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Ontogeny of insulin-receptor interaction: correlation with circulatory insulin levels★

ANIL K. BALAPURE, A. K. RASTOGI† and M. K. SAHIB

Division of Biochemistry, † Division of Biophysics, Central Drug Research Institute, Lucknow 226 001

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Abstract. Interaction of [^{125}I]-insulin with intact hepatocytes and its correlation with circulatory insulin level was examined. The hepatocytes from new-born rats bound lowest amount of [^{125}I]-insulin (1.39±0.41 pM/mg cell protein) when circulatory insulin level was high (8±1.5 μU/ml). Hepatocytes from 7 day and 21 day old animals demonstrated a more or less similar relationship, Cells from 31 day old animals exhibited maximum insulin binding, activity (5.13±0.18.pM/mg cell protein) against a low serum insulin level (4.25±0.25 μU/ml). Scatchard analysis of insulin binding shows that the affinity is higher in the hepatocytes from new-born animals than in the hepatocytes of 31 day old animals. Higher binding observed in the latter case may be due to a greater number of binding sites. Hepatocytes from one year old rats bound very little insulin (2.50±0.36 pM/mg cell protein) against a high circulatory insulin level (9.25±0.85 μU/ml). In view of these results, it appears that the down-regulation hypothesis holds true during ontogeny too.

Keywords. Ontogeny; insulin receptor; serum insulin; hepatocytes.

Introduction

Insulin plays a key role in the homeostasis of circulatory glucose (Lehninger, 1978). Physiological action of insulin on the target tissues owes to its interaction with the specific membrane receptors (Heise *et al.,* 1982). Recent years have witnessed accumulation of sizeable information on insulin receptor interaction. However, our knowledge regarding the regulation of insulin receptors and their bioavailability is scant (Caro and Amatruda, 1980). Circulatory insulin is believed to influence bioavailability of its own receptors on the target tissues (Bar *et al.,* 1976; Davidson and Kaplan, 1977). It was considered of interest therefore, to investigate the ontogenesis of insulin-receptor interaction and find correlation, if any, with the circulatory insulin level. The present communication describes the same using intact rat liver-cells as a model.

Materials and methods

Chemicals and animals

Collagenase, Type V, porcine insulin (23. 6 I.U./mg) and bovine serum albumin (BSA) were the products of Sigma Chemical Co., St. Louis, Missouri, USA. [^{125}I]-

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Abbreviations used: BSA, Bovine serum albumin; L-15, lebowitz-15; PBS, phosphate buffer saline.

Insulin (sp. activity 100 μCi/μg) was purchased from Bhabha Atomic Research Centre, Bombay. HEPES (N-2-hydroxyethyl piperazine N′ethanesulfonic acid) was from Sisco Research Laboratories, Bombay. Lebowitz-15 (L-15) medium was the product of GIBCO, USA. Albino rats of Charles Foster strain were used in the present study.

Preparation of hepatocytes

Hepatocytes from 21 day, 31 day and one year old animals were prepared by collagenase digestion according to Seglen (1976) and Berry and Friend (1969), using six animals in each case. In the case of new-born and 7 day old rats, the cells were obtained by direct incubation of minced liver with collagenase in phosphate buffer saline (PBS) containing 1 mM $CaCl_2$ supplemented with 25 mM HEPES (pH 7.4), 0.1% BSAand0. 1% glucose at 37°C. The number of animals used for the isolation of hepatocytes for new-born and 7 day old group was thirty each. The cells obtained were washed four times by suspending in PBS and centrifugation at 100 g for 2 min. Subsequently the hepatocytes were suspended in L-l5 medium supplemented with 7 mM glucose, 25 mM HEPES, pH 7.4 for 30 min before commencement of the experiment. Viability of the hepatocytes was monitored by following exclusion of 0.05% trypan blue and it was found to be 90-95%.

Receptor assay

This was carried out according to procedure described elsewhere (Balapure *et al.,* 1980). Suitable aliquots of hepatocytes from rats of different ages were incubated with 1-3 × 10^4 cpm of [^{125}I]-insulin (sp. act. 100 μCi/μg),purified on Sephadex G-50 column, in PBS containing 1 % BSA (buffer A). The incubation was carried out at 24°C for 90 min in a total volume of 1 ml with constant shaking. Native insulin in the concentration of 10^{-6}M was added to determine non-specific binding. After the incubation was over, the tubes were centrifuged at 100 g at 4°C for 4 min to separate the cell pellet and the supernatant. The cell pellet was washed again with 3 ml of chilled buffer A and centrifuged as before. The supernatants were pooled. Bound and free radioactivity was counted in a Packard Autogamma Scintillation Spectrometer Model 5230.

Radioimmunoassay of circulatory serum insulin

Blood was drawn from rats of different age groups by decapitation and allowed to clot. Serum was collected to estimate insulin levels by radioimmunoassay using double antibody procedure essentially according to Morgan and Lazarow (1963).

Protein estimation

Protein was estimated in the cell pellet according to Lowry *et al.* (1951) using BSA as standard.

Results

Ontogenesis of circulatory serum insulin levels

Figure 1 summarises the results obtained. A high serum insulin level was observed in new-born rats which declined progressively with age during the first three

weeks of post-natal life. The insulin level again rose in 31 day old rats and the highest value was observed in a year old rats.

