Uncoupling between the insulin-receptor cycle and the cellular degradation of the hormone in cultured foetal hepatocytes

Effect of drugs and temperature that inhibit insulin degradation

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Sequential changes in the numbers of cell-surface receptors induced by a transitory exposure to insulin in cultured 18-day foetal-rat hepatocytes were investigated in the presence of drugs and at a temperature of 22 °C, which inhibit cellular insulin degradation. Chloroquine (70 μ M) and monensin (3 μ M) did not greatly change the initial rate of internalization of cell-surface receptor sites after exposure to 10 nM-insulin, but led to a steady state after 20 min, which represented 40% of the initial binding, compared with 5 min and 60% in the absence of the drug. Moreover, these drugs strongly decreased the proportion of receptor sites recovered at the cell surface after subsequent removal of the hormone. They were ineffective when insulin was not present. The removal of monensin together with the hormone allowed partial restoration of cell-surface receptor sites and degradation of cell-associated insulin to start again at the initial speed, indicating a reversible effect of the drug. During this phase, the drug concentration-dependence for the two effects showed that receptor recycling was restored with concentrations of monensin not as low as for insulin degradation. The effect of vinblastine (50–100 μ M) was similar to that of chloroquine and monensin, whereas no modification in the internalization and recovery processes was observed in the presence of bacitracin concentrations (1–3 mM) that inhibit insulin degradation by 70%. A temperature of 22 °C did not prevent the receptor internalization, but had a slowing effect on the recycling process, which appeared to vary in experiments where insulin degradation remained inhibited. The present study shows that the process of insulin degradation mediated by receptor endocytosis is not a prerequisite for insulin-receptor recycling in cultured foetal hepatocytes.

INTRODUCTION

Two classes of cell-surface receptors have been categorized on the basis of their major function, i.e. to transmit information to cells (class I; e.g. epidermal growth factor and polypeptide hormone receptors) and to mediate ligand uptake (class II; e.g. asialoglycoprotein receptor) (Kaplan, 1981). In both cases, receptors and ligands move after ligand binding from the plasma membrane into the endocytic compartment, where they undergo dissociation, sorting and distribution to other cellular compartments. The reutilization of the receptors after returning to the cell surface has been demonstrated, and seems more strictly related to class II receptor function (for reviews, see Ashwell & Harford, 1982; Brown *et al.*, 1983; Wileman *et al.*, 1985; Wiley, 1985; Evans, 1986). The hepatocyte insulin receptor could belong to either class of receptors, according to the well-known effects of insulin on cell metabolism and the insulin clearance function of the liver. A theoretical analysis based on the insulin clearance process in the rat has estimated a cycle time for the insulin receptor of 3–6 min (Halperin *et al.*, 1986). Moreover, large differences in the rates of insulin-receptor and insulin degradation have been found in cultured chick hepatocytes, suggesting sparing of the receptor and its reutilization (Krupp & Lane, 1982). A similar situation has been found in cultured foetal hepatocytes, where insulin degradation is predominantly mediated by

receptor endocytosis, with a reutilization time for the receptor of less than 10 min (Plas & Desbuquois, 1982; Pringault *et al.*, 1985). In this cell system, a kinetic study of the variations in the numbers of cell-surface receptor sites induced by a transitory exposure to insulin has confirmed a rapid insulin-receptor cycle (Soubigou *et al.*, 1986). Its repetitive character has been demonstrated and related to the maximal expression of the glycogenic response to insulin (Soubigou *et al.*, 1986). Cultured foetal hepatocytes were used in the present work to study the correlative modifications in the insulin-receptor cycle and the cellular degradation of insulin under the influence of drugs, and at a temperature of 22 °C, which are known to inhibit hepatocyte insulin degradation with retention of intact insulin inside the cell (Terris *et al.*, 1979; Plas & Desbuquois, 1982). The results show that intracellular insulin degradation can be inhibited without affecting insulin-receptor recycling, which indicates uncoupling between these two processes.

