Effect of Insulin Fragments on Biological Activity of Insulin and Desoctapeptide Insulin

I. POTENTIATION OF BIOLOGICAL ACTIVITIES*

* Portions of this paper (including "Materials and Methods," "Results," and Figs. 1-11) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 81M-311, cite authors, and include a check or money order for $6.00 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

K. Kikuchi†, J. Larner‡, R. J. Freer¶, and A. R. Day††

From the †Department of Pharmacology, University of Virginia School of Medicine, Charlottesville, Virginia 22908 and the ¶Department of Pharmacology, Medical College of Virginia, Richmond, Virginia 23219

Four derivatives of the insulin B-chain COOH-terminal pentapeptide Arg-Gly-Phe-Phe-Tyr (B22-26) were synthesized and shown to be inactive alone. In the presence of submaximal concentrations of insulin or desoctapeptide insulin, peptides at concentrations of $10^{-4}$ M and higher, markedly stimulated the actions of insulin on rat adipocytes including labeled glucose oxidation, activation of glycogen synthase, and stimulation of 2-deoxyglucose transport. The B-chain COOH-terminal heptapeptide, Gly-Phe-Phe-Tyr-Thr-Pro-Lys (B23-29) was inactive alone or in the presence of submaximal concentrations of insulin or desoctapeptide insulin, suggesting that arginine is required. Dose response curves of insulin, and desoctapeptide insulin for labeled glucose oxidation by rat adipocytes were shifted 1 log concentration unit to the left in the presence of peptide. Peptide shortened the lag time of labeled glucose oxidation and markedly enhanced the rate of $^{14}$CO$_2$ production following the lag time. Peptides also enhanced insulin-like activities of concana was and nonsuppressible insulin-like activity P.

Weitzel and co-workers have proposed that the COOH-terminal region of the B-chain, Arg-Gly-Phe-Phe-Tyr (B22-26) is the "active center" of the insulin molecule, because the peptide alone had insulin-like activity which increased in a stepwise fashion from tripeptide to maximal activity at pentapeptide (1). We recently reported, however, that desoctapeptide insulin lacking eight COOH-terminal amino acids of the B-chain as well as desalanine-desasparagine insulin possessed full biological activity in terms of the maximal response, and that, in the presence of the submaximal concentrations of insulin or desoctapeptide insulin, the COOH-terminal B-chain peptides (with or without Arg 22) weakly stimulate insulin action without altering insulin binding (2). We have now examined more extensively the biological activity of the B-chain COOH-terminal peptides. As reported here, at concentrations of $10^{-4}$ M and higher, the peptide B22-26 containing Arg 22 markedly enhanced the action of insulin and desoctapeptide insulin on isolated rat adipocytes. In addition, the peptide enhanced the biological activity of two insulin-like agents, concana was and nonsuppressible insulin-like activity P.

