Diabetologia (1991) 34: 307–313
0012186X9100071K

Diabetologia

Binding and biological effects of insulin, insulin analogues and insulin-like growth factors in rat aortic smooth muscle cells. Comparison of maximal growth promoting activities

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Summary. Binding and growth promoting effects of insulin, insulin analogues modified in the B chain, proinsulin, insulin-like growth factor-I and -II were studied in cultured rat aortic smooth muscle cells. Specific binding of ^{125}I-insulin was 0.9 ± 0.2% of total ^{125}I-insulin added, and the IC_{50}-value was estimated to 8.9 pmol/l. The insulin analogue B10 Asp tended to be more potent than insulin in displacing ^{125}I-insulin, B28 Asp was equipotent, B9 Asp/B27 Glu was approximately 100 times less potent and insulin-like growth factor-I more than 1000 times less potent than insulin. Specific binding of ^{125}I-insulin-like growth factor-I after 4 h incubation at 10 °C was five times higher than the specific binding of insulin (4.4 ± 0.4% of total ^{125}I-insulin-like growth factor-I added), and the IC_{50}-value was 0.3 nmol/l. Insulin was approximately 500 times less potent than insulin-like growth factor-I in displacing ^{125}I-insulin-like growth factor-I. The insulin analogue B10 Asp was slightly more potent and analogue B28 Asp was equipotent with insulin. Analogue B9 Asp/B27 Glu was ten times less potent and proinsulin was more than ten times less potent than insulin. The order of potency was similar for ^{3}H-thymidine incorporation into DNA: insulin-like growth factor-I > B10 Asp > insulin-like growth factor-II > insulin ≥ B28 Asp > B9 Asp/B27 Glu > proinsulin. The maximal effect of insulin-like growth factor-I on ^{3}H-thymidine incorporation was 71 ± 16% higher than the maximal effect of insulin. The maximal effect of insulin-like growth factor-II was at least as high as the effect of insulin-like growth factor-I. Furthermore, the maximal effect of B10 Asp was 62 ± 10% higher than the maximal effect of insulin. Insulin-like growth factor-I and B10 Asp tended to increase cell number more than insulin. In conclusion, this study shows that insulin analogues interact with different potencies with receptors for insulin and insulin-like growth factor-I in vascular smooth muscle cells and that insulin-like growth factors and the insulin analogue B10 Asp have more pronounced growth effects than insulin. Substitution of the amino acid Asp for His at position B10 in insulin makes the molecule more similar to insulin-like growth factor-I, chemically and probably also biologically.

Key words: Insulin, insulin analogues, insulin-like growth factors, proliferation, vascular smooth muscle cells

Recombinant DNA technology has made it possible to synthesize insulin in large amounts, and to alter the structure of the molecule. By changing the insulin structure, insulins with favourable physico-chemical properties can be obtained. Insulin analogues modified at certain positions in the B chain do not form dimers or hexamers in concentrations used for injection [1]. These insulin analogues are absorbed faster after subcutaneous injection than the present rapid-acting insulins [1]. However, before they are used to treat patients with diabetes it is important to carefully examine their interaction with receptors and their biological effects.

The insulin receptor is structurally homologous to the insulin-like growth factor-I (IGF-I) receptor [2, 3], and insulin and IGF-I crossreact with their respective receptors [4, 5]. IGF-I, IGF-II, multiplication stimulating activity (the rat IGF-II analogue) and insulin stimulate DNA synthesis in cultured vascular smooth muscle cells [4, 6–9]. A growth promoting action of insulin on the vascular smooth muscle cells has been suggested to be of importance for the development of atherosclerosis in diabetic patients [10, 11].

These considerations prompted the present investigation to compare the binding and growth promoting activities of insulin, the insulin analogues B10 Asp, B28 Asp and B9 Asp/B27 Glu, proinsulin and recombinant human IGF-I and IGF-II in cultured rat aortic smooth muscle cells.

