EVIDENCE FOR AN INSULIN RECEPTOR IN MUSCLE

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1. Introduction

Evidence for a specific insulin receptor at the fat cell membrane was provided by Kono [1, 2], who showed that trypsin digestion (1 mg/ml for 15–30 min) lead to a complete loss of the insulin effect on both glucose transport and lipogenesis, and on antilipolysis, whereas the lipolytic actions of epinephrine and ACTH remained unchanged. After about 30 min the responsiveness of adipocytes to insulin reappeared. This regeneration phenomenon of the receptor could be suppressed by cycloheximide and suggested that protein synthesis was involved in the restoration of the insulin receptor.

These observations were followed by a number of publications [3–10] confirming these effects and describing the properties of enzymatically modified cell membranes.

In contrast to these studies with adipocytes there exist only few reports suggesting such receptors in muscle [11] and so far none employing trypsin digestion. The purpose of the present investigation was to extend the observations of Kono to the rat diaphragm preparation. Furthermore, this technique was used in the course of studies on the insulin-like mechanism of monoguanido compounds [12–14].

2. Experimental

2.1. *Preparation of diaphragms*

Male Wistar rats (150 g) were decapitated after 48 hr fast. The diaphragm was quickly excised, and two equal sections were cut out from each halve with the aid of a metal stamp. Each piece was cut into 4 parts of the same size (60–80 mg per segment). 32 Diaphragm segments from 4 rats were pooled and distributed to a series of 8 incubation vessels so that each vessel contained 4 segments, each one from another animal. The segments were kept in Krebs–Henseleit bicarbonate buffer (KHB) usually without albumin at 20° for 20 min.

2.2. *Trypsin digestion*

After the preincubation period the vessels were transferred to a water bath at 37°. Trypsin was added in a concentration of 1 mg/ml (unless otherwise noted) and inactivated by soybean trypsin inhibitor (STI 1.5 mg/ml), generally after 10 min (inactivation period). After removal of the medium, the diaphragm segments were washed twice with STI-containing buffer and then with KHB-buffer alone. The final incubation period was started after addition of agents to the glucose containing buffer (2.5 mg/ml) with vigorous gassing of the medium with carbogen for 5 min and continued usually for 60 min under constant shaking (incubation time respectively regeneration time).

Diaphragms in parallel incubations without trypsin treatment were handled by exactly the same procedure, including the application of STI.

2.3. *Measurement of glucose and glycogen*

The reaction was terminated by cooling in an ice bath. Glucose concentration in the incubation medium was measured enzymatically [15].

Diaphragm segments were rinsed, gently blotted and homogenized in KOH (30%) for 20 min at 100°. Glycogen was precipitated by ethanol (90%) in the

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cold. After centrifugation, the sediment was dissolved in water, its glycogen content was determined using the anthrone reagent [16].

Crystalline bovine insulin and 1-phenethylbiguanide were from Farbwerke Hoechst, agmatin (4-aminobutyl)-guanidin and soybeantrypsin-inhibitor from Serva, galegin (3,3-dimethylallyl)-guanidin from Calbiochem, 1-butylbiguanide from Chemie Grünenthal and trypsin from Boehringer Mannheim. L-Arginine-*N*-benzyl-amide was synthesized by Dr. Guglielmi [12].

3. Results and discussion

Pretreatment of the muscle preparation with trypsin (1 mg/ml) for 10 min was sufficient to abolish the influence of insulin on glucose uptake. The effects of the monoguanido compounds agmatin, galegin and L-arginine-*N*-benzylamide and of the two biguanides, however, remained totally unchanged (table 1). Insulin caused the same rate of glucose uptake, independent of whether it was added alone or together with medium from a previous muscle incubation shortly after inactivation of trypsin and removal of STI-containing buffer. Traces of insulin degrading activity of trypsin can therefore not account for the loss of hormonal action. According to further studies on enzyme release from the diaphragm pieces during incubation, an increased inactivation of insulin in the trypsin treated samples seems to be unlikely. As further shown in table 2, STI has neither an effect on glucose transport and glycogenesis under basal nor under insulin-stimulated conditions.