MATERIALS AND METHODS

Materials

Monensin, vinblastine and bacitracin were purchased from Sigma. Chloroquine (sulphate form) was a gift from Specia Laboratories, Paris, France. The source of other materials has been specified previously (Plas & Nunez, 1976; Soubigou *et al.*, 1986).

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Culture procedure

Primary cultures of hepatocytes were obtained from 18-day rat foetuses (Sprague-Dawley) as described previously (Plas *et al.*, 1973). The culture medium consisted of NCTC 109 medium (Evans *et al.*, 1964) supplemented with 10% (v/v) foetal-calf serum, 10 μ M-cortisol and 10 mM-Hepes, pH 7.3. Experiments were performed after 2 days of culture in the presence of cortisol, when the glycogenic effect of insulin is fully expressed (Plas & Nunez, 1976).

Cell-surface insulin binding

Cell-surface insulin binding after a transitory exposure to native hormone was determined by the method previously described (Soubigou *et al.*, 1986), with the following modifications. Cultured foetal hepatocytes were first exposed to 10 nM native insulin in culture medium at 37 °C for various times (up to 30 min) in the presence of the drug to be tested or its solvent [0.24% ethanol (final concn.) in the medium for monensin]. The medium was then replaced by an insulin-free culture medium in the presence or absence of the drug after first washing with the same medium, and cells were incubated at 37 °C for the times indicated for each experiment. At the end of the incubation, cell-surface insulin binding was measured at 4 °C as follows: cultures were washed once with binding medium containing 10 nM- 125 I-insulin and incubated for 4 h in the same medium (exchange binding assay). Binding medium consisted of glucose-free NCTC 109 medium + 1 mM-KCN, pH 7.8. After washing of cells four times with ice-cold Hanks solution, cell-associated radioactivity was measured as previously described (Plas & Desbuquois, 1982). In a second protocol, cell-surface bound insulin was first removed by a 5 min incubation in barbital/acetate buffer, pH 3.0 (Olefsky & Kao, 1982) before incubation in binding medium. The amount of 125 I-insulin specifically bound at the cell surface was found reproducibly identical by both protocols. In cells incubated with 10 nM- 125 I-insulin at 37 °C, cell-surface-bound insulin removed by the acid-extraction technique (Haigler *et al.*, 1980; Olefsky & Kao, 1982) represented, for example, 28% and 39% after 10 min in the presence and absence of 3 μ M-monensin, respectively. The same proportion of radioactivity was released during a 2 h chase incubation at 4 °C in the presence of 10 nM native hormone, indicating that dissociation of cell-surface-bound insulin effectively occurred during the conditions of the exchange binding assay.

Cell-associated insulin and its degradation

Cells were incubated for 10 or 30 min in culture medium at 37 °C in the presence of 10 nM- 125 I-insulin, and then for various times after removal of the hormone. At the end of the incubation, after collection of the medium, cells were washed four times with ice-cold Hanks solution. The cell-associated radioactivity was then extracted and tested for its ability to be precipitated by 10% (w/v) trichloroacetic acid as previously described (Plas & Desbuquois, 1982). The soluble fraction represents a degraded form of insulin, in agreement with the results obtained with gel filtration on Sephadex G-75 and re-binding to liver membranes (Plas & Desbuquois, 1982). The rate of insulin degradation was determined by measuring the release of degradative products into the medium by using the trichloroacetic acid-precipitation

Fig. 1. Effect of chloroquine on cell-surface insulin binding after transitory exposure to the native hormone, and on cell-associated insulin