Materials and methods

Isolation and culturing of cells

Rat aortic smooth muscle cells were isolated and cultured according to a modified method of Nilsson et al. [12]. Small pieces of the aortas were digested for 1 h in 0.1% collagenase in Ham's F-12 medium

[13], at 37 °C and then for 18–20 h in fresh collagenase/F-12. The cell suspension was filtered through a nylon filter (pore size 48 μm, Schweizerische Seidengaze Fabrik AG, Zürich, Switzerland), and the cells were washed in F-12 medium. The cells were transferred to a solution of the following composition: F-12 medium, 10 mmol/l HEPES, 10 mmol/l TES, 50 μg/ml ascorbic acid, 50 μg/ml gentamicin sulphate, 2 μg/ml Fungizone and 10% newborn calf serum (pH 7.4). Cells were plated in 75 cm^2 culturing flasks and kept at 37 °C in a humidified atmosphere of 5% CO_2 in air. The medium was changed three times a week and the cells were harvested using a solution of trypsin (0.25%) and EDTA (0.02%). Cells from the first to fourth generation were used in the experiments. The cells were characterized as smooth muscle cells by morphological criteria [14] and with a murine antibody which recognizes a unique epitope of α-smooth muscle actin [15]. The cells were randomly tested for mycoplasma infection with Mycotrim TC (Hana Media Inc., Berkeley, Calif., USA). All experiments were performed on near-confluent cultures which were kept for 24 h in serum-free MCDB 104 medium before the experiments.

Binding studies

Near-confluent cell cultures, cultured in six-well plates, were incubated for 4 h at 10 °C in HEPES binding buffer (pH 7.8) with the following composition (mmol/l): HEPES 100, NaCl 120, KCl 5, $MgSO_4$ 1.2, glucose 8 and 0.1% bovine serum albumin with the addition of 25,000 cpm ^{125}I-IGF-I (12.5 pmol/l) or ^{125}I-insulin (43.9 pmol/l) and unlabelled polypeptides at indicated concentrations. The cells were washed four times with ice-cold phosphate-buffered saline, and then solubilized in 0.1% sodium dodecyl sulphate (SDS). The radioactivity was measured in a gamma counter. Unspecific binding of ^{125}I-insulin or ^{125}I-IGF-I was defined as binding in the presence of 0.1 μmol/l unlabelled insulin or IGF-I, respectively.

^{3}H-thymidine incorporation into DNA

DNA-synthesis was quantified by measuring ^{3}H-thymidine incorporation into DNA according to a modified method of Nilsson and Thyberg [16]. The cells, grown in 24-well plates, were incubated for 24 h in F-12 medium at 37 °C with the addition of 1 μCi/ml ^{3}H-thymidine and in the absence or presence of polypeptides at indicated concentrations. DNA was precipitated with ice cold 5% trichloroacetic acid and then solubilized in 0.5 ml 0.1 mol/l KOH. Part of the solution (0.2 ml) was added to Insta-Gel, and the radioactivity was counted in a liquid scintillation counter (Rackbeta 1217, LKB Wallac, Turku, Finland). The data were expressed as per cent increase in ^{3}H-thymidine incorporation over control cells, incubated in the absence of polypeptides or serum. The time-course studies were performed as above. Polypeptides were added after 24 h in serum-free medium and after 6, 12, 16 and 22 h 2 μCi/ml ^{3}H-thymidine was added, and the cells incubated for an additional 2 h.

Autoradiography

DNA synthesis was also measured by autoradiography. The cells were grown on glass coverslips, while in other respects they were treated as described above. After 24 h of incubation the cells were fixed in a solution of 50 mmol/l sucrose/3% glutaraldehyde, dehydrated and autoradiographed with Kodak NTB_2 film. After an exposure time of 6 days (4°C) the cells were dyed in methylene blue, as described by Nilsson et al. [17].

Cell proliferation studies

Near-confluent cell cultures were deprived of serum for 24 h, and then incubated with serum or polypeptides in F-12 medium for 48 h. The cells were trypsinized and counted in a Bürker chamber.

IGF-I and insulin degradation

Degradation of ^{125}I-IGF-I and ^{125}I-insulin was measured after 24 h incubation with cells treated exactly as in the DNA-synthesis experiments, in the absence or presence of different concentrations of unlabelled IGF-I or insulin. After 24 h the medium was collected and precipitated with an equal volume of 25% ice-cold trichloroacetic acid [18]. Trichloroacetic acid soluble radioactivity was considered as degraded peptide.

