Identification and characterization of insulin receptors on foetal-mouse brain-cortical cells

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The occurrence of insulin receptors was investigated in freshly dissociated brain-cortical cells from mouse embryos. By analogy with classical insulin-binding cell types, binding of ^{125}I -insulin to foetal brain-cortical cells was time- and pH-dependent, only partially reversible, and competed for by unlabelled insulin and closely related peptides. Desalanine-desasparagine-insulin, pig proinsulin, hagfish insulin and turkey insulin were respectively 2%, 4%, 2% and 200% as potent as bovine insulin in inhibiting ^{125}I -insulin binding to brain-cortical cells, which corresponds to their relative biological potencies in classical insulin-target cells; no competition was observed with glucagon and nerve growth factor, even at high concentrations. Scatchard analysis of competitive-binding data resulted in curvilinear plots with a high-affinity binding of $K_a = 3.6 \times 10^8 \text{ M}^{-1}$. Insulin binding to foetal brain-cortical cells differed, however, in two distinct aspects from that to classical insulin-binding cell types. Firstly, dilution of ^{125}I -insulin-bound cells in the presence of unlabelled insulin did not accelerate dissociation of the labelled hormone. Secondly, exposure of brain-cortical cells to insulin before the binding assay enhanced insulin binding, suggesting up-regulation of insulin receptors in response to insulin. In conclusion, foetal-mouse brain-cortical cells bear specific binding sites for insulin. Their insulin receptor shows a marked specificity and affinity for insulin, but differs in at least two properties from most classical insulin receptors. These differences in hormone-receptor interaction could reflect structural differences between insulin receptors on embryonic and differentiated cells.

One of us has previously shown that insulin promotes survival and differentiation of foetal neuronal cells in culture (Hooghe-Peters *et al.*, 1981). The initial step in the biological action of insulin is the binding of the hormone to its receptor on the plasma membrane of the target cell (Czech, 1981). Although the metabolic effects of insulin and the characteristics of its receptor have been studied extensively in many tissues (Kahn, 1976; Ginsberg, 1977; Jacobs & Cuatrecasas, 1981), the role of insulin and its receptor in the central nervous system is not well understood (Havran-kova *et al.*, 1978; Pacold & Blackard, 1979; Van

Houten & Posner, 1979; Raizada *et al.*, 1980; Rosenzweig *et al.*, 1980; Snyder & Kim, 1980; Eng & Yalow, 1981; Frank & Pardridge, 1981; Havran-kova *et al.*, 1981; De Pablo *et al.*, 1982; Hendricks & Roth, 1982; Kappy & Raizada, 1982; Pillion *et al.*, 1982; Baskin *et al.*, 1983; Landau *et al.*, 1983; Sara *et al.*, 1983). It is generally assumed that insulin does not cross the blood-brain barrier in adults. However, evidence has been presented that pancreatic insulin might have access to the brain compartment (Margolis & Altszuler, 1967; Woods & Porte, 1977); in addition, some groups of investigators have suggested that the brain might provide its own insulin, giving rise to a plasma-independent concentration of insulin in the brain (Havran-kova & Roth, 1979; Dorn *et al.*, 1982; Raizada, 1983).

Abbreviation used: Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid.

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Since we observe insulin effects on foetal neuronal cells early in culture, the question is whether insulin receptors are already expressed early in brain development. Therefore we have designed studies to examine the presence of insulin receptors on freshly dissociated brain-cortical cells from mouse embryos and to characterize them with particular emphasis on their comparison with previously described insulin receptors.

This study was presented in part at the 23rd Annual Meeting of the American Society for Cell Biology in San Antonio, TX, U.S.A., November 29–December 3, 1983 (Van Schravendijk *et al.*, 1983).

Materials and methods

Peptides

Bovine insulin (lot 615-D62-5) was obtained from Lilly Research Laboratories (Indianapolis, IN, U.S.A.); pig insulin (lot 8311160), pig pro-insulin (lot 23272) and pig glucagon (lot 429251) were obtained from Novo (Copenhagen, Denmark).

