 mmol/l.

The incubation of rat serum with pregnant human serum produced a marked attenuation of IGFBP-3 and the disappearance of IGFBP-4 bands. These changes were blocked by addition of EDTA (fig., panel 3) but not by increasing glucose concentrations.

Finally, varying the incubation times with increasing concentrations of glucose had no observable effect on ^{125}I-IGF binding to IGFBPs.

Recently, the glucose-dependent glycation of IGFBPs has been reported to induce an enhancement of IGF binding in rat serum, thus reducing the amount of bioactive free IGFs[6]. It has been proposed that a glycation near the IGF binding sites of IGFBP-3 might modify its binding properties[6]. We previously reported that the appearance of IGFBPs on western ligand blotting is decreased by the action of circulating proteases for IGFBPs and that their activity is inversely related to the nutritional intake of severely ill patients, being reduced after the start of parenteral nutrition[9]. An effect of glucose on the activity of such proteases could have explained our results and those reported by Cortizo and Gagliardino[6].

This study, however, does not support the role of glucose as regulator of IGF binding to IGFBPs. In our experiments, glucose failed to influence IGF binding either in rat or in human serum, despite the occurrence of protein glycation as demonstrated by the increase in fructosamine levels. Exposure to increasing concentrations of glucose also failed to alter the IGFBP-1 band intensity, thus suggesting that the reduction of IGF bioactivity observed in insulin-dependent diabetes mellitus is not due to a glucose-dependent increase of IGF binding to IGFBP-1.

The discrepancy between our results and those of Cortizo and Gagliardino, who used a very similar western ligand blotting procedure, might be due to the different strain of rat used in the experiments. We did not measure the radioactivity of nitrocellulose strips corresponding to IGFBP bands, since, even with different exposure times, no appreciable glucose-dependent change in the intensity of the bands was observed.

Pregnant human serum contains a specific cation-dependent proteolytic activity which causes attenuation or disappearance of IGFBP bands on western ligand blots[10]. In our study, glucose failed to protect IGFBPs from protease action, which, as expected, was inhibited by the addition of EDTA. With a prolonged 48 h incubation we observed a slight protease activity in non-pregnant human serum and considerably more in non-pregnant rat serum, thus confirming the presence of such circulating proteolytic activity in the normal non-pregnant state[11].

In conclusion, our study did not confirm the previously reported glucose-dependent increase of IGF binding to IGFBP-3 in rat serum, showed no glucose stimulating effect on IGF binding in human serum, and demonstrated that glucose has no protective action against protease activity.

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