IMPROVEMENT OF INSULIN ACTION BY BRADYKININ IN THE ALLOXAN-DIABETIC RAT

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1. Introduction

The acceleration of the entry of glucose into muscle which occurs upon contraction depends on the presence of small concentrations of insulin [1]. This suggests that the 'muscle activity factor' which is released from exercising muscle [2] exhibits its action on glucose utilization by improving insulin sensitivity of muscle tissue. During the last few years appreciable experimental evidence has been accumulated suggesting that kinins might represent the still unidentified humoral factor. Thus, it was shown in studies on the human forearm that the increase of glucose uptake during work load [3] or hypoxia [4] was completely abolished after treatment with a protease inhibitor which prevents the formation of kinins from kininogen. This effect could be overcome by the administration of synthetic bradykinin. Moreover, bradykinin exhibited insulin-like activity on glucose uptake also in the resting forearm muscle in the normal [5] and in the maturity onset diabetic patient [6]. From these and further studies pointing to an involvement of kinins in the physiological action of insulin on muscular glucose uptake [7,8] it seemed of interest to investigate the possibility whether kinins might also be useful therapeutically by improving the efficiency of insulin in the treatment of diabetes.

2. Materials and methods

Male Sprague Dawley rats (170–200 g) fed ad libitum on a chow diet were used. Diabetes was induced by intravenous injection of 10 mg alloxan/100 g body wt (Merck AG, Darmstadt). After 24 h, treatment was started applying 3 U Depot Insulin Hoechst CS (Hoechst AG, Frankfurt) subcutaneously (S.C.) at 12 h intervals for 3 days. Thereafter, before injection of insulin, body weights were registered and blood samples taken from the tail vein without anaesthesia for enzymatic determination of glucose at 8 a.m. In one group, the animals received no further insulin but were treated every 12 h for 3 days by S.C. injection of synthetic bradykinin (Sandoz AG, Nürnberg) at increasing doses as indicated in table 1 (group A). Another group received further insulin therapy continued for 6 days as indicated in table 1 (group B). Group C in table 1 was treated by a combination of 3 U insulin plus varying amounts of bradykinin mixed immediatedly before use and injected every 12 h. Thereby, daily glucose concentrations were registered in both groups as indicated in fig.1. Under these circumstances application of bradykinin was stopped in one group and started in the other as indicated in fig.1. Group D received the two drugs not mixed in a syringe but injected separately at two different sites. In further experiments insulin therapy was combined with s.c. application of papaverine at 2 doses (2 × 0.75 mg/day and 2 × 5.25 mg/day; Dept. Pharmacol., Schwabing Hospital, Munich) and of eledoisin (2 × 5 μg/day and 2 × 7.5 μg/day; Serva AG, Heidelberg). After 3 days of treatment blood glucose was determined at 8 a.m.

Another set of experiments was designed to study the effect of combined insulin–bradykinin treatment also on the plasma levels of free fatty acids, β-hydroxybutyrate and alanine and on liver glycogen. For that

This work is dedicated to Professor Dr Dr G. Bodechtel on the occasion of his eightieth birthday

purpose the rats after the treatment indicated in table 2 were anaesthetized with phenobarbital sodium (Nembutal®, Abott, Belgium, 50 mg/kg intraperitoneally) and their arterial blood taken from the aorta for analyses. Procedures and precision of the tests have been given in [9]. After blood sampling the livers were rapidly freeze clamped and glycogen was determined enzymatically according to [10]. Standard statistical methods were employed using Student's *t*-test for paired and unpaired samples when applicable [11].

3. Results

The data obtained under bradykinin without insulin, under insulin at different doses (2 X 3 U, 2 X 4 U, 2 X 5 U) and insulin (2 X 3 U) mixed with bradykinin at different doses (2 X 2.5 μg, 2 X 5 μg, 2 X 10 μg) and under insulin (2 X 3 U) and bradykinin (2 X 20 μg) applied separately are indicated in table 1. The effects of 2 X 3 U insulin mixed with 2 X 10 μg bradykinin on carbohydrate-, free fatty acid-, ketone- and amino acid metabolism are given in table 2. There was no increase of the radioimmunologically measurable insulin concentration (insulin, 2.36 ± 0.93 μU/ml; insulin + bradykinin, 0.65 ± 0.21 μU/ml), but a reduction which cannot be explained so far. The time course of blood glucose under the influence of insulin and of insulin mixed with bradykinin is indicated in fig.1. Addition of papaverine and of eledoisine at different doses exhibited no effect on glucose concentration:
Insulin (2 X 3 U) at 21.0 ± 0.9 mmol/l;
Insulin (2 X 3 U) + papaverine (2 X 0.75 mg) at 20.96 ± 0.71 mmol/l;
Insulin (2 X 3 U) + papaverine (2 X 5.25 mg) at 21.8 ± 0.6 mmol/l;
Insulin (2 X 3 U) + eledoisin (2 X 2.5 μg) at 19.01 ± 2.08 mmol/l;
Insulin (2 X 3 U) + eledoisin (2 X 7.5 μg) at 22.03 ± 0.95 mmol/l.