Turkey insulin, hagfish insulin and desalanine-desasparagine-insulin were generously given by Dr. Jean Simon (INRA, Nouzilly, France), Dr. Stefan Emdin (Umea, Sweden) and Dr. Fred H. Carpenter (Berkeley, CA, U.S.A.). ^{125}I -labelled pig insulin, homogeneously labelled on Tyr-A14, with a specific radioactivity of $358\ \mu\text{Ci}/\mu\text{g}$, was prepared by lactoperoxidase iodination, purified by high-performance liquid chromatography (h.p.l.c.) using a RP-1/C 18 reverse-phase system (Frank, 1980) and was kindly given by Dr. B. H. Frank (Eli Lilly Research Laboratories). $[\text{A14-}^{125}\text{I-Tyr}]$ insulin was also prepared in our laboratory, by using a RP-1/C 18 μ -Bondapack column and Waters h.p.l.c. equipment (Markussen & Larsen, 1980). Both tracers had strictly identical binding properties (Keefer *et al.*, 1982).

Nerve growth factor (lot 92F-00561) from mouse submaxillary glands was from Sigma (St. Louis, MO, U.S.A.). All other materials were reagent grade.

Cortical cells

Dissociated cells from the brain cortex of C57/BL mice were obtained as previously described (Ransom *et al.*, 1977; Hooghe-Peters *et al.*, 1979). Briefly, cortices were dissected from 14–15-day embryos and cleaned from any adhering tissue. Excised tissue was cut into small pieces and further mechanically dissociated in the Ca^{2+} -free Krebs medium used for the binding assay (pH 7.5).

Most experiments were carried out on freshly dissociated cells, suspended at 10^7 cells/ml; at this stage, the cells are not fully differentiated and

consist of both neuronal and glial precursor cells (Rakic, 1982). Cells remained structurally uncoupled, and appeared more than 95% viable under phase-contrast microscopy when analysed at the end of each experiment.

Human cultured lymphocytes

Human cultured IM-9 lymphocytes were maintained at 37°C in RPMI-1640 medium, supplemented with 10% (v/v) foetal-calf serum, penicillin (100 units/ml) and streptomycin ($100\ \mu\text{g}/\text{ml}$), as previously described (De Meyts, 1976). Medium was changed three times a week.

Binding assay

Incubations were performed in Ca^{2+} -free Krebs–Ringer medium buffered with Hepes and bicarbonate, containing 1 mM-EGTA and glucose (0.1 g/dl) (pH 7.5) and supplemented with 1% (w/v) bovine serum albumin (fraction V, RIA grade; Sigma), bacitracin (0.8 mg/ml; Sigma) and Trasylol (1000 kallikrein-inhibition units/ml; Bayer, Leverkusen, Germany) (assay buffer).

The binding assay was initiated by adding $400\ \mu\text{l}$ of cell suspension to polystyrene silicone-treated tubes containing $50\ \mu\text{l}$ of ^{125}I -insulin (35–200 pM) and $50\ \mu\text{l}$ of assay buffer with or without unlabelled insulin or other competitors.

After incubation for 120 min at 15°C , duplicate $200\ \mu\text{l}$ samples were withdrawn from the binding assay tube, mixed with $200\ \mu\text{l}$ of ice-cold assay buffer and centrifuged at $10000g$ for 45 s in a Beckman Microfuge B. Tube tips were cut off, and the pellet was counted in a Kontron gamma counter, with 65% counting efficiency. Specific binding was calculated as the difference in ^{125}I -insulin binding in the presence and in the absence of unlabelled insulin ($10\ \mu\text{g}/\text{ml}$). For comparison, IM-9 cells were examined in the assay buffer used for neural cells, instead of the media previously used (De Meyts, 1976).

Kinetics of association and dissociation were studied as previously described for other cell types (De Meyts, 1976) and as specified in Figure legends.

Results

^{125}I -insulin binding to brain-cortical cells in suspension

Insulin binding measured as a function of cell concentration was linear up to 10^7 cells/ml (Fig. 1). Hormone binding was maximal at pH 7.5 and 8.0, 20% lower at pH 7.0 and 40% lower at pH 6.5 (Fig. 2). Binding experiments were therefore routinely performed at pH 7.5. Under these conditions, hormone degradation, measured as trichloroacetic acid-soluble radioactivity, never exceeded 10% at the end of the binding experiment.

Fig. 1. ^{125}I -insulin binding to brain-cortical cells from mouse embryos

Cells (5.6×10^5 – $9 \times 10^6/\text{ml}$) were incubated with ^{125}I -insulin (34pM), with and without unlabelled insulin ($1.7\text{ }\mu\text{M}$). After 2 h at 15°C , the ratio of specifically bound/free labelled insulin was determined. Results for duplicate samples differed by less than 5%. The line was computed by linear regression; the correlation coefficient was over 0.99.