MATERIALS AND METHODS AND RESULTS

Analogues of the pentapeptide Arg-Gly-Phe-Phe-Tyr (B22-26) were inactive alone. With suboptimal concentrations of insulin or desoctapeptide insulin, peptides ($10^{-4}$ to $10^{-3}$ M) stimulated glucose oxidation, 2-deoxyglucose transport, and glycogen synthase activation. We reported recently that desoctapeptide insulin possessed full biological activity of insulin but with markedly impaired binding to the insulin receptor (2). Although inactive alone, Arg-Gly-Phe-Phe-Tyr-NH$_2$ (B22-26) as the $\beta$-alanyl derivative and Gly-Phe-Phe-Tyr-Thr-Pro-Lys (B23-29) both weakly stimulated insulin action at $10^{-3}$ M without affecting the binding of labeled insulin. Peptide actions described here are due to a different mechanism. All B22-26 pentapeptide derivatives potentiated insulin and desoctapeptide insulin activity but with differing potency. The order of potency was B22-26 acid > N-acetyl pentapeptide amide > pentapeptide amide > $\beta$-alanyl pentapeptide amide. The heptapeptide Gly-Phe-Phe-Tyr-Thr-Pro-Lys ($10^{-4}$ to $10^{-3}$ M) was inactive in the absence or presence of insulin. Although the B22-26 pentapeptide acid was less active than the B22-26 pentapeptide amide, the acid form showed significant biological potency (Figs. 4 and 7). Therefore, the fact that the heptapeptide was biologically inactive was not due to it lacking the amide group at the COOH-terminal. These results suggest that B22 arginine is essential and is in accord with experiments of Weitzel et al. (9). By demonstrating a graded response to peptides of increasing size from tripeptide up to pentapeptide for maximal activity, Weitzel and co-workers provided convincing evidence, for the specific sequence and pentapeptide size requirement for full activity (see 9-11). However, in our experiments, peptides alone were inactive, and were active only in the presence of submaximal insulin or desoctapeptide insulin. The insulin-like action of peptides alone described by Weitzel et al. (9) is more notable in whole animals or in tissues rather than in adipocytes, despite the fact that adipocytes are among the cells most...
sensitive to the action of insulin (3). These data can be reasonably interpreted if we assume that the insulin-like activity of the peptides reported by Weitzel et al. (10) was not due to their inherent activity per se, but rather due to the stimulation by the peptides of the action of insulin or of degraded insulin present in the animals or tissues but not present in the cells. In our experiments, peptides alone occasionally demonstrated weak insulin-like actions in the absence of added insulin, but only when exceedingly high concentrations of adipocytes were used, conditions favoring the presence of traces of contaminating endogenous insulin or degraded insulin. The fact that anti-insulin serum completely cancelled desoctapeptide insulin was shortened and the rate of glucose oxidation after the lag phase increased in direct relationship to increasing concentrations of insulin or desoctapeptide insulin. This lag period may thus reflect the time required to attain equilibrium of insulin binding or of receptor activation and/or the time required to accumulate insulin mediator (12, 13). The fact that the lag phase was seen with insulin and desoctapeptide insulin, supports again the conclusion that desoctapeptide insulin acts through the insulin receptor.

Peptide increased the insulin-like actions of both concanavalin A and nonsuppressible insulin-like activity P. Two general possibilities can be considered. One is that concanavalin A and nonsuppressible insulin-like activity P both act through the insulin receptor itself (14). The other is that they interact with other receptors specific for concanavalin A and nonsuppressible insulin-like activity P, respectively, which contain a common structure to the insulin receptor. Concanavalin A and nonsuppressible insulin-like activity P both have been reported to have a peptide sequence similar to the COOH-terminal of the B-chain of insulin (15, 16). This suggests the possibility that concanavalin A or nonsuppressible insulin-like activity-P specific receptors, if they exist, may contain a structure similar to the insulin receptor which can interact with the COOH-terminal peptide of the B-chain of insulin.

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REFERENCES
SUPPLEMENTAL MATERIAL TO

EFFECT OF INSULIN FRAGMENTS ON BIOLOGICAL ACTIVITY OF INSULIN AND DEOXY-
PEPTIDE INSULIN

1. Potentiation of Biological Activities

1.1. Biological Activity of Insulin Fragments

Running Title: Biological Activity of Insulin Fragments

MATERIALS AND METHODS

Materials were prepared from modified adipose tissue of rats weighing 120 g to 150 g. (Final useful material derived from pooled samples of between 200 and 250 rats). Glucagon was purchased from Sigma Chemical Company. Cytochrome c was purchased from Calbiochem-Behring Corporation. Crystalline insulin was obtained from Novo Scientific Company. Glucagon was purified as described previously [1].

RESULTS

We recently reported the C-terminal B-chain peptides isolated from bovine insulin containing 10 458 and 10 484 as potent stimulators of glucose oxidation in vitro. These peptides are approximately 1/10 the size of the intact hormone, yet they are capable of stimulating glucose oxidation in isolated perfused liver preparations with minimal side effects. In the present study, we have examined the effect of these peptides on glucose oxidation in vivo. The results indicate that the C-terminal B-chain peptides are effective in vivo as well.

