SUPPORTING INFORMATION

Insulin Analogs with Modifications at Position B26. Divergence of Binding Affinity and Biological Activity.

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Binding affinity of [D-AlaB26]-insulin and of human insulin.

[D-AlaB26]-insulin analog was prepared according to the methodology described in the main manuscript. The binding affinities of [D-AlaB26]-insulin and of human insulin (Table) were determined in a separate experiment using the methodology described in the main manuscript. The binding curves are shown in Figure. The relative binding affinity of the analogue [D-AlaB26]-insulin, 17% compared to human insulin, is in a very good agreement with the result of Kurapkat et al. (*1*) who published 18% potency. This result again shows that substitutions, which give very potent DTI-NH_2 analogs, [NMeAlaB26]-DTI-NH_2 (this study) or [D-AlaB26]-DTI-NH_2 (*1*), do not result in potent full length analogs.

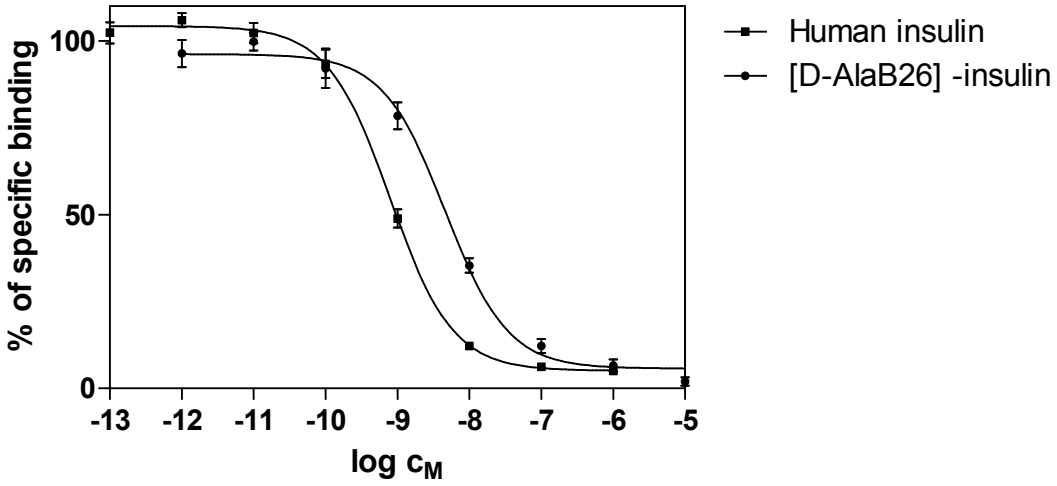
Table. Values of IC_{50}[a] and relative receptor binding affinities of human insulin and [D-AlaB26]-insulin. The experimental values were determined with ^{125}I-monoiodotyrosylA14-insulin.

Peptide	IC_{50}[a] ± SEM [nM] (*n*)	potency[b] [%]
Human insulin	0.79 ± 0.08	(6) 100
[D-AlaB26]-insulin	4.66 ± 0.41	(5) 17

[a]IC_{50} values represent concentrations of insulin or the analog that cause half-maximal inhibition of binding of human ^{125}I-monoiodotyrosylA14-insulin to the insulin receptor. Each value represents the mean ± SEM of multiple determinations (*n*).

[b]Relative receptor binding affinity is defined as (IC_{50} of human insulin/IC_{50} of analog) x 100.

Figure. Inhibition of binding of human ^{125}I-insulin to adipose plasma membranes by human insulin (■) and [D-AlaB26]-insulin (●).Quantitative information is provided in Table.



Reference

1. Kurapkat G., Siedentop M., Gattner H.-G., Hagelstein M., Brandenburg D., Grötzinger J. and Wollmer A., *Protein Sci.* **8**, 499-508 (1999).