Fig. 3. Association kinetics of ^{125}I -insulin to brain-cortical cells

Cells ($1.3 \times 10^7/\text{ml}$) were incubated with ^{125}I -insulin (210pM). At the time indicated, cell-bound radioactivity was measured and expressed as a percentage of total radioactivity bound at 120 min ($\bullet$). Non-specific binding was determined after 5 and 120 min ($\blacksquare$); specific binding ($\circ$) was obtained as described in the Materials and methods section.

Fig. 2. ^{125}I -insulin binding to brain-cortical cells as a function of pH

Cells ($7 \times 10^6/\text{ml}$) were incubated with ^{125}I -insulin (37pM), with and without unlabelled insulin ($1.7\text{ }\mu\text{M}$) at pH 6.5, 7.0, 7.5 and 8.0. After 2 h at 15°C , ratios of bound/free labelled insulin were determined in both the absence ($\square$) and the presence ($\blacksquare$) of unlabelled insulin. Samples were run in duplicate, and error bars connect the values of individual data points.

Binding kinetics of ^{125}I -insulin

Binding of ^{125}I -insulin to brain-cortical cells increased with time, with half-maximal specific values observed after 20 min at 15°C . A true steady state was not completely reached after 2 h (Fig. 3).

This binding was reversible after addition of

unlabelled insulin as well as after 'infinite' dilution.

Addition of unlabelled insulin to the incubation medium ($1.5\text{ }\mu\text{M}$ final concn.) after 10 min of association resulted in a 65% dissociation of specifically bound ^{125}I -insulin over the following 30 min (Fig. 4). When the hormone was added after 120 min of association, only 45% of labelled insulin was dissociated in 30 min (Fig. 4). In both conditions, dissociation kinetics was not first-order.

When dissociation of bound ^{125}I -insulin was measured after dilution of the cells in the absence of any additional insulin, its kinetics was also non-first-order (Fig. 5). Under these conditions, the half-life of ^{125}I -insulin dissociation was 35 min (± 3 min), which is comparable with the values measured after addition of unlabelled insulin to the incubation mixture after 2 h of association (Fig. 4).

The presence of unlabelled insulin in the dilution medium ($0.17\text{ }\mu\text{M}$) did not enhance dissociation of ^{125}I -insulin from brain-cortical cells (Fig. 5a), in contrast with its accelerating effect on hormone dissociation from IM-9 lymphocytes (Fig. 5b), as was previously shown by De Meyts *et al.* (1973).

Competition by insulin and Scatchard analysis

Cells were incubated with ^{125}I -insulin in the absence or presence of increasing concentrations of unlabelled insulin (Fig. 6). The facts that a true equilibrium was not reached, and that the reaction was not fully reversible, preclude meaningful equilibrium analysis. However, when for comparison with other systems these data were analysed as

Fig. 4. Insulin-induced dissociation of ^{125}I -insulin from brain-cortical cells

Cortical cells ($1.1 \times 10^7/\text{ml}$) were first incubated with ^{125}I -insulin (160 pM) for 10 min (O) or for 120 min (●) and then exposed to unlabelled insulin ($1.5 \mu\text{M}$) up to 30 min. Specific binding was determined at 0, 10, 20 and 30 min after addition of the hormone. All values were expressed as a percentage of binding measured at the end of association. Results for duplicate samples differed by less than 2%.

described by Scatchard (1949), curvilinear plots were obtained (Fig. 6, inset).

The apparent affinity constants obtained by using a model for two classes of sites were obtained by computer analysis of the data with a do-file (F. Grigorescu, R. P. C. Rodgers & P. De Meyts, unpublished work) in the MLAB curve-fitting program (Knott, 1979). The high-affinity binding constant K_1 is $3.6 (\pm 0.2) \times 10^8 \text{ M}^{-1}$, corresponding to $Q_1 = 4650 (\pm 1250)$ receptors per cell, and the low-affinity constant K_2 is $9.5 (\pm 3.8) \times 10^6 \text{ M}^{-1}$, corresponding to $Q_2 = 33000 (\pm 10500)$ receptors per cell. In the absence of an appropriate binding model, these values have only an indicative significance.