(a) Foetal hepatocytes were first incubated in the presence of 10 nM native insulin alone or together with 70 μ M-chloroquine at 37 °C for various times up to 40 min, insulin and/or the drug being removed or not after 10 min. Then cell-surface insulin binding was measured after incubation for 4 h at 4 °C in binding medium in the presence of 10 nM- 125 I-insulin, by using the exchange binding assay as described in the Materials and methods section. (b) In parallel cultures, incubation conditions were similar, except that cells were first exposed to 10 nM- 125 I-insulin and that the amount of specifically cell-associated radioactivity was measured after the kinetics of exchange for 4 h at 4 °C conducted in the presence of 10 nM native insulin. Cell-surface-bound insulin (a) and cell-associated insulin (b) in cultures incubated with insulin (●), and then after removal of insulin (○), with insulin together with chloroquine (▲), and then after removal of insulin (□), or removal of insulin and chloroquine (◆), are shown.

method. Corrections were made for the non-specific association of 10 nM- 125 I-insulin with cells by performing parallel incubations in the presence of 10 μ M-insulin.

Definitions

Each protocol involved at least three experiments performed on different cell preparations. Each symbol on the graphs corresponds to a different culture. The results are expressed per culture. The cell population of a culture dish is of the order of 1.3×10^6 hepatocytes, which corresponds to 500 μ g of protein and to 3.6 mg wet wt. of liver.

Fig. 2. Effect of monensin on cell-surface insulin binding after transitory exposure to the native hormone, and on cell-associated insulin and its degradation

(a) Foetal hepatocytes were first incubated in the presence of 10 nM native insulin with or without 3 μ M-monensin at 37 °C for up to 60 min, insulin and/or the drug being removed or not after 30 min. The cell-surface binding was measured by the exchange binding assay. (b and c). In parallel cultures, incubation conditions were similar, except that cells were exposed to 10 nM-¹²⁵I-insulin instead of native hormone, and that the amount of specifically cell-associated radioactivity and the percentage of insulin degradation were measured as described in the Materials and methods section. Cell-surface bound insulin (a), cell-associated insulin (b) and its percentage degradation (c) are shown.

RESULTS

Effect of chloroquine on cell-surface ¹²⁵I-insulin binding after transitory exposure to insulin

Foetal hepatocytes were first exposed to 10 nM native insulin in the presence or absence of 70 μ M-chloroquine for various times at 37 °C, and cell-surface insulin binding was measured after incubation for 4 h at 4 °C in the presence of 10 nM-¹²⁵I-insulin by using the exchange binding assay. Cell-surface binding decreased sharply during the first minutes of insulin exposure, with a similar initial rate in the presence and absence of chloroquine (41, 48, 59 and 42, 46, 56 fmol of insulin bound/2 min, respectively, in three experiments performed with different cell preparations). In both cases, a slowing of the decrease was afterwards observed, which occurred later in the presence of the drug. This led to a steady state achieved after 20 min and representing 40% of the initial value in the presence of chloroquine, compared with 5 min and 60% in its absence (Fig. 1a). When native insulin was removed after 10 min exposure to the hormone alone, restoration of cell-surface insulin binding was nearly complete within 20 min (80%), but only partial (30%) when the drug was present throughout the incubation period. This partial restoration was improved by the removal of both native insulin and chloroquine (55%). Finally, chloroquine alone in the absence of insulin did not cause any modification in cell-surface binding.

In parallel cultures, cells were first exposed to 10 nM-¹²⁵I-insulin with or without 70 μ M-chloroquine at 37 °C. Cell-associated radioactivity was measured after incubation for 4 h at 4 °C in the presence of 10 nM native insulin and corresponded to internalized insulin not exchangeable at 4 °C. The insulin-binding steady state was reached later in the presence of chloroquine (30 min) than in its absence (5 min), and was increased 2-fold in the presence of the drug (Fig. 1b). As previously described (Pringault *et al.*, 1985), this increase was associated with an inhibitory effect of the drug on the intracellular degradation of insulin and the subsequent release of insulin-degradation products in the medium. For example, the amount of degraded insulin released into the medium was 260 and 85 fmol/culture after 40 min of incubation in the absence and presence of 70 μ M-chloroquine respectively. Also, the dissociation rate of insulin measured after removal of the labelled hormone was slowed in the presence of 70 μ M-chloroquine.

