Diabetologia 4, 322–329 (1968)

Influence of Insulin on Cyclic 3′,5′-AMP Phosphodiesterase Activity in Liver, Skeletal Muscle, Adipose Tissue, and Kidney *

G. Senft **, G. Schultz, K. Munske and M. Hoffmann
Department of Pharmacology, Freie Universität Berlin

Received: March 5, 1968

Non-Standard Abbreviations

Glucose-6-phosphate, G 6 P; UDP-glucose, UDPG; non-esterified, free fatty acids, FFA; cyclic adenosine-3′,5′-monophosphate, 3′,5′-AMP; 3′,5′-AMP phosphodiesterase, PDE.

Enzymes

Glucose oxidase, β-D-glucose: 0_2 oxidoreductase (E.C. 1.1.3.4); 5'-nucleotidase, 5'-ribonucleotide phosphohydrolase (E.C. 3.1.3.5); alkaline phosphatase, orthophosphoric monoester phosphohydrolase (E.C. 3.1.3.1); adenosine deaminase, adenosine aminohydrolase (E.C. 3.5.4.4); glycogen phosphorylase, α-1,4-glucan: orthophosphate glucosyltransferase (E.C. 2.4.1.1); glycogen synthetase, UDP-glucose: α-1,4-glucan α-4-glucosyltransferase (E.C. 2.4.1.11).

Summary. Influence of insulin on liver glycogen metabolism and on lipolysis appears to be mediated by a decreased intracellular 3′,5′-AMP concentration. Reduced formation of 3′,5′-AMP had been shown in adipose tissue incubated with insulin. The influence of insulin on 3′,5′-AMP degradation has been investigated. — 3′,5′-AMP phosphodiesterase (PDE) activity was reduced in liver, adipose tissue and, insignificantly, in skeletal muscle of insulin deficient, i.e. alloxan diabetic or starved rats. I.V. injection of a low dose of insulin (0.5 U/kg) or stimulation of endogenous insulin secretion by injection of glucose led to a rapid increase of PDE activity in these tissues. 15 min after insulin injection liver PDE activity was increased. The maximal effect occurred after 30—45 min. Renal PDE activity was not decreased in alloxan diabetes, insulin injection has been found ineffective. — *In vitro*, there was an activating effect of crystalline insulin on PDE purified from beef heart. Insulin concentration required for duplication of enzyme activity was of the order of $2 \cdot 10^{-5}$ M. Treatment with actinomycin D nearly prevented stimulation of liver PDE by insulin. This may indicate that the action of insulin on PDE activity is essentially based on an increased enzyme synthesis. — Owing to the influence of insulin secretion on liver and adipose tissue 3′,5′-AMP concentration, glycogen metabolism and lipolysis can be quickly adapted to food intake.

Influence de l'insuline sur l'activité de la 3,′5′-AMP-phosphodiestérase dans le foie, le muscle strié, le tissu adipeux et le rein

Résumé. L'influence de l'insuline sur le métabolisme du glycogène hépatique et sur la lipolyse semble s'exercer par l'intermédiaire d'une diminution de la concentration de 3,′5′-AMP intracellulaire. On a montré une diminution de la formation de 3′5′-AMP dans le tissu adipeux incubé avec de l'insuline. L'influence de l'insuline sur la dégradation du 3,′5′-AMP est étudiée. — L'activité de la 3,′5′-AMP-phosphodiestérase (PDE) est diminuée dans le foie, le tissu adipeux et, de façon non-significative, dans le muscle strié des rats qui manquent d'insuline, c-à-d les rats rendus diabétiques par l'alloxane ou les rats privés de nourriture. L'injection intraveineuse d'une faible dose d'insuline (0.5 U/kg) ou la stimulation de la sécrétion d'insuline endogène par une injection de glucose provoquent une augmentation rapide de l'activité de la phosphodiestérase dans ces tissus. 15 min après l'injection d'insuline, l'activité de la phosphodiestérase du foie est augmentée. L'effet maximum est atteint après 30—45 min. L'activité de la phosphodiestérase rénale n'est pas diminuée dans le diabète alloxanique, l'injection d'insuline s'est avérée inefficace. *In vitro*, l'insuline cristalline a un effet activant sur la phosphodiestérase purifiée du coeur de boeuf. La concentration d'insuline requise pour doubler l'activité de l'enzyme est de l'ordre de $2 \cdot 10^{-5}$ M. Le traitement avec actinomycin D empêche la stimulation par l'insuline de la PDE dans le foie. Ceci peut indiquer que l'action de l'insuline sur l'activité de la phosphodiestérase est essentiellement basée sur une synthèse accrue de l'enzyme. A cause de l'influence de la sécrétion d'insuline sur la concentration en 3,′5′-AMP du foie et du tissu adipeux, le métabolisme du glycogène et la lipolyse peuvent s'adapter rapidement à la prise de nourriture.