Production of recombinant polypeptides

Recombinant human IGF-I was produced in yeast as the authentic peptide [19]. The batches used (2104, 2111) had a specific activity of approximately 14,000 units/mg according to placental radioreceptor assay (1 unit is defined as the activity of 1 ml of a normal human serum pool). Recombinant human IGF-II, identical to IGF-II isolated from human plasma, was produced as a fusion protein in *Escherichia coli*. The fusion protein was secreted and after cleavage the peptide was purified to > 95% purity. Insulin analogues were produced as described by Brange et al. [1]. The analogues used were analogue B10His → Asp, analogue B28Pro → Asp and analogue B9Ser → Asp/B27Thr → Glu.

Chemicals

Human semisynthetic insulin and mono-^{125}I-(Tyr A14)-human insulin (1500 Ci/mmol) were obtained from Novo (Copenhagen, Denmark). [6-^{3}H]thymidine (5 Ci/mmol) and ^{125}I-IGF-I (2000 Ci/mmol) were from Amersham (Amersham, Bucks, UK). Bovine serum albumin, HEPES, TES, ascorbic acid, collagenase and gentamicin sulphate were from Sigma (St. Louis, Mo., USA). Medium F12, newborn calf serum and medium MCDB 104 were from Gibco (Paisley, UK). Fungizone was from ER Squibb & Sons (Princeton, NJ, USA). Trypsin was from Difco Labs (Detroit, Mich., USA). Insta-Gel was obtained from Packard (Groningen, The Netherlands) and NTB_2 film was obtained from Kodak (Stockholm, Sweden). All other chemicals were of reagent grade.

Statistical analysis

Values are given as mean ± SEM. Levels of significance were calculated by analysis of variance or Student's *t*-test. Concentration-effect curves were treated statistically by fitting the experimental data points into the logistic function:

$$\text{Observed effect} = \text{Maximal effect} \times (D)^q/(D^q + EC50^q)$$

by means of non-linear least-square regression [20], using Marquardt's algorithm for least-square regression [21]. D represents the molar concentration of the peptide and q is a constant which is dependent on the slope of the curve. The logarithmic transformation of EC_{50} or IC_{50} (pD_2) was used when standard errors were calculated according to Waud [20].

Results

Binding studies

The specific binding of ^{125}I-insulin was 0.9 ± 0.2% of total ^{125}I-insulin added. The concentration needed to give half-maximal displacement (IC_{50}) was estimated to 8.9 pmol/l ($pD_2 = 11.0 \pm 0.1$) (Fig. 1). The insulin analogue B10 Asp

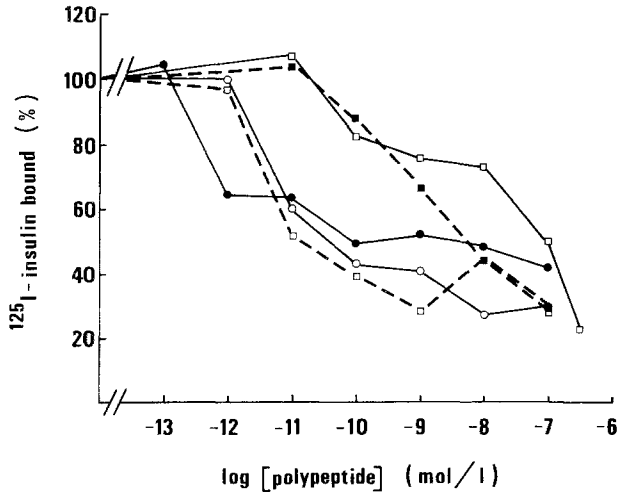

Fig. 1. Displacement of ^{125}I-insulin (43.9 pmol/l) by unlabelled insulin (○—○, $n = 5$), insulin-like growth factor-I (□—□, $n = 5$), analogue B10 Asp (●—●, $n = 8$), analogue B28 Asp (□---□, $n = 5$) and analogue B9 Asp/B27 Glu (■---■, $n = 5$). Near-confluent cells were incubated with polypeptides for 4 h (10 °C) in Hepes-binding buffer. Values are given as mean. SEM was 2.6–25.0% of the mean value