Competition by insulin analogues and other hormones

^{125}I -insulin binding was measured with and without increasing concentrations of unlabelled peptides (Fig. 7). Hagfish or turkey insulin, desalanine-desasparagine-insulin and proinsulin competed for ^{125}I -insulin binding, and the degree of competition paralleled their capability to induce insulin-like effects in classical bioassays *in vitro* for insulin (Ginsberg, 1977): turkey insulin > bovine insulin > proinsulin > desalanine-desasparagine-insulin.

Fig. 5. Dissociation kinetics of ^{125}I -insulin from brain-cortical cells (a) and from IM-9 lymphocytes (b)

Cortical cells ($1.3 \times 10^8/\text{ml}$) or IM-9 lymphocytes ($8.6 \times 10^6/\text{ml}$) were first incubated with ^{125}I -insulin (200 pM) for 2 h at 15°C , then centrifuged (800g for 2 min at 4°C) and resuspended in assay buffer, before being distributed as 50 μl samples in 2 ml of assay buffer. Incubation was continued (15°C) in the presence (O) or absence (●) of unlabelled insulin ($0.17 \mu\text{M}$). Cell-bound hormone was measured at different intervals and expressed as a percentage of binding at the end of the association incubation. Data were not corrected for non-specific binding, and each point is the mean value of a duplicate determination; duplicates differed by less than 5%.

Fig. 6. Competition for ^{125}I -insulin binding by unlabelled insulin

Cortical cells ($9 \times 10^6/\text{ml}$) were incubated for 2 h at 15°C with ^{125}I -insulin (170 pM) in the presence of different concentrations of unlabelled insulin ($1.7\text{ }\mu\text{M}$ – 8.3 pM). The ratio bound/total radioactivity was determined for each concentration of unlabelled insulin. Non-specific binding was not subtracted. All samples were run in duplicate; data points represent individual values. Inset: Scatchard plot of the same binding data. Bound/free radioactivity was plotted as a function of total insulin binding to the cortical cells. Data were corrected for non-specific binding.

Nerve growth factor and glucagon did not compete with insulin binding, even at high concentrations.

Effect of preincubation with insulin on ^{125}I -insulin binding to foetal brain-cortical cells

Minced brain cortices were preincubated in culture medium (Ransom *et al.*, 1977; Hooghe-Peters *et al.*, 1979) at 37°C with and without $1.7\text{ }\mu\text{M}$ -insulin (Table 1) before being mechanically dissociated as described in the Materials and methods section, and then tested for their ability to bind ^{125}I -insulin in the binding assay. In four out of five experiments, an increased binding was noted to cells that had been preincubated with insulin.

Discussion

The observation that insulin increases survival and promotes growth of foetal brain cells in culture (Hooghe-Peters *et al.*, 1981) led us to investigate the presence of insulin receptors on these cells.

In the present study, we demonstrate that specific insulin receptors are expressed on freshly dissociated brain-cortical cells from mouse embryos. Insulin binding fulfils the criteria of a true hormone-receptor interaction (De Meyts, 1976), as it was rapid, reversible and competed for by low hormone concentrations.

Fig. 7. Competition for ^{125}I -insulin binding by insulin analogues, nerve growth factor (NGF) and glucagon

Cortical cells were incubated with labelled insulin and the indicated concentrations of unlabelled peptides (abbreviation: DAA-insulin, desalanine-desaspargine-insulin). Binding of ^{125}I -insulin is expressed as a percentage of binding in the absence of any other peptide. Correction was made for non-specific binding.

Table 1. Effect of 22h preincubation with insulin on ^{125}I -insulin binding on foetal brain-cortical cells

Values for bound insulin are corrected for non-specific binding, and are means $\pm$ S.E.M. ($n = 4$). $\dagger P < 0.05$ by unpaired Student's *t* test.

Expt. no.	Bound ^{125}I -insulin (fmol/ 10^7 cells)	
	Control	Insulin ($1.7\text{ }\mu\text{M}$)
1	1.21 ± 0.05	1.52 ± 0.09
2	1.26 ± 0.02	2.17 ± 0.01
3	1.14 ± 0.02	1.00 ± 0.05
4	1.23 ± 0.05	1.81 ± 0.09
5	1.16 ± 0.04	1.81 ± 0.02
Mean ...	1.20 ± 0.02	$1.66 \pm 0.17\dagger$

The biological specificity of the receptor appears from the inhibition of insulin binding by insulin analogues; the degree of this competition was proportional to the ability of these analogues to induce insulin-like effects in classical target cells. In contrast, nerve growth factor and glucagon did not compete for insulin binding. This specificity

profile of insulin binding is comparable with that described for other cell types, and thus supports the existence of specific insulin receptors on foetal brain-cortical cells. Also in support of a typical insulin receptor is the pH-dependency of hormone binding, with an optimum at pH 7.5 and 8.0 (Goldfine, 1981).