Effect of monensin on the insulin-receptor cycle

Experiments carried out with 3 μ M-monensin instead of chloroquine showed a similar pattern for variations in cell-surface ¹²⁵I-insulin binding after a transitory exposure to the native hormone, i.e. no detectable effect of monensin on the rate of the initial decrease, a lower steady-state value in the presence of the drug, followed by a partial restoration after removal of both insulin and monensin. After exposure for 30 min to 10 nM-insulin and 3 μ M-monensin, removal of both agents produced a

(c) in cultures incubated with insulin (●), and then after removal of insulin (○), with insulin together with monensin (▲) and then after removal of insulin (□), or removal of monensin (■), or removal of both insulin and monensin (△), are shown.

50% restoration of cell-surface binding within 10 min, whereas no restoration occurred after removal of the hormone with the drug still present (Fig. 2a). The amount of total cell-associated ^{125}I -insulin after 30 min at 37 °C was increased by 34% in the presence of monensin (Fig. 2b), with a concomitant decrease in the percentage of cell-bound insulin degraded from 25 to 8% (Fig. 2c). The dissociation rate of ^{125}I -insulin pre-bound in the presence of monensin, which was slow when the drug was maintained after removal of the hormone, was clearly accelerated by the simultaneous withdrawal of the drug: 35 and 70% of the radioactivity was dissociated within 20 min after maintenance or removal of monensin respectively, compared with 90% when insulin was pre-bound and allowed to dissociate in the absence of the drug. The reversible effect of monensin was also seen when examining the percentage of cell-bound insulin degraded during the dissociation step. After removal of monensin, this percentage increased rapidly from 8 to 20% within 10 min and more slowly thereafter to reach 25% after 30 min, whereas it remained at 6% when monensin was still present.

The reversibility of the monensin effect was further investigated in experiments using a 10 or 30 min incubation at 37 °C in the presence of 10 nM-insulin and 3 μM -monensin, and a 20 min incubation in the presence of decreasing monensin concentrations after removal of the hormone (from 10 to 0.6 μM). Cell-surface insulin binding was similarly decreased after 10 or 30 min in the presence of insulin and monensin, to be partially restored after removal of both agents, by 80 and 40% with cells incubated in the presence of insulin and the drug for 10 and 30 min respectively (Fig. 3a). When monensin was still present at a concentration of 10 or 3 μM after removal of the hormone, restoration was prevented. At a lower concentration close to 1 μM , monensin allowed recovery to the value found after complete removal of the drug. By contrast, restoration of cell-associated insulin degradation was only partial for concentrations of monensin as low as 0.6 μM , the half-maximal effect being obtained with 0.8 μM (Fig. 3b). A similar progressive pattern was obtained for restoration of dissociation of pre-bound insulin.

Effect of vinblastine and bacitracin on the insulin-receptor cycle

Cells were first exposed to 10 nM-insulin and vinblastine (30–100 μM) or bacitracin (1–3 mM) for 30 min at 37 °C. Insulin was then removed and the drugs were maintained at the same concentration for a further 20 min. Variations in cell-surface insulin binding induced by the hormone in the presence of vinblastine were very close to those obtained with chloroquine or monensin, i.e. an accentuation of the decrease in cell-surface binding after exposure to insulin and an inhibition of the recovery after removal of the hormone (Fig. 4a). With bacitracin, no modification in the insulin-receptor cycle could be detected, either on the rate of internalization or on that of recovery after removal of the hormone (Fig. 4b). The concentrations of vinblastine and bacitracin used in these experiments have previously been shown to inhibit cellular insulin degradation (Plas & Desbuquois, 1982; Pringault *et al.*, 1985). Similar results were obtained after incubation for 30 min in the presence of 10 nM- ^{125}I -insulin, when the percentage of cell-bound insulin degraded was found to be 7, 9 and 32%, and the

Fig. 3. Dependence on monensin concentration of the recovery of cell-surface insulin binding after previous insulin exposure in the presence of the drug, and of cell-associated insulin and its degradation