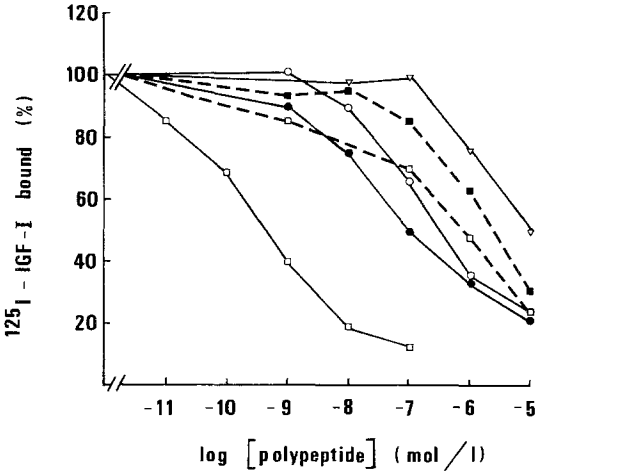

Fig. 2. Displacement of ^{125}I-insulin-like growth factor I (IGF-I) (12.5 pmol/l) by unlabelled IGF-I (□—□), insulin (○—○), analogue B10 Asp (●—●), analogue B28 Asp (□---□), analogue B9 Asp/B27 Glu (■---■) and proinsulin (▽—▽). Near-confluent cells were incubated with polypeptides for 4 h (10 °C) in Hepes-binding buffer. Values are given as mean ($n = 4$). SEM was 2.7–17.1% of the mean value

tended to be more potent than insulin ($IC_{50} = 0.8$ pmol/l, $pD_2 = 12.1 \pm 3.7$), B28 Asp was equipotent with insulin ($IC_{50} = 5.4$ pmol/l, $pD_2 = 11.3 \pm 0.2$), B9 Asp/B27 Glu was approximately 100 times less potent than insulin ($IC_{50} = 1.2$ nmol/l, $pD_2 = 8.9 \pm 0.5$) whereas IGF-I was more than 1000 times less potent than insulin in displacing ^{125}I-insulin (Fig. 1).

The specific binding of ^{125}I-IGF-I was approximately five times higher than the specific binding of insulin (4.4 ± 0.4% of total ^{125}I-IGF-I added). The IC_{50}-value was 0.3 nmol/l ($pD_2 = 9.5 \pm 0.1$) as shown in Figure 2. Unlabelled insulin was approximately 500 times less potent than IGF-I ($IC_{50} = 149$ nmol/l, $pD_2 = 6.8 \pm 0.3$), and proinsulin was approximately 10,000 times less potent. Analogue B10 Asp was slightly more potent than insulin ($IC_{50} = 76$ nmol/l, $pD_2 = 7.1 \pm 0.5$), analogue B28 Asp was equipotent, and analogue B9 Asp/B27 Glu was approximately ten times less potent than insulin to displace ^{125}I-IGF-I (Fig. 2).

DNA synthesis

Incorporation of ^{3}H-thymidine into control cells, incubated for 24 h in the absence of polypeptides or serum was 5.1 ± 0.4 nmol thymidine/g protein (mean ± SEM, $n = 6$). When the effects of IGF-I, IGF-II and insulin were compared, the mean maximal effect of IGF-I was found to be 71.0 ± 16.4% higher than the maximal effect of insulin. The maximal effect of IGF-II was at least as high as the maximal effect of IGF-I (Fig. 3). Insulin ($EC_{50} = 29$ nmol/l, $pD_2 = 7.5 \pm 0.3$) and IGF-II ($EC_{50} = 15$ nmol/l, $pD_2 = 7.8 \pm 0.2$) were more than 100 times less potent than IGF-I ($EC_{50} = 0.1$ nmol/l, $pD_2 = 9.9 \pm 0.1$). The combined effects of IGF-I and IGF-II, the combined effects of IGF-I and insulin or the combined effects of IGF-II and insulin were not additive (Fig. 3).

Analogue B10 Asp ($EC_{50} = 3.0$ nmol/l, $pD_2 = 8.5 \pm 0.3$) was more potent than human insulin ($EC_{50} = 21$ nmol/l, $pD_2 = 7.7 \pm 0.3$) (Fig. 4) and induced a 62.1 ± 10.3% higher maximal effect than insulin as shown in Figure 5. B28 Asp was equipotent with insulin ($EC_{50} = 70$ nmol/l, $pD_2 = 7.1 \pm 0.1$) and the maximal effect on DNA synthesis was equal (Fig. 4, insert). The insulin analogue B9 Asp/B27 Glu was approximately eight times less potent