Der Einfluß von Insulin auf die 3′,5′-AMP-Phosphodiesterase-Aktivität in Leber, Skeletmuskulatur, Fettgewebe und Niere

Zusammenfassung. An der Steigerung der Glykogensynthese der Leber und der Verminderung der Lipolyse durch Insulin ist eine Abnahme der 3′,5′-AMP-Konzentration wesentlich beteiligt. Die 3′,5′-AMP-Bildung ist in Fettgewebe, das mit Insulin inkubiert wird, vermindert. Insulin beeinflußt jedoch auch den 3′,5′-AMP-Abbau. — Die 3′,5′-AMP-Phosphodiesterase (PDE)-Aktivität des Fettgewebes, der Leber und, in geringerem Grade, der Skeletmuskulatur ist im Insulinmangel vermindert, d.h. bei alloxandiabetischen oder hungernden Ratten. I.v. Injektion von 0,5 E/kg Insulin oder eine erhöhte Abgabe von Insulin aus dem Pankreas nach Glucoseinjektion führen in diesen Geweben zu einem raschen Anstieg der PDE-Aktivität. Dieser ist in der Leber schon 15 min nach Insulingabe nachweisbar und erreicht nach 30—45 min sein Maximum. In der Niere ist kein Einfluß von Insulin auf die PDE-Aktivität nachweisbar. — Aus Rinderherz isolierte PDE wird *in vitro* durch Insulin aktiviert, jedoch werden $2 \cdot 10^{-5}$ M zur Verdopplung der Aktivität benötigt. Actinomycin D verhindert die Steigerung der Leber-PDE-Aktivität nach Insulininjektion. So kann die Wirkung des Hormons im wesentlichen auf eine gesteigerte PDE-Synthese zurückgeführt werden. — Durch diesen Einfluß der Insulininkretion auf die 3′,5′-AMP-Konzentration in Leber und Fettgewebe können Glykogenstoffwechsel und Lipolyse rasch an die Nahrungsaufnahme angepaßt werden.

Key-words: Insulin, 3′,5′-AMP phosphodiesterase, glycogen metabolism, lipolysis, insulin secretion, antilipolytic action of insulin, glycogen synthesis and insulin, cyclic adenosine 3′,5′-monophosphate.

* This study was supported by the Deutsche Forschungsgemeinschaft.

** Deceased October 31, 1967.

The glycogenolytic and lipolytic actions of several hormones, e.g. catecholamines and glucagon, are mediated by an increased formation of cyclic adenosine 3′,5′-monophosphate [52]. Insulin antagonizes the lipolytic effect of epinephrine [3, 40] and favours hepatic glycogen synthesis [51, 50]. These actions of insulin appear to be mediated by a decreased intracellular 3′,5′-AMP concentration. This conception is based upon the following observations. In adipose tissue from rats (in fat pads as well as in isolated fat cells), insulin added to the incubation medium leads to a rapid decrease of 3′,5′-AMP concentration, expecially if it is elevated by an increased formation and reduced degradation of 3′,5′-AMP [3,3a,5]. In rat liver 3′,5′-AMP concentration rises markedly in insulin deficiency, i. e. in alloxan diabetes [23a] or when insulin activity is neutralized by addition of insulin antibodies [11]; the level of liver 3′,5′-AMP is reduced by treating the diabetic animals with insulin [23a].