Fig. 1. Effect of the B30-37 peptide on [3H]glucose oxidation in the absence and presence of insulin. Cells were incubated at 37°C for 1 h in a medium containing 0.1 mM 2-DG and 10 458-glucose with the indicated concentrations of the B30-37 peptide and/or insulin. The results are presented as a percentage of the control (100%) and are the mean of at least three experiments.

Fig. 2. Effect of the B30-37 peptide on [3H]glucose oxidation in the presence and absence of insulin. Cells were incubated at 37°C for 1 h in a medium containing 0.1 mM 2-DG and 10 458-glucose with the indicated concentrations of the B30-37 peptide and/or insulin. The results are presented as a percentage of the control (100%) and are the mean of at least three experiments.

Peptide solutions were prepared by adding one drop of 3M HCl to one ml of the peptide in water and adjusting the volume to 10 ml with water. The solutions were incubated at 37°C for 1 h, after which the peptide solution was added to the assay mixture for insulin action. The amount of the assay mixture was varied from 0.1 ml to 1 ml. Detailed studies on this point will be discussed in a subsequent paper.
Fig. 1. Time course of [14C]-glucose oxidation in the presence of insulin, desaminopeptide insulin, or insulin plus the peptide. (A) Time course of the presence of varying concentrations of insulin. Cells were incubated with insulin (A, 10⁻¹⁰ M, B, 10⁻⁹ M, C, 10⁻⁸ M) as described in Fig. 1 for the indicated time. After the appropriate time, the incubations were terminated and [14C]-glucose oxidase activity measured. All values obtained after incubation were subtracted from the basal values obtained after incubation without insulin and nanomolar. (B) Time course of the presence of varying concentrations of desaminopeptide insulin. All results were as C, 10⁻⁸ M, basal values were subtracted from the basal values obtained after incubation without insulin and nanomolar. (C) Time course of the presence of varying concentrations of desaminopeptide insulin. All results were as C, 10⁻⁸ M, basal values were subtracted from the basal values obtained after incubation without insulin and nanomolar. (D) Time course of the presence of varying concentrations of desaminopeptide insulin. All results were as C, 10⁻⁸ M, basal values were subtracted from the basal values obtained after incubation without insulin and nanomolar.

When peptide was added after preincubation of adipocytes with subthreshold (32° for 3 h) Glucose oxidation was unaltered and still stimulated when additional insulin (50 units) was added (Fig. 6).

Fig. 2. Time course of [14C]-glucose oxidation in the absence and presence of the peptide after preincubation with adrenaline in the presence of insulin. Cells were preincubated with adrenaline (0.2 M) as described in Fig. 1 for the indicated time. [14C]-Glucose oxidation was measured for 1 hour in the presence of both insulin and adrenaline. The results were as described in Fig. 5.

With subthreshold levels of desaminopeptide insulin, peptide markedly stimulated glucose oxidation (Fig. 7).

Fig. 3. Stimulations of glycogen synthesis activity by peptide in the presence of insulin. A) Amino acid-free glucosed medium (pH 6.8) was incubated in Eagle’s-Ringer phosphate buffer without glucose for 1 h at 37°C without (C) or with 0.2 M adrenaline without (A) or with 0.2 M adrenaline and 0.5 M 10⁻⁷ M desaminopeptide insulin (B) and then centrifuged for 15 min in a clinical centrifuge. Medium was removed, 0.5 ml of culture buffer (100 mM NaF and 10 mM Tris, pH 7.4) was added, cells rapidly homogenized at 4°C, then centrifuged at 10,000 × g for 15 min, a supernatant was prepared for malate activity. A control was obtained from incubation of cells with glucose. The results were as described in Fig. 5.

