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Insulin internalization in human erythrocytes

Dear Sir,

In a recent Letter to the Editor, Bihr et al. [1] concluded from their studies that "(1) insulin binding is not necessarily followed by internalization of insulin; (2) only cells with nuclei internalize insulin; (3) insulin may well exert different effects on intracellular metabolism depending upon nuclei content; and (4) reticulocytes that can internalize insulin might be of importance concerning the rate of insulin degradation in circulating blood."

In contrast with these suggestions, we take the following view. Bihr et al. used electron microscopy to show insulin internalization in blood cells [1]. Prior to this technique, biochemical methods involving receptor down-regulation in vitro pioneered studies of insulin internalization [2]. Unlike electron microscopy, the biochemical methods can evaluate various physiological actions involved in insulin receptor down-regulation and insulin internalization [3]. These biochemical studies are equally important and should be considered simultaneously before arriving at any conclusion from electron microscopic studies of insulin internalization.

The first conclusion by Bihr et al was apparently based upon electron microscopic observations of insulin bound to human erythrocytes. They did not specify the temperature and incubation time for insulin binding prior to the observation.

However, binding is similar at 0°C and 4°C for both nucleated cells [4] and human erythrocytes. We have shown that 91%–98% of bound insulin at 4°C is extractable and the extractable insulin is intact [5]. At 15°C, after 3.5 h of binding, 80% of bound insulin is extractable and 60% of the extractable insulin is intact, while at 37°C, with increasing time of incubation, the extractability of cell-bound insulin and the proportion of intact, undegraded, extractable insulin decreases. Since the degradation of insulin in these erythrocytes can only be by cytosolic enzyme [7], degradation is a measure of internalization. Thus, these findings clearly show that insulin internalization is time- and temperature-dependent in human erythrocytes.

In contrast with the second conclusion of Bihr et al. [1], our published data [5–8] provided the following evidence of insulin internalization by human erythrocytes at 37°C: (a) human erythrocyte cell membranes lack an insulin degrading enzyme [6]; (b) there is a very specific and potent insulin degrading enzyme in cell cytosol (7.8); (c) there is a time-dependent continuous increase in insulin association

with the cells at 37°C [6]; and (d) about 56% of the cell-associated insulin is degraded at the end of 5 h of incubation at 37°C [6]. Moreover, according to biochemical studies performed to evaluate insulin receptor down-regulation (and thereby assess insulin internalization) in IM-9 lymphocytes (nucleated cells), contradictory results were obtained by Baldwin et al. [9], that is, insulin internalization was not observed.

Reticulocytes are known not to have nuclei. They are capable of limited protein synthesis due to performed mRNA, although they cannot synthesize mRNA [10]. Thus, Bihr et al. should not have seen insulin internalization in reticulocytes. Therefore, their fourth conclusion is contradictory to their second.

Bihr et al. [1] have drawn their conclusions without referring to the contradictory state of current published reports. We have demonstrated insulin internalization in mature human erythrocytes, which do not possess nuclei.

Yours sincerely,

S. G. Nerurkar and K. K. Gambhir

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Adenine nucleotide metabolism in diabetes

Dear Sir,

In a recent paper in this journal, Bakhle and Chelliah [1] demonstrated a modest decrease in the uptake and metabolism of adenosine diphosphate (ADP) in perfused lungs removed from rats rendered