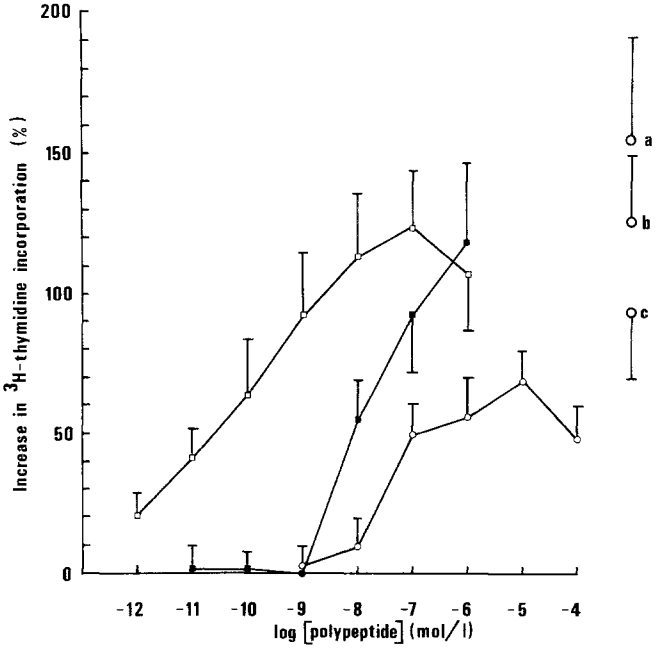

Fig. 3. Effect of insulin-like growth factor (IGF-I) (□), IGF-II (■) and insulin (○) on ^{3}H-thymidine incorporation in rat aortic smooth muscle cells. Near-confluent cell cultures were incubated at 37 °C for 24 h in F-12 medium in the presence of ^{3}H-thymidine and IGF-I, IGF-II and/or insulin. Combinations of 0.1 μmol/l IGF-I and 0.1 μmol/l IGF-II (a), 1.0 μmol/l insulin and 0.1 μmol/l IGF-I (b) and 1.0 μmol/l insulin and 0.1 μmol/l IGF-II (c) are indicated. Values are mean ± SEM ($n = 4$)

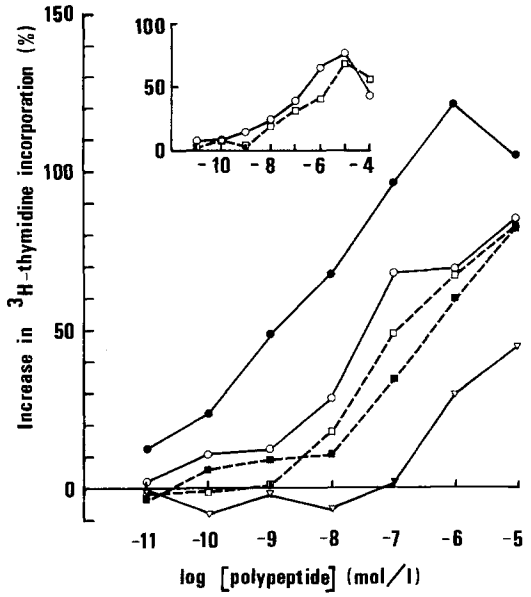

Fig. 4. Effect of insulin (○—○), analogue B10 Asp (●—●), analogue B28 Asp (□···□), analogue B9 Asp/B27 Glu (■···■) and proinsulin (▽—▽) on ^{3}H-thymidine incorporation in rat aortic smooth muscle cells. Near-confluent cell cultures were incubated at 37 °C for 24 h in F-12 medium in the presence of ^{3}H-thymidine and polypeptides. Values are calculated as mean. SEM was less than 21% ($n = 10$). Insert: Comparison of maximal effects on ^{3}H-thymidine incorporation induced by insulin (○—○) and B28 Asp (□···□). Values are mean of seven different observations

than insulin ($EC_{50} = 178$ nmol/l, $pD_2 = 6.7 \pm 0.2$), while human proinsulin was more than 100 times less potent than insulin (Fig. 4).

Time-course studies showed that the effects of both IGF-I and insulin on DNA synthesis were most pronounced when measured 14 h after addition of the polypeptides. IGF-I (0.1 μmol/l) had a higher maximal effect on DNA synthesis than insulin (10 μmol/l) throughout (Fig. 6).