(a) Foetal hepatocytes were first incubated for 10 or 30 min in the presence of 10 nM-insulin and 3 μM -monensin at 37 °C, then insulin was removed and cells were incubated for 20 min in the absence or presence of various concentrations of monensin (10–0.6 μM). Cell-surface binding was measured by the exchange binding assay. (b) In parallel cultures, incubation conditions were similar, except that cells were exposed to 10 nM- ^{125}I -insulin instead of native hormone for 30 min, and that the amount of specifically cell-associated radioactivity and the percentage of insulin degradation were measured. Cell-surface bound insulin for a first incubation period of 10 (Δ) or 30 ($\bullet$) min, cell-associated hormone ($\blacksquare$) and its percentage of degradation ($\blacklozenge$), in cultures incubated in the presence of various concentrations of monensin after removal of the hormone, are shown. In (a), the initial cell-surface binding is represented by the broken horizontal line.

amount of degraded insulin in the medium to be 60, 65 and 190 fmol/culture, in the presence of 100 μM -vinblastine, 3 mM-bacitracin and in the absence of drug, respectively.

Influence of temperature on the insulin-receptor recycling after receptor internalization at 37 or 22 °C

Cells were first incubated in the presence of 10 nM-insulin for 10 min at 37 °C. Then insulin was removed and cells were maintained in insulin-free culture medium for 10 or 20 min at 37, 22 or 4 °C. After the initial decrease in cell-surface insulin binding induced by exposure to 10 nM-insulin, the recovery was complete during the subsequent 10–20 min incubation in the absence of the hormone at 37 °C, but was partial at 22 °C and non-existent at 4 °C (Fig. 5a). After 10 min at 22 °C,

Fig. 4. Effect of vinblastine and bacitracin on cell-surface binding after transitory exposure to the native hormone

Foetal hepatocytes were incubated for 30 min in the presence of 10 nM-insulin and the concentration of the drug indicated in the abscissa; then insulin was removed and cells were incubated for a further 20 min in the presence of the same drug concentration. Cell-surface binding was measured by the exchange binding assay. Cell-surface bound insulin in cultures incubated with vinblastine (a) or bacitracin (b) together with insulin (●), and then after removal of insulin (○), are shown. The initial cell-surface binding is represented by the broken line.

for example, this recovery represented an average of 73% of the initial binding with large variations in different experiments where the rate of insulin dissociation and the percentage of cell-bound insulin degraded, remained inhibited (Figs. 5b and 5c). Receptor internalization was not greatly modified by lowering the temperature from 37 to 22 °C. After internalization at 22 °C, a temperature-dependence for receptor recycling similar to that observed when previous internalization occurred at 37 °C was obtained, but with a slight attenuation of the recovery rate (results not shown).

DISCUSSION

A reversible translocation of insulin receptors from the cell surface to a compartment not accessible to insulin at 4 °C occurs after a transitory exposure to the hormone in cultured foetal hepatocytes (Soubigou *et al.*, 1986). In the present study, this insulin-induced phenomenon was modified in the presence of chloroquine, a weak base, and monensin, a carboxylic acid ionophore, which raise the pH of the lysosomal and endocytic compartment (Yamashiro & Maxfield, 1984), and markedly impair insulin degradation in cultured foetal hepatocytes (Plas & Desbuquois, 1982; the present paper). If the initial rate of insulin-receptor internalization was not greatly changed, that of receptor externalization was strongly inhibited in the presence of the drugs, especially monensin. A new steady state of receptor distribution was obtained, despite complete inhibition of receptor externalization, probably because receptor internalization had also slowed. One possible explanation is that two subpopulations of insulin receptors exist, both able to bind insulin but only one implicated in the ligand-induced monensin-dependent receptor cycle. Also, in isolated adult hepatocytes, monensin accentuates the distribution of insulin-receptor sites inside the cell without affecting internalization; only 60% of insulin receptors appear to be monensin-sensitive (Whittaker