In analogy to other studies (Freychet, 1976; Ginsberg, 1977), the Scatchard plot of the competitive-binding data was curvilinear with an upward concavity. This curvilinearity is compatible with the presence of several classes of independent binding sites and/or with negative co-operativity within one class of binding sites (De Meyts *et al.*, 1976).

Insulin binding to freshly dissociated foetal brain-cortical cells differed, however, in two distinct aspects from that observed in most studies on insulin receptors.

In most systems studied so far, dissociation of bound insulin was enhanced by the presence of unlabelled insulin in the dilution medium (De Meyts *et al.*, 1973), supporting the view that the curved Scatchard plot is due to negative co-operativity (De Meyts, 1980). Such a phenomenon was not observed with foetal brain-cortical cells; moreover, the dissociation curve in dilution experiments was similar to that obtained without dilution but after addition of unlabelled insulin after a 2 h association. These results indicate that the dissociation rate of insulin receptors on foetal brain-cortical cells is not regulated by the degree of receptor occupancy. So, unless there is some effect of occupancy on the association rate constant, the curvilinear Scatchard plot is probably due to receptor heterogeneity in foetal brain-cortical cells.

Competitive-binding data were therefore analysed in terms of a two-site model (Knott, 1979). The low- and high-affinity constants for insulin binding to foetal brain-cortical cells are comparable with those obtained with other cell types. It should, however, be noted that the affinity constant for the low-affinity sites might be close to 'non-specific binding', and that the curvilinearity of the Scatchard plot might therefore also be caused by an underestimation of non-specific binding.

The high-affinity component of binding ($K_a = 3.6 \times 10^8 \text{ M}^{-1}$) is lower than that found on hepatocytes ($K_a = 2 \times 10^9 \text{ M}^{-1}$) (Gammeltoft *et al.*, 1978; Fehlmann *et al.*, 1981) and even than the K_e calculated for IM-9 lymphocytes by using a model for negative co-operativity ($K_e = 1.1 \times 10^9 \text{ M}^{-1}$; Ginsberg, 1977); it is similar to that of adipocytes ($K_a = 3 \times 10^8 \text{ M}^{-1}$; Gammeltoft & Gliemann, 1973; Gammeltoft *et al.*, 1978), although a higher affinity has also been described for these cells (Cuatrecasas, 1971; Gliemann *et al.*, 1975). In

contrast, cultured HTC and H35 hepatoma cells presented insulin receptors of a single class with a high-affinity binding constant which is lower than that found with foetal brain-cortical cells (Iwamoto *et al.*, 1981).

Some caution is, however, required in the interpretation of these data, as binding in our and several other systems does not reach true equilibrium and is not fully reversible. Moreover, although the presence of high- and low-affinity receptors is suggested by the non-first-order dissociation kinetics, their relative proportion does not correspond to that predicted from Scatchard analysis: indeed, whereas the largest amount of tracer would be expected to bind to high-affinity sites, more than 60% of the tracer was found to dissociate quickly after 10 min of association; in addition, after 120 min of association, a significantly larger fraction of hormone dissociated much slower than after the shorter incubation. Besides indicating a receptor heterogeneity, the non-first-order kinetics might thus also express isomerization of the receptor to a tighter-binding state (Corin & Donner, 1982), or an interaction with another molecular component leading to a change in affinity of the receptor (Harmon *et al.*, 1981), or a cellular uptake of insulin (Kahn & Baird, 1978), or a combination of these possibilities.

The number of binding sites per cell was determined by computer curve fitting of the competitive-binding data, and averaged 4650 high-affinity sites per cell among a total of 33000 binding sites per cell. It should be noted that these values do not take into account the cellular heterogeneity in the foetal brain cell preparations (Rakic, 1982) and might therefore underestimate the actual number of insulin receptors per cell if they are not homogeneously distributed over all cells.