Autoradiography

The localization of ^{3}H-thymidine was exclusively nuclear. Labelling indices were calculated as per cent ^{3}H-thymidine-labelled nuclei of total nuclei. IGF-I and insulin significantly increased labelled nuclei compared to control, and the effect of IGF-I was slightly higher than that of insulin. The effects of serum (0.5%) and IGF-I or insulin were additive (Table 1).

Cell proliferation studies

Insulin and IGF-I increased the cell number in the absence of serum, and the effects of insulin and IGF-I were additive to the effect of 0.5% serum (Table 2). IGF-I and B10 Asp increased cell number slightly more than insulin, although the difference was not statistically significant when using analysis of variance (Table 2).

Degradation of IGF-I and insulin

The degradation of ^{125}I-IGF-I was 47.6 ± 2.8% after 24 h incubation with the cells, whereas the degradation of ^{125}I-insulin was 67.8 ± 1.2% ($n = 8$). The degradation of ^{125}I-IGF-I and ^{125}I-insulin was similar in the presence of different concentrations of unlabelled IGF-I (0.1 nmol/l or 0.1 μmol/l) or insulin (0.1 nmol/l or 1.0 μmol/l), respectively.

Discussion

The results of the present study show that the maximal growth promoting activities of IGF-I, IGF-II and the insulin analogue B10 Asp in vascular smooth muscle are higher than that of insulin.

The binding studies indicate that specific insulin and IGF-I receptors are present in cultured rat aortic smooth muscle cells, and that IGF-I receptors are more abundant than insulin receptors. This is in agreement with results obtained from cultured vascular smooth muscle cells from other species and a cell line derived from embryonic rat

Table 1. Labelling index measurements. The cells were deprived of serum for 24 h, and then incubated in serum, insulin, insulin-like growth factor (IGF-I) or serum-free medium (control) for 24 h. Labelling index was calculated as per cent ^{3}H-thymidine-labelled nuclei visualized by autoradiography of total nuclei. Values are given as mean ± SEM ($n = 3$). Levels of significance (compared to control) were calculated using analysis of variance

Treatment	Labelling index	Level of significance
Control	13.6 ± 0.5	
Insulin 1.0 μmol/l	25.8 ± 1.2	$p \leq 0.05$
IGF-I 0.1 μmol/l	33.9 ± 2.3	$p \leq 0.001$
Serum 0.5%	21.9 ± 2.4	
Insulin 1.0 μmol/l + serum 0.5%	42.7 ± 4.2	$p \leq 0.001$
IGF-I 0.1 μmol/l + serum 0.5%	52.3 ± 4.7	$p \leq 0.001$
Serum 5%	79.5 ± 4.9	$p \leq 0.001$

Table 2. Cell number measurements. The cell cultures were deprived of serum for 24 h, and then incubated in serum, insulin, insulin-like growth factor (IGF-I) or serum-free medium (control) for 48 h. The cells were counted in a Bürker chamber. Values are given as mean ± SEM. Levels of significance (compared to control) were calculated using Student's t-test. Two different experiments are presented in the table

Treatment	Number of cells (% increase)	Level of significance	n
Insulin 1.0 μmol/l	23.6 ± 10.3		6
IGF-I 0.1 μmol/l	55.2 ± 14.1	$p \leq 0.05$	6
0.5% serum	32.0 ± 10.1	$p \leq 0.05$	6
Insulin 1.0 μmol/l + 0.5% serum	73.4 ± 19.2	$p \leq 0.05$	6
IGF-I 0.1 μmol/l + 0.5% serum	87.7 ± 9.9	$p \leq 0.001$	6
5% serum	125.6 ± 11.6	$p \leq 0.01$	6
Insulin 10 μmol/l	22.7 ± 4.0	$p \leq 0.001$	8
B10 Asp 10 μmol/l	30.6 ± 3.9	$p \leq 0.001$	8
IGF-I 0.1 μmol/l	33.2 ± 4.1	$p \leq 0.001$	8

