Binding and Degradation of Insulin by Isolated Cells from Adult Rat Heart at 37 °C

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The presence of highly specific insulin receptors on isolated muscle cells from adult rat heart has been recently demonstrated by us (1). We have now studied the kinetics of ^{125}I-labelled insulin binding and the degradation of receptor-bound insulin at physiological temperature.
Association of ^{125}I-labelled insulin (10^{-10} mol/l) was found to be rapid reaching a maximum by 60 min; further incubation resulted in a decrease in specific binding. Dissociation of bound hormone was monitored by a 50fold dilution of the cell suspension in the absence or presence of native insulin (10^{-8} mol/l). The hormone dissociated in a multiexponential way with a half-time of 25 min. The presence of native insulin markedly enhanced the dissociation rate, suggesting the presence of negative cooperativity among the receptor sites. 90 % of cell bound radioactivity was dissociated by addition of an excess of unlabelled insulin after 10 min of association. This dissociable fraction decreased to about 70 % after 60 min of association, but remained unaltered after 180 min of association.

In a second series of experiments we have studied the chemical nature of cell-bound radioactivity at different times of association using gel filtration on Sephadex G-50. After 10 min only intact ^{125}I-labelled insulin could be detected on the cells. Up to 180 min of association an increasing amount of radioactivity eluted in the void volume, mainly representing nonspecific binding. After 60 min 4 % of bound radioactivity consisted of low molecular weight fragments; this fraction remained unaltered even after 180 min of association. In contrast, after 180 min about 20 % of ^{125}I-labelled insulin in the incubation medium was found to be trichloroacetic acid -soluble.

In conclusion: (a) After reaching equilibrium binding (60 min) no "compartmentalization" of receptor-bound insulin does take place in cardiac myocytes. (b) No accumulation of insulin degradation products can be observed after 60 min. The major part of low molecular weight fragments is released into the medium. (c) The kinetic data suggest that negative cooperativity among insulin receptors may be of physiological significance in the heart muscle.

1. Eckel, J. and Reinauer, H. (1980) Biochim. Biophys. Acta, in press.