In general, similar results were obtained when all incubation steps were performed in the presence of albumin (fig. 1). Enzyme treatment of muscle exerted comparable effects on glucose transport and formation of glycogen. In both metabolic events decarboxylated arginine sustains its biological activity. As more than 90% of the increased glucose uptake in the presence of insulin could be accounted for as tissue glycogen [17], the loss of hormonal action on both parameters after enzymatic treatment of the tissue is not unexpected.

Prolonged incubation with insulin up to 90 min and 120 min after trypsin inactivation led to a continuously increasing uptake of glucose (not shown) and formation of glycogen (table 3). These returning metabolic processes amount to about 25–50% of the maximum effect of untreated preparations after 90 min. Under assay conditions without albumin, the loss

Table 1

Effect of insulin, monoguanide and biguanide compounds on glucose uptake of control and trypsin treated hemidiaphragm section.

Additions	No. of experiments	Trypsin treatment	Glucose uptake (μmole/g in per cent of control)	Δ	*P*
None	9	–	100		
		+			
Insulin 100 μU/ml	9	–	514 ± 26.4	−388	< 0.0005
		+	126 ± 15.1		
Agmatin 1 mM	5	–	325 ± 20.1	−14	N.S.
		+	311 ± 21.8		
Galegin 1 mM	5	–	368 ± 25.2	+7	N.S.
		+	375 ± 27.7		
ABA 1 mM	4	–	210 ± 16.6	−18	N.S.
		+	192 ± 15.2		
1-Butylbiguanide 1 mM	2	–	296	−2	N.S.
		+	294		
1-Phenylethyl-biguanide 1 mM	2	–	272	+11	N.S.
		+	283		

ABA refers to L-arginine-*N*-benzylamide. Incubation time 60 min.

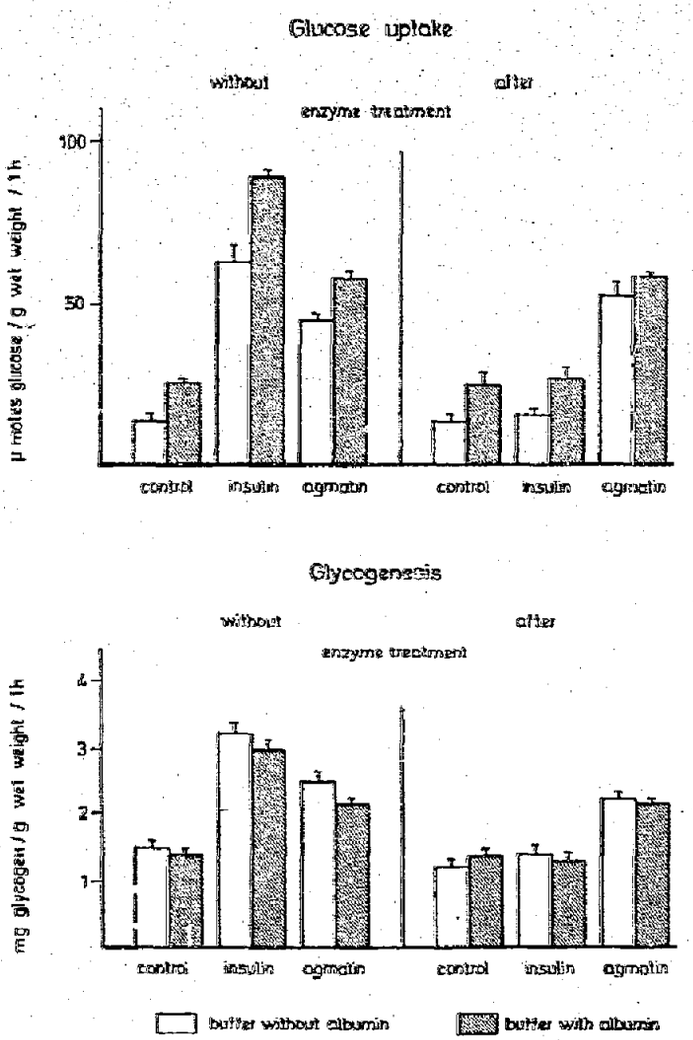

Fig. 1. Glucose uptake and glycogen synthesis in diaphragm sections with and without trypsin treatment. Effect of insulin (100 µU/ml) and agmatin (0.7 mM) in presence and absence of 2% human serum albumin from the Swiss Red Cross, Bern. Columns represent mean ± S.E.M. of three incubated samples. Trypsin treatment was for 5 min with 2 mg/ml.