4. Discussion

Addition of bradykinin to insulin revealed dose-dependent improvement of diabetes concerning not only carbohydrate but also fat and amino acid metabolism (tables 1,2). It was manifest within 12 h and

Table 1
Effect of bradykinin and insulin on carbohydrate-metabolism

Group	Experiment		Treatment (days)	N^a	Blood glucose (mmol/l)	
	Insulin (U/24 h)	Bradykinin (μg/24 h)			Basal[b]	After treatment
A	–	2 × 10	3	6	21.2 ± 3.2	36.8 ± 1.1[c]
	–	2 × 80	3	5	18.0 ± 1.7	30.6 ± 4.4[c]
	–	2 × 160	3	5	20.7 ± 1.6	38.5 ± 1.9[c]
	–	2 × 200	3	5	20.7 ± 2.3	34.6 ± 1.8[c]
B	2 × 3	–	6	44	20.1 ± 1.4	22.5 ± 1.1
	2 × 5	–	6	18	21.0 ± 2.0	16.2 ± 1.6[c,d,e]
	2 × 6	–	6	15	24.9 ± 2.0	7.6 ± 1.7[c,d]
C[f]	2 × 3	2 × 2.5	6	7	20.7 ± 3.0	13.6 ± 2.6[c,d]
	2 × 3	2 × 5	6	6	21.0 ± 2.1	6.1 ± 1.0[c,d]
	2 × 3	2 × 10	6	42	20.8 ± 1.5	6.3 ± 0.5[c,d]
D[g]	2 × 3	2 × 20	6	13	21.0 ± 1.7	14.2 ± 2.0[c,d]

Mean ± SEM: [a]Number of rats; [b]Blood sugar after the first 3-day insulin treatment after alloxan; [c]Significantly different to basal ($p < 0.05$, paired *t*-test); [d]Significantly different to 6 U insulin/day ($p < 0.05$, unpaired *t*-test); [e]Significantly different to 6 U insulin + 10 μg bradykinin/day ($p < 0.05$, unpaired *t*-test); [f]Drugs administered in mixture; [g]Drugs administered separately

Table 2
Improvement of the diabetic carbohydrate-, fat-, ketone- and amino acid metabolism by bradykinin

	N	Insulin[a]	N	Insulin + Bradykinin[b]	P
Glucose[c]	20	14.3 ± 1.7	12	5.9 ± 0.6	<0.0005
Free fatty acids[c]	20	0.39 ± 0.04	9	0.23 ± 0.04	<0.01
β-Hydroxybutyrate[c]	18	0.45 ± 0.06	12	0.29 ± 0.05	<0.05
Alanine[c]	19	0.35 ± 0.02	12	0.29 ± 0.02	<0.05
Liver glycogen[d]	17	8.7 ± 0.9	10	4.5 ± 0.6	<0.0025
Liver weight[e]	19	10.6 ± 0.3	11	9.2 ± 0.3	<0.0025
Increase of body weight[f]	20	130 ± 1	18	138 ± 1	<0.0005

Mean ± SEM: [a]2 × 3 U/24 h for 6 days; [b]3 U insulin mixed with 10 μg twice daily for 6 days; [c]Arterial concentration (mmol/l); [d]g/100 g wet wt; [e]g wet wt; [f]%

disappeared in 24–36 h after the end of treatment (fig.1). The doses used were far beyond those known to cause lowering of blood pressure (reviewed [12]). Comparing the dose with that of insulin it turned out to be almost equimolar. Since bradykinin exhibited no increased secretion of insulin (see section 3) four possible ways were left how it might have worked:

(i) By increased mobilization of insulin via accelerated subcutaneous blood flow [12] and/or via improved vascular permeability [12];

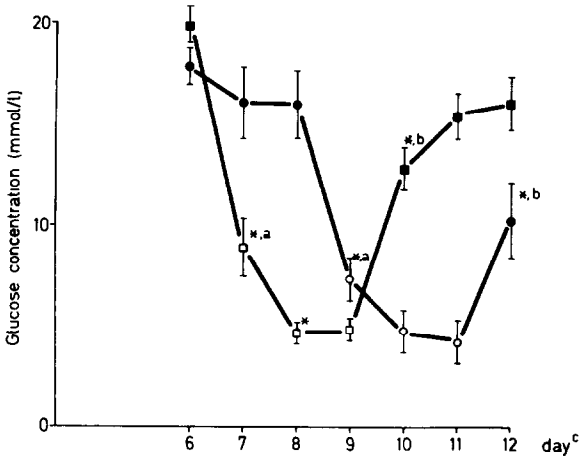

Fig.1. Effect of bradykinin (BK) on blood glucose in the alloxan-diabetic, insulin-treated rat. Mean ± SEM of 12 animals in each group under insulin (2 × 3 U) (●,■) and insulin plus BK (2 × 3 U plus 2 × 5 μg) (□,○). (a) 12 h after start with BK; (b) 36 h after stop with BK; (c) days of insulin treatment. (*) significantly different to preceding value ($p < 0.01$, paired t-test).

(ii) By reduction of local insulin degradation;
(iii) By an alteration of insulin's structure improving its physiological action;
(iv) By faster glucose uptake into muscle tissue as found [3–8].

The first and second notion were not very probable since papaverine and eledoisin which exhibit identical action on blood flow [12] and vascular permeability [12] and similar chemical properties [12] did not affect metabolism (see section 3). Concerning the third possibility, there was no evidence from the literature that a peptide might alter insulin's structure revealing increased physiological action of the hormone, although the finding that bradykinin was beginning to work at equimolar doses pointed to that view. Furthermore, the first and second notion were not very likely since kinins were also working when applied separately (table 1).

The last mechanism seems to be the most probable although it is difficult to imagine how kinins which are rapidly degraded [13] might entirely get to muscle tissue. However, considering that insulin works as an important kininase inhibitor [14], bradykinin was probably prevented from degradation if applied together with the hormone. This notion was also underlined by its smaller effect when applied separately from insulin (table 1). Since plasma insulin rises considerably after its subcutaneous application, kinins would also be preserved on their way to muscle tissue. Finally, these data also fit into the notion that these tissue hormones are involved in the control of substrate metabolism by improving insulin sensitivity.

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