Decrease of the intracellular 3′,5′-AMP concentration caused by insulin and increased 3′,5′-AMP concentration in insulin deficiency may be explained by two hormonal actions. An insulin-induced decrease of 3′,5′-AMP formation has been found in adipose tissue incubated with insulin [24]. It is shown in the present paper[1] that the degradation of 3′,5′-AMP to 5′-AMP is also influenced by insulin. In adipose tissue, liver and skeletal muscle, insulin increased 3′,5′-AMP phosphodiesterase activity. Renal PDE was not affected by insulin.

Material and Methods

Body weight of the male Wistar rats used was in the range 150—200 g. Standard pellet diet (Altromin®) and drinking water were removed before experiments unless otherwise noted.

Studies in diabetic animals were performed 48 h after i.v. injection of 80 mg/kg alloxan tetrahydrate dissolved in 0.9% NaCl. Only such animals were used whose blood glucose concentration exceeded 200 mg/100 ml. Crystalline, glucagon-free insulin from beef and hog (Farbwerke Hoechst AG[2]) diluted in 0.9% NaCl solution was injected i.v., crystalline bovine insulin (26 U/mg, Farbwerke Hoechst AG[2]) dissolved in buffer was used for experiments *in vitro*.

Actinomycin D (Dactinomycin®, Merck, Sharp, and Dohme[2]) dissolved in 0.9% NaCl solution was injected i.p. 3 h before decapitation.

Solvents were injected into respective controls.

Blood glucose determination was performed by use of glucose oxidase [22]. Protein was assayed according to LOWRY et al. [31] in the fraction of homogenate used for determination of enzyme activity. Human serum albumin served for standardization. Isolation and assay of tissue DNA were carried out as described by NEUBERT et al. [35].

For determination of 3,′5′-AMP phosphodiesterase activity, tissues dissected immediately after decapitation were homogenized for about 30 sec, 1 volume in approximately 10 volumes of a 0.15 M glycylglycine buffer, pH 7.5, containing 6 mM $MgCl_2$, by use of an Ultra-Turrax (JAHNKE and KUNKEL). Homogenates were centrifuged $1000 \cdot g$ for 10 min at 0°C. 5′-AMP formed from 3′,5′-AMP under the influence of PDE in the same buffer at 25°C, was dephosphorylated using a 5′-nucleotidase in excess (alkaline phosphatase from calf intestine, Boehringer u. Soehne, Mannheim). This enzyme was also used to minimize adenosine phosphomonoesters before starting measurements by addition of 3′,5′-AMP ($[S] = 8 \cdot 10^{-5}$M 3′,5′-AMP unless otherwise specified). The conversion of adenosine to inosine, catalyzed by adenosine deaminase in excess (from calf intestine, Boehringer u. Soehne), was measured by recording the difference of the nucleotide absorbances at 265 mμ. $\Delta E = 8.1 \cdot 10^6$ cm²/mole [34, 21] was taken as a basis for the calculation of PDE activity. Experiments in which crystalline insulin was added to PDE purified from beef heart [6], were carried out using the same buffer and the same enzymes.

Data are given as mean ± S.E.M., and compared using Student's *t*-test and the *t*-tables of PÄTAU [36].

Results

In alloxan-diabetic animals liver PDE activity was reduced. I.v. injection of 0.5 U insulin/kg led to an increase of the enzyme activity within 15 min after injection. The stimulation of PDE activity was maximal between 30 and 45 min after injection of insulin. In rats given insulin for 4 h, liver PDE activity was no longer elevated (Fig. 1).

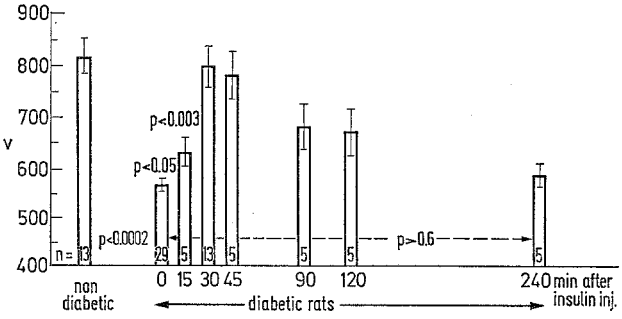

Fig. 1. Decreased liver 3′,5′-AMP phosphodiesterase activity $\left(\frac{n \text{ moles}}{\text{min} \cdot \text{g w.w.}}\right)$ in alloxan diabetic rats. Stimulation of enzyme activity by injection of insulin (0.5 U/kg i.v.)