et al., 1986). As described for several non-hormonal ligands, dissociation of the internalized insulin-receptor complex, sorting and cellular distribution of hormone and receptor probably occur in the acidic endocytic compartment with the drug-induced disruption of the endosomal function by elevating pH responsible for the inhibition of receptor recycling and ligand degradation (for reviews see Brown *et al.*, 1983; Yamashiro & Maxfield, 1984; Wileman *et al.*, 1985; Wiley, 1985; Evans, 1986). Moreover, data obtained on the monensin concentration-dependency of the reversibility of the drug effect show that the inhibition of receptor recycling was reversed with monensin concentrations still effective on insulin degradation. This suggests different locations of the monensin effect, leading to inhibition of receptor recycling and insulin degradation, which thus appear independent processes.

The inhibitory effect of vinblastine on the processes of insulin-receptor recycling and insulin degradation, with no great modifications of the rate of receptor internalization, resembled that obtained with chloroquine and monensin. Disappearance of cytoplasmic microtubules and appearance of paracrystals have been observed with the same range of vinblastine concentrations as are found to inhibit insulin degradation, indicating that vinblastine could act as a cytoskeleton inhibitor (Pringault *et al.*, 1985). Whether the effect of vinblastine takes place after the dissociation of the ligand-receptor complex, as suggested for the hepatocyte asialoglycoprotein receptor (Oka & Weigel, 1983), is not known. By contrast, bacitracin concentrations which inhibit insulin degradation were unable to modify the rate of receptor internalization and that of receptor recycling. Bacitracin is known to inhibit both glutathione-insulin transhydrogenase and insulin-proteinase (Roth, 1981; Duckworth & Kitabchi, 1981). In isolated or cultured adult hepatocytes, bacitracin has been thought to inhibit cellular degradation of insulin, predominantly at the plasma-membrane level with or

Fig. 5. Effect of temperature on recovery of cell-surface insulin binding after previous short exposure to the hormone at 37 or 22 °C, and on cell-associated insulin and its degradation at 37 °C

(a) Foetal hepatocytes were incubated for 10 min in the presence of 10 nM native insulin at 37 °C (or 22 °C); then insulin was removed and cells were incubated for a further 10 or 20 min at 37, 22 or 4 °C. Cell-surface binding was measured by the exchange binding assay. (b and c) In parallel cultures, incubation conditions were similar, except that cells were exposed to 10 nM-¹²⁵I-insulin instead of native hormone and that the amount of specifically cell-associated radioactivity and the percentage of insulin degradation were measured. Cell-surface bound insulin (a), cell-associated insulin (b) and its percentage of degradation (c) in cultures incubated with insulin at 37 °C (●), or at 22 °C (▲), or with insulin at 37 °C and then after removal of the hormone at 37 °C (○), 22 °C (△) and 4 °C (□), are shown. Results are presented as means ± S.D. for five experiments performed with different cell preparations.

without impairing internalization, after either primary binding or retroendocytosis (Juul & Jones, 1982; Bonser *et al.*, 1983; Peavy *et al.*, 1985; Blackard *et al.*, 1985; Fleig *et al.*, 1986). In cultured foetal hepatocytes, where insulin degradation occurs predominantly by a chloroquine-sensitive pathway, the kinetics of the early

inhibition of insulin degradation by bacitracin and chloroquine are similar (Plas & Desbuquois, 1982). These results suggest that bacitracin acts after the dissociation of the insulin-receptor complex, and clearly show the uncoupling between the degradation of the hormone and the rapid recycling of its receptor.

Another way to inhibit hepatocyte insulin degradation is to lower the temperature to 22 °C (Terris *et al.*, 1979). Insulin-receptor internalization was preserved at 22 °C, whereas receptor recovery was more sensitive to temperature and could be inhibited at 22 °C. The consistency of the results for the inhibition of insulin degradation at 22 °C contrasted with the considerable experimental variability in the corresponding slowing of the rate of receptor recycling. This again indicates that no relationship exists between the extent of insulin degradation and the rate of receptor recycling, although large and abrupt temperature changes must affect many cellular processes. Also, a critical step for insulin-receptor recycling could be dependent on temperature around 22 °C. In this respect, low temperature has been shown to inhibit selectively fusion between pinocytotic vesicles and lysosomes during heterophagy of ¹²⁵I-asialofetuin by the perfused rat liver, despite continued ligand endocytosis (Dunn *et al.*, 1980).