Table 2

Effect of soybean trypsin inhibitor on glucose uptake and glycogen formation of control and trypsin-treated hemidiaphragm sections.

	Glucose uptake (µmole/g wet wt.)		Glycogen formation (mg/g wet wt.)	
	Trypsin digestion			
	–	+	–	+
Control	16.4	15.0	1.8	1.7
STI 1 mg/ml	16.6	16.2	1.8	1.75
Insulin 100 µU/ml	82.5	21.6	4.05	2.16
Insulin 100 µU/ml + STI 1 mg/ml	78.9	22.0	3.95	2.18
Insulin 100 µU/ml + medium control	80.7	N.D.	3.8	N.D.

The effect of added medium plus insulin from a previous incubation after trypsin inactivation is shown in the last line (insulin + muscle control). Each number represents the mean of three samples. Incubation time 60 min.

of responsiveness of muscle to insulin as measured by glycogen formation and glucose uptake was complete during the first 30 min. In analogy to the fat cell preparation [2] restoration of the insulin effector system can be suppressed by cycloheximide.

Whereas maximal glucose uptake and formation of glycogen in untreated tissue was usually seen between 100 and 500 µU/ml of insulin, the dose–response curve with trypsin treated muscle tissue shows an apparent shift to supraphysiological concentrations (table 4).

A similar phenomenon can be noticed in trypsin-treated adipocytes [9, 18] and for its explanation the existence of "spare receptors" was postulated. Since the mechanism of the insulin-like action of monoguanide and biguanide compounds appears to be completely independent of the insulin receptor it is likely that trypsin only modifies the acceptor of the hormonal signal and/or its transmission to metabolic systems, such as glucose transport, leaving the metabolic systems intact. Low concentrations of trypsin (2 µg/ml) have a pronounced insulin-like effect on muscle [19] possibly by stimulation of insulin receptors (and destroying a few), but 10^3-fold higher concentrations used in these experiments seem to degrade within 10 min part of a renewable peptide thus rendering it inert for insulin binding. With these concentrations, insulin-like effects of trypsin have not been observed during the inactivation period. Thus there is evidence for an insulin receptor in the muscle membrane with properties which are comparable to those characterized by Kono in fat cells.

Table 3
Restoration of insulin effect on glycogen formation after previous trypsin treatment.

Time (min)	15	30	60	90	120
Control	0.84 ± 0.11	0.87 ± 0.13	0.89 ± 0.16	1.04 ± 0.15 $P < 0.01$	1.27 ± 0.18 $P < 0.0025$
Insulin	0.78 ± 0.16	0.9 ± 0.14	1.13 ± 0.17	1.5 ± 0.17	2.2 ± 0.21
Insulin + cycloheximide				1.11 ± 0.13	1.38 ± 0.18

Values (mg glycogen per g wet weight) indicate mean of 3 experiments ± S.E.M. After a regeneration time of 90 min, respectively 120 min the reappearing insulin effect becomes significant (paired comparison).

Table 4
Effect of different concentrations of insulin on receptor responsiveness after 60 min regeneration period.

Insulin (mU/ml)	Glucose uptake (μmole/g wet wt.)	Glycogenesis (mg/g wet wt.)
–	15.6 ± 0.8	1.3 ± 0.22
0.1	18.3 ± 0.7	1.45 ± 0.20
1.0	22.8 ± 1.05	1.9 ± 0.26
10.0	32.7 ± 1.4	2.6 ± 0.44

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