3′,5′-AMP degradation in the rat liver was also influenced by alimentary variations of the endogenous insulin secretion. Plasma insulin concentrations in rats fasted for 2.5 days can be assumed to be low [17,

[1] Results were partially presented in form of a short communication (41) and at the 30th Meeting of the German Pharmacological Society (42).

[2] The authors wish to thank Dr. A. FLAMME, Farbwerke Hoechst AG, and Dr. H. F. HOFMANN, Merck, Sharp, and Dohme, who kindly supplied these substances,

47]. In comparison with the liver-PDE activity of fed rats, 3',5'-AMP degradation was reduced in fasted animals. Stimulation of insulin secretion by i.v. injection of glucose [44, 45, 7] caused an increase of PDE activity. In fed rats a further elevation of enzyme activity could be achieved when insulin secretion was stimulated by glucose injection (Fig. 2).

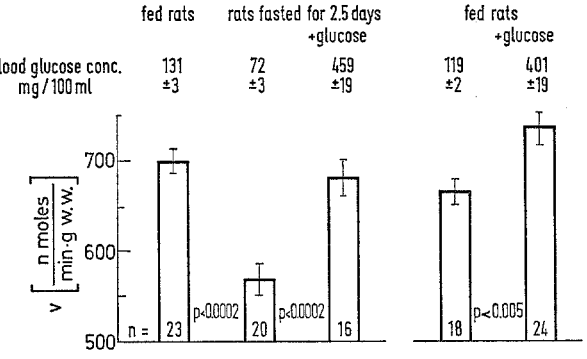

Fig. 2. Influence of blood glucose concentration altered by feeding, starvation and refeeding on the activity of liver 3',5'-AMP phosphodiesterase. Glucose (4 g/kg) was injected 45 and 30 min prior to decapitation

Table 1. *Influence of alloxan diabetes and insulin injection (0.5 U/kg i.v., 30 min) on 3',5'-AMP phosphodiesterase activity in rat skeletal muscle*

	controls	diabetic rats	
	$n = 28$	$n = 20$	+ insulin $n = 17$
$\frac{n \text{ moles}}{\text{min} \cdot \text{g w.w.}}$	80.6 ±4.0	72.3 ±6.6	100.6 ±4.2
		p = 0.3	p < 0.001

In adipose tissue, PDE activity calculated on a tissue protein or DNA basis, was reduced in alloxan diabetic animals compared with fed rats not suffering from insulin deficiency. On a wet weight basis there was no decrease of enzyme activity. The cause is a reduced fat content of adipose tissue in diabetic rats. This reduction is revealed in a decreased ratio wet weight/protein content of the tissue. I.v. injection of 0.5 U insulin/kg led to an increased PDE activity as measured after 45 min (Table 2). In fasted rats with low insulin plasma concentration, PDE activity in adipose tissue was also reduced provided that the activity is calculated on a protein or DNA basis. The increased PDE activity referred to the wet weight of adipose tissue, was due to a strong reduction of the fat content evident from the decreased ratio mg wet weight/mg protein after starvation. Stimulation of insulin secretion by injection of glucose into fasted rats, also led to an increased PDE activity in adipose tissue (Table 2).

In the kidney of alloxan-diabetic rats, PDE activity was not reduced (Table 3). Stimulation of enzyme activity could be achieved neither by injection of

Table 3. *The lack of an influence of alloxan diabetes and of insulin injection (5 U/kg i.v., 45 min) on renal 3',5'-AMP phosphodiesterase activity*

	controls	diabetic rats	
	$n = 9$	$n = 13$	+ insulin $n = 13$
$\frac{n \text{ moles}}{\text{min} \cdot \text{mg prot.}}$	6.37 ±0.23	6.39 ±0.27	6.30 ±0.23
		p > 0.5	p > 0.5