Results reported in the present study show that: (i) receptor cycling was not modified under conditions where insulin degradation was totally inhibited by bacitracin, (ii) in the experiments on monensin reversibility, insulin degradation was still inhibited by drug concentrations that allow receptor-recycling inhibition to be raised, (iii) a temperature around 22 °C regulated receptor recycling while clearly inhibiting insulin degradation. Another aspect is the possible link between the insulin-receptor recycling and the dissociation of the insulin-receptor complex. Insulin-receptor recycling measured by using insulin covalently attached to the receptor is progressive, and occurs within 4 h in isolated and cultured adult hepatocytes (Fehlmann *et al.*, 1982; Carpentier *et al.*, 1986a). The irreversible binding of the ligand to its receptors could affect the recycling process either by slowing down the normal route usually followed by the receptor or by by-passing another pathway of the receptor (Carpentier *et al.*, 1986b). These adult hepatocyte systems differ from cultured foetal hepatocytes in the fact that exposure to native insulin alone cannot induce a decrease in cell-surface binding, simultaneous exposure to insulin and monensin being necessary to obtain this effect (Bonser & Garcia-Webb, 1984; Whittaker *et al.*, 1986). This is at variance with the situation *in vivo*, where a single injection of insulin into rats is followed by a rapid and large translocation of liver insulin receptors from the plasma membranes to the Golgi subcellular fractions (Posner *et al.*, 1980; Desbuquois *et al.*, 1982). Besides, cultured foetal hepatocytes, unlike cultured adult hepatocytes (Blackard *et al.*, 1978; Caro & Amatruda, 1980; Kalant *et al.*, 1984), show no evidence for receptor down-regulation after prolonged exposure to the hormone (Menuelle & Plas, 1981; Caliendo & Patel, 1983; Pringault & Plas, 1983). Such a process, however, has been revealed after simultaneous exposure to insulin and drugs, such as chloroquine, via a possible inhibition of the dissociation of the insulin-receptor complex and receptor reutilization (Pringault *et al.*, 1985). The present study demonstrates that the efficiency of the insulin-receptor recycling in

cultured foetal hepatocytes is not related to the ligand-degradation process, but the role of the dissociation of the insulin-receptor complex needs further investigation.

