The Effect of Aprotinin on the Absorption of Subcutaneously Injected Regular Insulin in Normal Subjects

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SUMMARY

The effect of aprotinin on the absorption of regular insulin was assessed in normal man. Ten units of Actrapid insulin were subcutaneously injected together with 1.4 mg aprotinin (i.e., 0.5 ml of Trasylol) or an equivalent volume of physiologic saline (controls) into the thighs of overnight-fasted normal subjects. Aprotinin caused an increase in the rate of insulin entry into the circulation; the absolute amount of insulin that was detected in the circulation during the course of the experiment was also higher. In addition, the onset of the hypoglycemic action of exogenous insulin was significantly accelerated when insulin was administered together with aprotinin. These data suggest that aprotinin increases the absorption rate of subcutaneously injected insulin from its depot into the circulation, possibly by an inhibition of the local degradation of exogenous insulin at the injection site. DIABETES *29*:81–83, January 1980.

We have recently reported that a considerable proportion of subcutaneously injected insulin is degraded at the site of injection, i.e., before it even reaches the circulation.[1] In addition, we have demonstrated that the degradative activity of rat adipose tissue in vitro is at least partially specific to insulin and that the degradation of insulin by adipose tissue slices in vitro can be inhibited by the bovine pancreatic protease inhibitor aprotinin.[2] This study indicates that the absorption into the circulation of subcutaneously injected insulin in normal man is accelerated when the hormone is administered together with aprotinin.

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MATERIALS AND METHODS

The experiments were carried out in accord with the Helsinki Declaration of 1975 in four male volunteers of normal body weight (mean weight 73 kg, range 67–78 kg; mean height 178 cm, range 170–186 cm), mean age 24 yr (range 21–27 yr), and free of acute or chronic illnesses, who had never been injected with insulin or aprotinin before. The individuals reported to the laboratory on two consecutive mornings after an overnight fast. Throughout the experiments they rested in a supine position. A Teflon catheter was inserted into an antecubital vein, kept patent with 0.9% saline, and was used to draw venous blood samples at indicated time intervals (Figure 1). At time zero, 10 U of Actrapid insulin (Novo Industri, Copenhagen, Denmark) was subcutaneously injected into the thigh. Immediately before administration, insulin was mixed within the injection syringe with 0.5 ml 0.9% saline or with 0.5 ml aprotinin solution (Trasylol, Bayer AG, Leverkusen, West Germany), obtained in ampules of isotonic saline solution at 20,000 kallikrein inhibitor units (KIU) per ml, equivalent to 10,000 KIU. Separate sets of experiments showed that the rate of entry of 10 U exogenous insulin into the circulation was independent of the concentration of the injected solution over the range from 40 to 12 U insulin/ml. In two subjects insulin mixed with saline was injected on the first experimental day; in the two remaining subjects the insulin-Trasylol mixture was injected first.

Except for one individual presenting some minor symptoms of hypoglycemia during both experimental conditions at 1 h following the insulin administration, no untoward side effects of the test procedure became apparent.

In control experiments, the effect of aprotinin (10,000 KIU Trasylol) subcutaneously injected together with 0.25 ml 0.9% saline was examined in four healthy volunteers, matched for age, sex, height, and weight.

Blood glucose and serum insulin were measured as previously reported;[3] serum C-peptide levels were determined according to Kaneko et al.[4] using a commercially available kit system (Byk-Mallinckrodt, 6057 Dietzenbach, West Germany).

