Brief Notes and Comments

Comparison of the Lipolytic Effects of Insulin and Proinsulin on Isolated Fat Cells

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SUMMARY

Proinsulin, like insulin, has a biphasic effect on the lipolysis of isolated fat cells. Low concentrations of the peptides inhibit the response to submaximal doses of epinephrine, while higher concentrations of the peptides enhance the effect of supramaximal doses of epinephrine. Half-maximal doses are: (a) inhibition of lipolysis: insulin, 2.3 x 10^{-11}M; proinsulin, 6.9 x 10^{-10}M; (b) enhancement of lipolysis: insulin, 3.1 x 10^{-9}M; proinsulin, 8.1 x 10^{-8}M. The ratios of the potencies of the peptides are the same for both effects. These results suggest that both the inhibitory and the lipolytic effects of insulin are not caused by contaminants, and are mediated by similar receptors. DIABETES *24*:238-39, February, 1975.

Several investigators have described a biphasic effect of insulin on the lipolysis of isolated rat adipose tissue.[1-6] In the presence of epinephrine or corticotropin, concentrations of insulin less than about 10 ng/ml. (1.7 x 10^{-9}M) inhibit lipolysis in a dose-related fashion, but higher concentrations inhibit less well or even stimulate lipolysis.[1-6] Whether the lipolytic response should be attributed to a contaminant in commercial preparations of insulin remains an unsettled question, although indirect evidence[4,6] suggests that this is an effect of insulin itself.

We approached the question from another angle by comparing the effects of proinsulin with those of insulin. If the lipolytic response be mediated by insulin receptors, then (a) a high enough concentration of proinsulin, which binds to these receptors,[7,8] should produce the effect; and (b) the relative potencies of proinsulin and insulin should be the same for inhibition and for enhancement of lipolysis.

Porcine proinsulin (Elanco) was a gift from Dr. John Ensinck. The sources of other materials and the methods for incubations and for measurement of glycerol release were as previously described.[6] As in our earlier work,[9] dose-response curves were made linear by conversion of responses to probits,[10] and statistics calculated by standard methods.[11] Details of experimental conditions are given in the legend to table 1.

Table 1 shows that high concentrations of both insulin and proinsulin enhanced epinephrine-stimulated lipolysis, with the ratio of their potencies the same as for inhibition of lipolysis. For purposes of comparison, in our previous study[9] the 95 per cent confidence limits for the half-maximal doses (ED_{50}) for inhibition of lipolysis were: insulin, 2.6 x 10^{-11} to 3.3 x 10^{-11}M; proinsulin, 3.7 x 10^{-10} to 4.5 x 10^{-10}M. Our present data, obtained with different preparations of insulin and proinsulin, agree well with the previous results.

Our results are consistent with the hypothesis that both inhibition and stimulation of lipolysis are caused by insulin molecules. It seems less likely that a contaminant would have partitioned between proinsulin and insulin peaks during preparative gel filtration, exactly in proportion to the relative potencies of the two peptides. Moreover, our confidence limits for the ED_{50} for the lipolytic effect of insulin include the approximate value of 0.6 x 10^{-9}M reported by Kono and Barham.[4] If another substance produced these results, it would have had to have contaminated their preparation of insulin and ours to the same degree. The above arguments are not conclusive, but the weights of the probabilities

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TABLE 1

Measurement	Slope of Dose-Response Line $\left(\frac{\text{probits}}{\log_{10}[\text{peptide}]}\right)$	ED_{50}(M)	Molar Ratio of Potency: Proinsulin/Insulin
Inhibition of Lipolysis:			
Insulin	2.38 (2.0 - 2.8)	2.27×10^{-11} (1.9×10^{-11} to 2.7×10^{-11})	0.033 (0.026 - 0.041)
Proinsulin	2.32 (1.8 - 2.9)	6.93×10^{-10} (5.4×10^{-10} to 9.2×10^{-10})	
Enhancement of Lipolysis:			
Insulin	0.92 (0.23 - 1.6)	3.10×10^{-9} (0.26×10^{-9} to 8.2×10^{-9})	0.038 (0.009 to 0.13)
Proinsulin	0.84 (0.21 - 1.5)	8.13×10^{-8} (3.5×10^{-8} to 2.8×10^{-7})	

For measurement of inhibition of lipolysis, fat cells were incubated for one hour with 5.5×10^{-7}M epinephrine and either insulin, 4.4×10^{-12} to 7.0×10^{-11}M, or proinsulin, 1.6×10^{-10} to 2.0×10^{-8}M. Results were pooled from seventeen sets of triplicate incubations performed on five different days.

For measurements of enhancement of lipolysis, incubations were for two hours with 5.5×10^{-6}M epinephrine, and insulin, 7.0×10^{-10} to 7.0×10^{-5}M, or proinsulin, 8.8×10^{-9} to 2.2×10^{-7}M. Results were pooled from nine sets of triplicate incubations, performed on two different days.
ED_{50} = the concentrations of peptide which produced 50 per cent of the maximal response.
Molar ratio of potency: $\frac{\text{proinsulin}}{\text{insulin}} = \frac{ED_{50} \text{ for insulin}}{ED_{50} \text{ for proinsulin}}$
Results are shown as mean values, with 95 per cent confidence limits[11] in parentheses.

suggest that insulin itself can stimulate lipolysis. Of all the evidence adduced to date[4,6] none supports a contrary conclusion.

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[11]Ibid, pp. 135-44; 156-69.