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REFERENCES

- Ashwell, G. & Harford, J. (1982) *Annu. Rev. Biochem.* **51**, 531-554
- Blackard, W. G., Guzelian, P. S. & Small, M. E. (1978) *Endocrinology (Baltimore)* **103**, 548-553
- Blackard, W. G., Ludeman, C. & Stillman, J. (1985) *Am. J. Physiol.* **248**, E194-E202
- Bonser, A. M. & Garcia-Webb, P. (1984) *Biochem. Biophys. Res. Commun.* **119**, 706-712
- Bonser, A. M., Garcia-Webb, P. & Bhagat, C. I. (1983) *Biochim. Biophys. Acta* **762**, 390-397
- Brown, M. S., Anderson, R. G. W. & Goldstein, J. L. (1983) *Cell* **32**, 663-667
- Caliendo, A. M. & Patel, M. S. (1983) *Arch. Biochem. Biophys.* **227**, 552-561
- Caro, J. F. & Amatruda, J. M. (1980) *Science* **210**, 1029-1031
- Carpentier, J. L., Gazzano, H., Van Obberghen, E., Fehlmann, M., Freychet, P. & Orci, L. (1986a) *Mol. Cell. Endocrinol.* **47**, 243-255
- Carpentier, J. L., Gazzano, H., Van Obberghen, E., Fehlmann, M., Freychet, P. & Orci, L. (1986b) *J. Cell Biol.* **102**, 989-996
- Desbuquois, B., Lopez, S. & Burlet, H. (1982) *J. Biol. Chem.* **257**, 10852-10860
- Duckworth, W. C. & Kitabchi, A. E. (1981) *Endocr. Rev.* **2**, 210-233
- Dunn, W. A., Hubbard, A. L. & Aronson, N. N., Jr. (1980) *J. Biol. Chem.* **255**, 5971-5978
- Evans, V. J., Bryant, J. C., Kerr, H. A. & Schilling, E. L. (1964) *Exp. Cell Res.* **36**, 439-474
- Evans, W. H. (1986) *Biochem. Soc. Trans.* **14**, 170-172
- Fehlmann, M., Carpentier, J.-L., Van Obberghen, E., Freychet, P., Thamm, P., Saunders, D., Brandenburg, D. & Orci, L. (1982) *Proc. Natl. Acad. Sci. USA.* **79**, 5921-5925
- Fleig, W. E., Hoss, G., Nother-Fleig, G. & Ditschuneit, H. (1986) *Biochem. J.* **237**, 99-104
- Haigler, H. T., Maxfield, F. R., Willingham, M. C. & Pastan, I. (1980) *J. Biol. Chem.* **255**, 1239-1241
- Halperin, M. L., Cheema-Dhadli, S., Haynes, F. J. & Yip, C. C. (1986) *Clin. Invest. Med.* **9**, 141-143
- Juul, S. M. & Jones, R. H. (1982) *Biochem. J.* **206**, 295-299
- Kalant, N., Ozaki, S., Maekubo, H., Mitmaker, B. & Cohen-Khallas, M. (1984) *Endocrinology (Baltimore)* **114**, 37-43
- Kaplan, J. (1981) *Science* **212**, 14-20
- Krupp, M. N. & Lane, M. D. (1982) *J. Biol. Chem.* **257**, 1372-1377
- Menuelle, P. & Plas, C. (1981) *Diabetologia* **20**, 647-653
- Oka, J. A. & Weigel, P. H. (1983) *Biochem. Biophys. Acta* **763**, 368-376
- Olefsky, J. M. & Kao, M. (1982) *J. Biol. Chem.* **257**, 8667-8673
- Peavy, D. E., Hamel, F. G., Kincke, V. L. & Duckworth, W. C. (1985) *Diabetes* **34**, 217-221
- Plas, C. & Desbuquois, B. (1982) *Biochem. J.* **202**, 333-341
- Plas, C. & Nunez, J. (1976) *J. Biol. Chem.* **251**, 1431-1437
- Plas, C., Chapeville, F. & Jacquot, R. (1973) *Dev. Biol.* **32**, 82-91
- Posner, B. I., Patel, B., Verma, A. K. & Bergeron, J. J. M. (1980) *J. Biol. Chem.* **255**, 735-741
- Pringault, E. & Plas, C. (1983) *Biochem. J.* **212**, 529-537
- Pringault, E., Plas, C., Desbuquois, B. & Clauser, H. (1985) *Biol. Cell* **52**, 13-22
- Roth, R. A. (1981) *Biochem. Biophys. Res. Commun.* **98**, 431-438
- Soubigou, P., Pringault, E. & Plas, C. (1986) *Biochem. J.* **239**, 609-615
- Terris, S., Hofmann, C. & Steiner, D. F. (1979) *Can. J. Biochem.* **57**, 459-468
- Whittaker, J., Hammond, V. A., Taylor, R. & Alberti, K. G. M. M. (1986) *Biochem. J.* **234**, 463-468
- Wileman, T., Harding, C. & Stahl, P. (1985) *Biochem. J.* **232**, 1-14
- Wiley, H. S. (1985) *Curr. Top. Membr. Transp.* **24**, 369-412
- Yamashiro, D. J. & Maxfield, F. R. (1984) *J. Cell. Biochem.* **26**, 231-246

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