A second feature where insulin receptors on foetal brain-cortical cells differ from those on most other cell types is their regulation by the hormone itself. Down-regulation of receptors by the homologous ligand has been described in many systems; thus continuous exposure of lymphocytes to insulin or growth hormone (Gavin *et al.*, 1974; Lesniak & Roth, 1976), of hepatocytes to glucagon (Santos & Blazquez, 1982), of S49 lymphoma cells to isoprenaline (Shear *et al.*, 1976), or of gonadal tissue to lutropin ('LH') (Conti *et al.*, 1976, 1977; Chen & Payne, 1977), results in a decrease of the corresponding receptors on the cell surface. Foetal brain-cortical cells were devoid of this type of receptor regulation, but underwent, in contrast, an up-regulation of their insulin receptors in response to insulin. Whether this enhanced binding is due to an increment in receptor concentration, to an increased binding affinity, or to an altered processing of the hormone, remains to be elucidated.

These uncommon binding properties of the foetal brain-cortical cells have been observed previously in other cell types. For example, insulin did not enhance dissociation of ^{125}I -insulin bound to cultured HTC and H35 hepatoma cells (Iwamoto *et al.*, 1981); competition experiments on these cells led, however, to linear Scatchard plots and to the exclusion of a binding heterogeneity. Interestingly, insulin binding to these cell types has been related to growth-promoting effects (Koontz & Iwahashi, 1981). Massagué *et al.* (1982) have suggested that these effects are mediated by receptors which might have minor structural differences when compared with the classical insulin receptors; these differences would, however, go undetected in most types of receptor analysis. In view of the present results, it seems possible that the absence of an enhanced dissociation of labelled insulin from foetal brain-cortical cells after their dilution in the presence of unlabelled hormone reflects such a structural difference. Experimental evidence has been presented to support this hypothesis (Heidenreich *et al.*, 1983).

Along this line, a similar insensitivity to unlabelled insulin, in association with curvilinear Scatchard plots, was observed in chick-embryo fibroblasts (Rechler *et al.*, 1977), but not in human cultured fibroblasts (Rechler & Podskalny, 1976).

The second uncommon phenomenon observed in the present study, namely the insulin-induced increase in insulin binding, was also found with chondrosarcoma chondrocytes (Stevens *et al.*, 1983) and with developing 3T3-L1 fatty fibroblasts (Chang & Polakis, 1978). Those findings, together with the results presented here, suggest that the up-regulation of insulin receptors might be a common phenomenon in embryonic cells or in cells which are still in a stage of differentiation.

Since the present study was conducted on foetal cells, one might expect receptors for insulin-like growth factors to be present on mouse foetal brain-cortical cells (Sara *et al.*, 1983). This raises the question of the possible relation between insulin binding and the eventual binding of insulin-like growth factors to mouse foetal brain-cortical cells. Most receptors for insulin-like growth factors have been distinguished in specificity and in binding characteristics from insulin receptors (Megyesi *et al.*, 1974). However, insulin was a potent competitor for binding of multiplication-stimulating activity to human and chick-embryo fibroblasts (Rechler *et al.*, 1975, 1976).

In the present study we have shown that insulin binds to a specific receptor in foetal brain-cortical cells, but with some atypical binding characteristics. It might be relevant that insulin binding to chick-embryo fibroblasts, which also show atypi-

cal insulin-binding properties, underwent a 10-fold higher competition by multiplication-stimulating activity as compared with the more classical insulin-binding cell types (Rechler *et al.*, 1977). Although multiplication-stimulating activity was only 5% as potent as insulin in these embryonic fibroblasts, it appears necessary to investigate a similar interaction in mouse foetal brain-cortical cells; an analogous competition would indeed provide another argument for the existence of a growth-promoting insulin receptor in foetal brain cells, in foetal fibroblasts and possibly in other non-classical insulin-binding cell types.

In conclusion, freshly dissociated brain-cortical cells from mouse embryos bear specific insulin receptors which present both similarities to and differences from most classical insulin receptors. It seems conceivable that the observed differences in hormone-receptor interaction reflect structural differences between insulin receptors on embryonic and differentiated cells.

Further studies are now required to test whether these particular properties of insulin receptors on brain-cortical cells determine their participation in cellular growth (Hooghe-Peters *et al.*, 1981) rather than in glucose handling (F. K. Gorus, E. L. Hooghe-Peters & D. G. Pipeleers, unpublished work).

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