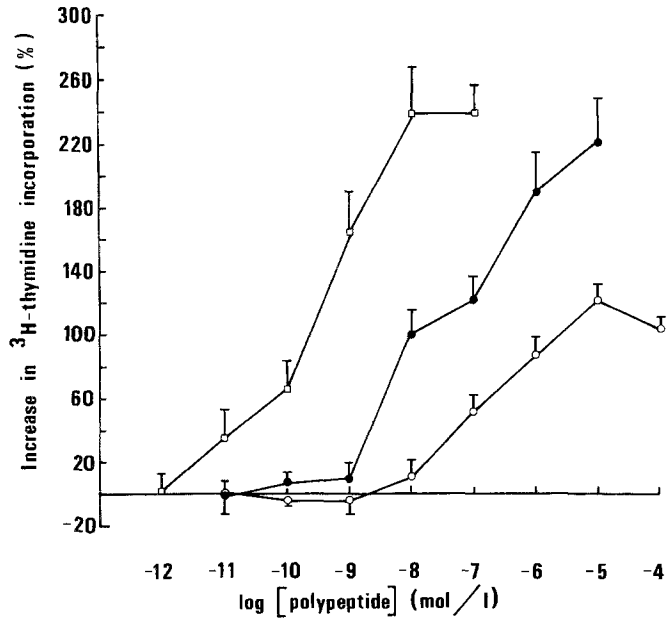

Fig. 5. Effect of insulin (○), analogue B10 Asp (●) and insulin-like growth factor-I (□) on ^{3}H-thymidine incorporation in rat aortic smooth muscle cells. Near-confluent cell cultures were incubated at 37 °C for 24 h in F-12 medium in the presence of ^{3}H-thymidine and polypeptides. Values are calculated as mean ± SEM ($n = 4$)

aorta [4, 8, 9]. The insulin analogues interacted with both insulin and IGF-I receptors with the following order of potency: B10 Asp > insulin ≥ B28 Asp > B9 Asp/B27 Glu. This order of potency has also been shown in a liver cell line of human origin [22].

The EC_{50}-values for IGF-II and insulin on DNA synthesis were approximately 100 times higher than the EC_{50}-value obtained with IGF-I. One should, however, bear in mind that this method for comparing the relative potency of agonists is most valid if the agonists are capable of eliciting the same maximal response [23]. IGF-I has been shown to be more potent than insulin and IGF-II/multiplication stimulating activity to stimulate DNA synthesis in vascular smooth muscle cells from various sources [4, 8, 9].

In the present study, the effect of a very high concentration of insulin was not additive to the effects of IGF-I or IGF-II. Furthermore, the effect of IGF-II was not additive to the effect of IGF-I. Receptor studies in the present study and in other studies have shown that insulin [4, 5] and IGF-II [9] interact with the IGF-I receptor in vascular smooth muscle cells in concentrations which stimulate DNA synthesis. Taken together, these data suggest that both insulin and IGF-II act via the IGF-I receptor to induce DNA synthesis in these cells. Since the insulin analogues interacted with the IGF-I receptor and were 30 – 1,800 times less potent than IGF-I to induce DNA synthesis it is probable that also these analogues act via the IGF-I receptor to induce DNA synthesis.

The higher maximal effect of IGF-I and IGF-II than insulin on thymidine incorporation is in agreement with a recent study performed on cultured fetal bovine aortic smooth muscle cells [9]. In the present study the insulin analogue B10 Asp was also found to give a higher maximal stimulation of thymidine incorporation than insulin. Furthermore, the effect of IGF-I on ^{3}H-thymidine-labelled nuclei and the effect of IGF-I and B10 Asp on cell number were slightly higher than the effect of insulin, indicating an authentic difference in maximal growth-promoting activity of IGF-I and B10 Asp in comparison with insulin.

How can the higher maximal growth promoting activities of IGF-I and B10 Asp be explained? One possibility could be dimerization/hexamerization of insulin, but not B10 Asp and IGF-I, at high concentrations (1–100 μmol/l), which would reduce the ability to bind to the IGF-I receptor. However, the insulin analogue B28 Asp, which does not form dimers/hexamers [1], did not reach a higher maximal effect on DNA synthesis than insulin. Degradation of insulin was approximately 20% higher than degradation of IGF-I, and the degradation was constant when different concentrations of insulin or IGF-I were added. Such pattern of degradation would result in a shift of the concentration-effect curve to the right, rather than a lower maximal effect of insulin. Furthermore, IGF-I had a higher maximal effect than insulin throughout the time-course study. A third explana-