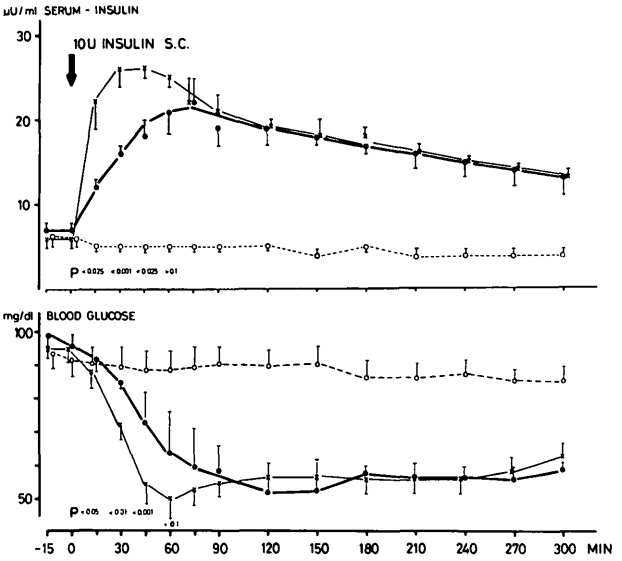

FIGURE 1. Effect of aprotinin on the increase of serum insulin and the fall of blood glucose levels after the subcutaneous injection of 10 U Actrapid insulin in four normal men. Means ± SEM are given. P values indicate statistical significance of differences for the time points indicated by paired comparison. (—×—): 0.5 ml aprotinin Trasylol, equivalent to 10,000 KIU, was added to 10 U of Actrapid insulin. (—•—): 0.5 ml 0.9% saline was added to 10 U of Actrapid insulin. (--O--): the results of control experiments are given, in which 0.5 ml aprotinin Trasylol, equivalent to 10,000 KIU, was subcutaneously injected together with 0.25 ml 0.9% saline in four matched control subjects.

RESULTS

The addition of aprotinin to subcutaneously injected insulin accelerated the rise of circulating insulin and induced significantly higher serum insulin levels at 15, 30, and 45 min after the administration of the hormone (Figure 1). In addition, the maximal serum insulin concentration reached during the experimental procedure was significantly higher when insulin was injected together with aprotinin, compared with the control injections (28 ± 2 SD versus 22 ± 2 SD µU/ml, $P < 0.01$ on paired comparison). As a consequence, the onset of the hypoglycemic action of the subcutaneously injected regular insulin was accelerated by the protease inhibitor; during the first hour following the insulin injection, blood glucose levels were significantly lower when insulin had been injected together with aprotinin, compared with the control experiments (Figure 1). Under both sets of experimental conditions, there was a continuous fall of circulating C-peptide levels, indicating the expected suppression of endogenous insulin secretion. No additional effect of aprotinin on the course of C-peptide levels was observed (Table 1).

In control experiments, any effect of aprotinin on blood glucose or serum insulin levels was ruled out (Figure 1).

DISCUSSION

This study demonstrates that the early rise of circulating insulin levels, following the subcutaneous injection of regular insulin in normal man, is accelerated and increased when the hormone is injected together with 10,000 KIU aprotinin, a bovine pancreatic protease inhibitor. This effect of aprotinin is corroborated by a significant amplification of the early hypoglycemic effect of subcutaneously injected insulin. On the other hand, there was no apparent effect of aprotinin on the suppression of endogenous insulin secretion, induced presumably via the rapid fall of glycemia, under the experimental conditions. Thus, it is assumed that the aprotinin-induced rise of circulating insulin levels in the first hour after the subcutaneous injection of regular insulin is due to an acceleration of insulin absorption into the circulation. This assumption is in agreement with the view that the early phase of the rise of exogenous insulin concentration in the circulation is primarily determined by the rate of absorption of insulin from its subcutaneous depot.[5–9] The molecular mechanisms involved in the absorption of subcutaneously injected insulin into the bloodstream remain to be elucidated. Aprotinin, however, could influence this process in a variety of possible ways, such as alterations of the local circulation or simply by working as an insulin carrier.

On the basis of our recent observations,[1,2] it might also be suggested that aprotinin increases the absorption rate of subcutaneously injected insulin, and amplifies its biological effect by inhibition of the local degradation of exogenous insulin at the injection site. If aprotinin were to likewise increase the biological effectiveness of subcutaneously injected insulin in diabetic patients, this observation should not be without therapeutic implications.

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TABLE 1
Effect of insulin, 10 U Actrapid, subcutaneously injected together with aprotinin, 10,000 KIU Trasylol or an equivalent volume of 0.9% saline, on circulating C-peptide levels in four healthy men (means ± SEM).

	Time (min)														
	−15	0	15	30	45	60	75	90	120	150	180	210	240	270	300
Controls (ng/ml) (insulin injected together with saline)	1.74 ± 0.10	1.73 ± 0.09	1.58 ± 0.01	1.43 ± 0.08	1.14 ± 0.06	0.94 ± 0.11	0.91 ± 0.07	0.86 ± 0.06	0.68 ± 0.05	0.57 ± 0.04	0.58 ± 0.05	0.51 ± 0.06	0.45 ± 0.05	0.43 ± 0.07	0.43 ± 0.06
Aprotinin experiments (ng/ml) (insulin injected together with Trasylol)	1.66 ± 0.08	1.65 ± 0.08	1.58 ± 0.02	1.28 ± 0.09	1.11 ± 0.15	0.89 ± 0.09	0.77 ± 0.08	0.74 ± 0.04	0.58 ± 0.04	0.59 ± 0.04	0.52 ± 0.02	0.51 ± 0.03	0.50 ± 0.03	0.48 ± 0.02	0.46 ± 0.03

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