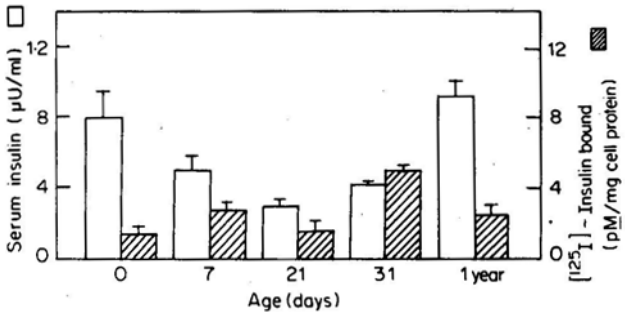

Figure 1. Radioimmunoassay of serum insulin □ and [^{125}I] -insulin binding to the hepatocyte insulin receptor ▨. Insulin level and [^{125}I]-insulin binding to the receptor was monitored according to Morgan and Lazarow (1963) and Balapure *et al.* (1980) respectively. Each point represents the Mean ± S.D. of six observations.

[^{125}I]-Insulin and hepatocyte insulin receptor interaction

Insulin-receptor interaction was detectable in hepatocytes from new-born rats as shown in figure 1. The same showed a small increase in the hepatocytes from 7 day old ones with a subsequent decline in 21 day old animals. Insulin binding activity again increased with a peak in 31 day old rats. Hepatocytes from, one year old rats showed low insulin binding activity, yet it was higher than that observed in those from new-born rats:

Scatchard analysis (Scatchard, 1949) of [^{125}I]-insulin binding was evaluated in hepatocytes from new-born and 31 day old rats. It was observed that association constant (K_a) for high and low affinity [^{125}I]-insulin binding sites were $10.4 \times 10^9 M^{-1}$ and $5 \times 10^9 M^{-1}$ respectively for the new-born rat hepatocytes (figure 2 inset). The corresponding values for the hepatocytes from 31 day old animals were $4.64 \times 10^9 M^{-1}$ and $0.86 \times 10^9 M^{-1}$ respectively (figure 2).

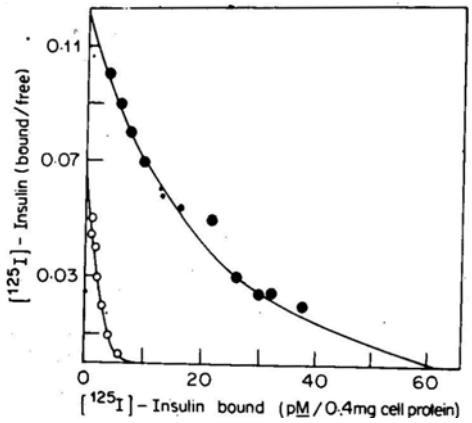

Figure 2. Scatchard plot of [^{125}I]-insulin binding to the hepatocyte insulin receptor from new born (inset, 1) and 31 day old (●) rats. Each point represents the average of three determinations.

Discussion

Ontogeny of insulin receptor from rat hepatocyte revealed an interesting pattern with low insulin binding in hepatocytes from new-born rats, rising to highest level in the hepatocytes from 31 day old rats to decline further with age to a low level in the cells derived from one year old rats. In contrast, the developmental profile of serum insulin level was almost inversely related to the pattern observed for the ontogeny of hepatocyte insulin receptor. High circulatory insulin level was observed in the serum from 31 day old rodents which increased further with age. It would appear that insulin in circulation and insulin receptors on hepatocytes bear a reciprocal relationship. Such a relationship between insulin and insulin receptors has been observed and defied as "down-regulation" (Gavin III *et al.,* 1974) under different *in vitro* as well as *in vivo* experimental conditions (Livingston *et al.,* 1978; Blackard *et al.,* 1978), barring a few exceptions (Amatruda *et al.,* 1975; Broer *et al.,* 1977; Livingston *et al.,* 1972; Misbin *et al.,* 1979; Smith and Digirolamo, 1980). That such a pattern is seen during development also is interesting and supports the view that a down regulation phenomenon could be operating during ontogeny as well.

Scatchard analysis of [^{125}I]-insulin-receptor binding was carried out to probe the reasons for the observed differences in insulin binding activity of hepatocytes from new-born (1.4 pM/10^6 cells) and 31 day old rats (5.1 pM/10^6 cells). It was observed that the association constants (K_a), both for the high and low affinity sites were higher for the hepatocytes from new-born rats. It would, therefore, appear that the higher insulin binding observed in the hepatocytes from 31 day old rats was due to an increase in the number of insulin receptors as opposed to enhanced affinity for insulin. This observation again supports the proposition that circulatory insulin level autoregulates the number of insulin receptors. It is not clear at the moment how insulin is able to regulate the bioavailability of insulin receptors. It has been suggested that insulin receptor interaction alone does not suffice to "down-regulate" insulin receptors (Amatruda *et al.,* 1975). Studies are in progress to evaluate the turnover of insulin receptors and its regulation by circulatory insulin. This will help in elucidating the mechanism of their bioavailability in normal and diseased conditions.

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