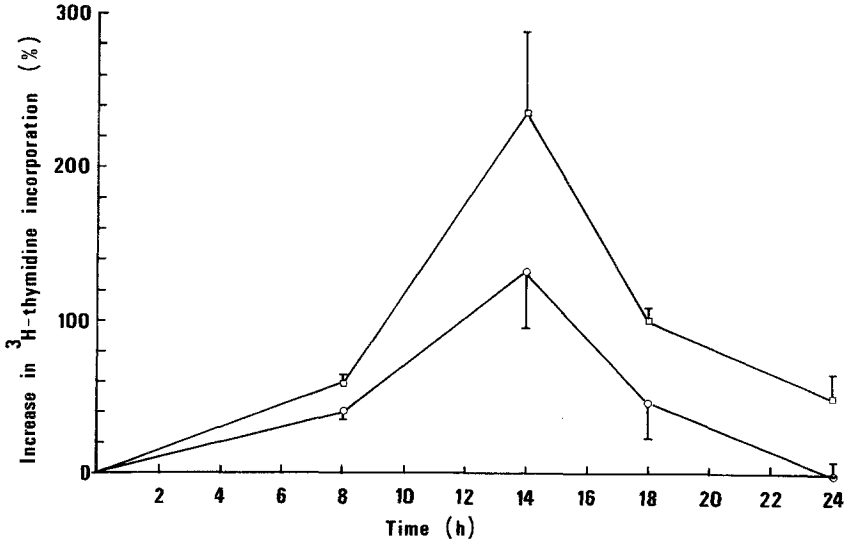

Fig. 6. Time-course study on the maximal effects of insulin (○) and insulin-like growth factor-I (□) on ^{3}H-thymidine incorporation in rat aortic smooth muscle cells. Near-confluent cell cultures were incubated in the presence of polypeptides at 37 °C for 6, 12, 16, or 22 h. ^{3}H-thymidine was present during an additional 2 h incubation. Values are mean ± SEM ($n = 4$)

tion could be that IGF-binding proteins secreted by the smooth muscle cells would increase the effect of IGF-I on DNA synthesis. A low-molecular weight IGF-binding protein purified from human amniotic fluid has been reported to increase the effect of IGF-I, but not of insulin, on DNA synthesis in aortic smooth muscle cells [24]. It was suggested that the increased effect of IGF-I in the presence of the binding protein was due to an enhanced binding of IGF-I to the receptor. Cultured vascular smooth muscle cells have been found to secrete IGF-binding proteins [27], but these binding proteins did not increase the cellular binding of IGF-I [28]. Several other studies have shown that the action of IGF-I and IGF-II is inhibited by low-molecular weight binding proteins [25, 26]. The difference in maximal growth promoting activities between insulin and B10 Asp and IGF-I, therefore, do not seem to be due to either dimerization/hexamerization or degradation and it seems unlikely that it is due to interaction with IGF-binding proteins.

A compound that produces a response less than maximum even when occupying nearly all of the receptors is termed a partial agonist [29]. One could speculate that insulin acts as a partial agonist on the IGF-I receptor in these cells, whereas IGF-I and IGF-II act as full agonists. The reason for this is unknown, but the fact that analogue B10 Asp also induced a higher maximum than insulin may give a clue. Human insulin, as well as porcine and bovine insulin, has a basic amino acid in position B10 (His), whereas IGFs have an acidic amino acid (Glu) in the corresponding position. Thus, changing the amino acid in position B10 from a basic to an acidic amino acid (Asp) in analogue B10 Asp makes the analogue chemically more similar to IGF-I. Residue B10 is located just outside the receptor-binding region [1]. It is possible that this residue, at least partly, is responsible for the lower maximal response on DNA synthesis induced by insulin in comparison with IGF-I.

Whether the difference in growth effects between insulin, IGF-I and the insulin analogue B10 Asp could be of any clinical importance is presently an open question.

Acknowledgements. We are grateful to NovoNordisk a/s, Denmark, for the generous gift of insulin analogues and human proinsulin. Financial support was given by Nordisk Insulinfond, the Swedish Diabetes Association, the Swedish Hoechst Diabetes Fund, Lion's Scholarship for Medical Research, the Swedish Society for Medical Research and the Swedish Medical Research Council (4952 and 4486).

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Received: 4 December 1990

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