Fig. 4. Effect of peptide on 2-deoxyglucose transport in the presence of varying concentrations of insulin or desaminopeptide insulin. Cells were preincubated at 37°C for 1 h without or with 0.5 M adrenaline and desaminopeptide insulin. For other conditions, see text. N, insulin, O, desaminopeptide insulin, +, desaminopeptide insulin plus peptide.

Peptide in the presence of increasing concentrations of desaminopeptide insulin elicits a further stimulated glucose oxidation in a concentration-dependent manner (Fig. 6A, B).

Fig. 5. Effect of peptide on [14C]-glucose oxidation in the presence of varying concentrations of insulin. Cells were preincubated without (A, A) or with 0.5 M adrenaline and desaminopeptide insulin (B, B) in the presence of varying concentrations of insulin. The results were as described in Fig. 1.

The stimulation of glucose oxidation by peptide was obliterated by 2-methyl-b-amanitine, but not the stimulation of insulin by peptide (Fig. 8).

Fig. 6. Effect of peptide on [14C]-glucose oxidation in the presence of varying concentrations of desaminopeptide insulin. Cells were preincubated at 37°C for 1 h without or with 0.5 M adrenaline and desaminopeptide insulin. For other conditions, see text. N, insulin, O, desaminopeptide insulin, +, desaminopeptide insulin plus peptide.

The stimulation of glucose oxidation by peptide was obliterated by 2-methyl-b-amanitine, but not the stimulation of insulin by peptide (Fig. 8).

Fig. 7. Effect of peptide on [14C]-glucose oxidation in the presence of varying concentrations of desaminopeptide insulin. Cells were preincubated without (A, A) or with 0.5 M adrenaline and desaminopeptide insulin (B, B) in the presence of varying concentrations of desaminopeptide insulin. The results were as described in Fig. 1.

The stimulation of glucose oxidation by peptide was obliterated by 2-methyl-b-amanitine, but not the stimulation of insulin by peptide (Fig. 8).

Fig. 8. Effect of peptide on [14C]-glucose oxidation in the presence of varying concentrations of desaminopeptide insulin. Cells were preincubated at 37°C for 1 h without or with 0.5 M adrenaline and desaminopeptide insulin. For other conditions, see text. N, insulin, O, desaminopeptide insulin, +, desaminopeptide insulin plus peptide.

The stimulation of glucose oxidation by peptide was obliterated by 2-methyl-b-amanitine, but not the stimulation of insulin by peptide (Fig. 8).

Fig. 9. Effect of peptide on [14C]-glucose oxidation in the presence of varying concentrations of desaminopeptide insulin. Cells were preincubated without (A, A) or with 0.5 M adrenaline and desaminopeptide insulin (B, B) in the presence of varying concentrations of desaminopeptide insulin. The results were as described in Fig. 1.

The stimulation of glucose oxidation by peptide was obliterated by 2-methyl-b-amanitine, but not the stimulation of insulin by peptide (Fig. 8).

Fig. 10. Effect of peptide on [14C]-glucose oxidation in the presence of varying concentrations of desaminopeptide insulin. Cells were preincubated without (A, A) or with 0.5 M adrenaline and desaminopeptide insulin (B, B) in the presence of varying concentrations of desaminopeptide insulin. The results were as described in Fig. 1.

The stimulation of glucose oxidation by peptide was obliterated by 2-methyl-b-amanitine, but not the stimulation of insulin by peptide (Fig. 8).

Fig. 11. Effect of peptide on [14C]-glucose oxidation in the presence of varying concentrations of desaminopeptide insulin. Cells were preincubated without (A, A) or with 0.5 M adrenaline and desaminopeptide insulin (B, B) in the presence of varying concentrations of desaminopeptide insulin. The results were as described in Fig. 1.

The stimulation of glucose oxidation by peptide was obliterated by 2-methyl-b-amanitine, but not the stimulation of insulin by peptide (Fig. 8).