BIOLOGICAL ACTIVITY OF SOME INSULIN DERIVATIVES

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Received February 21st, 1980

The ability of insulin and some of its derivatives to compete with ^{125}I-insulin for the binding site in the membrane fraction prepared from the mammary gland of lactating mice is reported. The binding affinity decreased in the following order: insulin > desoctapeptide-insulin > tert-butyloxycarbonyl$_3$-insulin > tert-butyloxycarbonyl$_2$-desoctapeptide-insulin. Insulin hexamethyl ester and its desoctapeptide in concentrations $5{\cdot}5 \cdot 10^{-11} - 2 \cdot 10^{-5}$M did not inhibit the binding of ^{125}I-insulin. The comparison of the ability to decrease the blood glucose level showed that tert-butyloxycarbonyl$_3$-insulin had 5% of the activity of insulin and that the other derivatives had less than 0·5% of that of insulin.

In our previous paper[1], we described the immunoreactivity of some insulin derivatives containing protected functional groups. In this paper, we present further characteristics of these compounds, *i.e.* of desoctapeptide-insulin, tert-butyloxycarbonyl$_3$-insulin, insulin hexamethyl ester and their desoctapeptide derivatives. We studied the interaction of the compounds with the membrane fraction prepared from mammary glands of lactating mice and their effect on the blood glucose level in rabbits.

EXPERIMENTAL

The preparation of desoctapeptide-insulin (B_{1-21}), tert-butyloxycarbonyl$_3$-insulin (BOC_3-insulin), its desoctapeptide derivative (BOC_2-desoctapeptide-insulin), insulin hexamethyl ester and its desoctapeptide derivative was described earlier[1]. Crystalline bovine Zn-insulin (23·4 I.U./mg) was purchased from the British Drug House, England; insulin (a mixture of bovine and porcine insulin) was obtained from Léčiva, Prague. Na^{125}I was supplied by Isotop, Budapest, Hungary, Sephadex G-100 and DEAE-Sephadex A-25 were purchased from Pharmacia, Uppsala, Sweden.

For determining the decrease of the blood glucose level, we used rabbits weighing 2·5—3·5 kg. The membrane fraction of mammary glands was prepared from lactating female mice of the H strain (Srbsko, Czechoslovakia), weighing 30—40 g, which were sacrificed 10—14 days post partum.

Radioactive labelling of insulin: Insulin-HCl, prepared according to Young and Carpenter[2] from Zn-insulin, was labelled by ^{125}I according to a method using Chloramine T (ref.[2,3]). Radioactively labelled insulin was purified either by chromatography on a column of Sephadex G-100 or by ion exchange chromatography on a column of DEAE-Sephadex A-25 (ref.[4]). The specific activity of labelled insulin was 25—50 mCi/mg.

The preparation of the crude membrane fraction from mammary glands of lactating mice was described in our previous paper[3]. The membrane fraction was incubated[3] with either labelled insulin alone or in combination with unlabelled insulin or its derivative; the concentration of the polypeptide was in the range of $5 \cdot 10^{-11}$—$2{\cdot}7 \cdot 10^{-5}$M. The ability of the individual derivatives to compete with labelled insulin in binding to the membrane fraction was compared with that of unlabelled insulin. Corrections were made for non-specific binding.

The decrease of the blood glucose level in fasting rabbits was estimated after the application of the individual derivatives and compared with the effect of insulin. Each derivative was assayed on 6 rabbits; the results are given as arithmetic mean values. The blood glucose level was determined by the orthotoluidine method[5,6]. The activity of the derivatives was expressed in percentages of the activity of insulin.

RESULTS AND DISCUSSION

We were able to detect the presence of only one type of binding site with high affinity ($K_{aff} = 1{\cdot}03 \cdot 10^{10}$ mol^{-1} l) in the crude membrane fraction. The binding capacity of the membrane fraction for insulin was $1{\cdot}06 \cdot 10^{-4}$ mol of insulin/mg (ref.[3]). The binding of insulin to the membrane fraction was inhibited by only one of the derivatives tested. The results are presented in Table I. Desoctapeptide insulin displaced 50% of ^{125}I-insulin at concentrations that were higher by two orders of ten than the concentration of native insulin that had the same inhibitory effect. In the case of BOC_3-insulin and insulin hexamethyl ester, 50% inhibition of the binding of radioactive insulin could not be achieved in the concentration range used. Apart from data

TABLE I
Biological Activity of Some Insulin Derivatives

Derivative	Mouse mammary gland (% of insulin binding)	Immunoreactivity[a] (% of insulin binding)	Decrease of blood glucose level (% of insulin activity)
Desoctapeptide-insulin	0·60	12·4	10·3
BOC_3-insulin	0·01	25·0	5·6
Desoctapeptide--BOC_2-insulin	0·01	0·56	0·5
Insulin hexamethyl ester	0·01	0·001	0·01
Desoctapeptide-insulin pentamethyl ester	0·01	0·001	0·01

[a] According to reference[1].

on the cross-reactivity of the derivatives studied, Table I presents values expressing their ability to decrease the blood glucose level in rabbits. The removal of the terminal octapeptide decreased this biological activity by one order of ten (Carpenter and Baum[7] observed that this derivative had 2% of the activity of oxytocin, Halban and Offord[8] state 1·5%). The substitution of the primary amino groups by tert-butyloxycarbonyl resulted in a 20fold decrease of biological activity. All the other modifications practically eliminated biological activity. As can be seen from Table I, the modifications had the most pronounced effect on the affinity to the receptors in the mammary gland. The removal of the octapeptide led to a more than 100fold decrease of affinity; all the other modifications, *i.e.* acylation and esterification practically eliminated affinity.

REFERENCES

1. Barth T., Šiševa A., Sirakov L., Slaninová J., Ditzov S.: This Journal *43*, 2967 (1978).
2. Young J., Carpenter F. H.: J. Biol. Chem. *236*, 743 (1961).
3. Apostolova J., Sirakov L. M., Barth T.: This Journal *41*, 3830 (1976).
4. Sodoyez I. C., Sodoyez-Goffaux F., Goff M. M., Zimmerman A. E., Arquilla E. R.: J. Biol. Chem. *250*, 4268 (1975).
5. Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. L.: J. Biol. Chem. *193*, 265 (1951).
6. Dubowski K. M.: Clin. Chem. *8*, 215 (1962).
7. Carpenter F. H., Baum W. E.: J. Biol. Chem. *237*, 409 (1967).
8. Halban P. A., Offord R. E.: Biochem. J. *151*, 219 (1975).

Translated by L. Servitová.