Table 2. *Influence of insulin on 3,5-AMP phosphodiesterase activity in adipose tissue*

	diabetic rats (80 mg/kg alloxan i.v., 2 days)		fed rats	rats fasted for 4—5 days	
		+ insulin (0.5 U/kg i.v., 45 min)			+ glucose (4 g/kg i.v., 45 min)
	$n=14$	$n=14$	$n=16$	$n=51$	$n=23$
$\frac{n \text{ moles}}{\text{g w.w.} \cdot \text{min}}$	38.7 ± 3.2	49.6 ± 4.7	35.2 ± 2.8	68.8 ±11.2	
	$p=0.06$		$p<0.001$		
$\frac{\text{mg w.w.}}{\text{mg prot.}}$	67.0	72.3	84.6	36.8	
$\frac{n \text{ moles}}{\text{mg prot.} \cdot \text{min}}$	2.60 ±0.13	3.59 ±0.31	2.98 ±0.18	2.54 ±0.10	5.02 ±0.33
	$p<0.01$		$p<0.04$	$p<0.0002$	
$\frac{n \text{ moles}}{\text{mg DNA} \cdot \text{min}}$	643 ± 59	895 ± 97	953 ± 87	565 ± 46	
	$p<0.04$		$p<0.0002$		
		$p<0.0005$		$n=14$	

In skeletal muscle of diabetic rats, PDE activity was insignificantly lower than in non-diabetic animals. 0.5 U insulin/kg injected i.v. 30 min before decapitation led to an increase of muscle PDE activity (Table 1).

0.5 U insulin/kg [21] nor by injection of 5.0 U insulin/kg (Table 3).

In order to investigate the mechanism of the stimulatory effect of insulin on PDE activity, crystalline bovine insulin was added to PDE purified from

beef heart. Before use, the enzyme was dialyzed overnight against distilled water to remove constituents from the preparation, e.g. imidazole. The results are plotted according to LINEWEAVER and BURK [30]. As shown by Fig. 3, purified PDE was activated by insulin. The insulin concentration required for duplication of PDE activity was of the order of $2 \cdot 10^{-5}$ M,

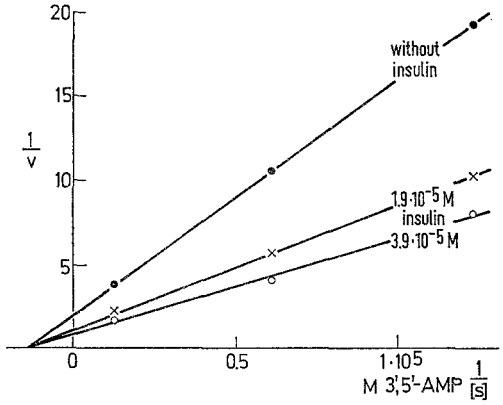

Fig. 3. Activation of 3′,5′-AMP phosphodiesterase (purified from beef heart) by insulin (crystalline beef insulin)

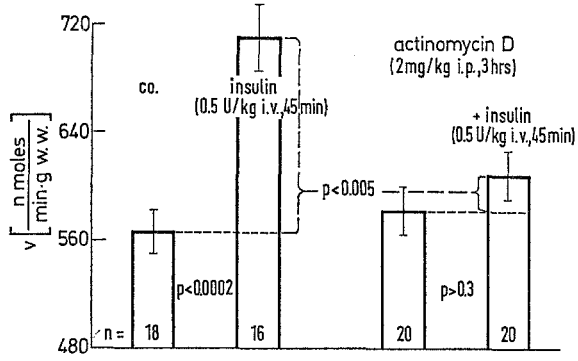

Fig. 4. Prevention of insulin-induced increase of liver 3′,5′-AMP phosphodiesterase activity by actinomycin D (alloxan diabetic rats)

i.e. about 3 U insulin per ml. With respect to the high insulin concentration needed for stimulation of PDE activity *in vitro*, it was examined whether insulin may influence PDE activity by stimulation of enzyme synthesis. For this purpose, actinomycin D, which interferes with DNA-dependent RNA synthesis [13], was employed. The increase of liver PDE activity induced by i.v. injection of 0.5 U insulin/kg, was almost eliminated in the animals pretreated with actinomycin D. This compound was injected i.p. in a dose of 2 mg/kg, 3 h before decapitation (Fig. 4).

Discussion

In adipose tissue insulin-induced reduction of 3′,5′-AMP concentration can be explained by two hormonal actions. A decreased formation of 3′,5′-AMP has been shown in adipose tissue incubated with insulin [24]. Increased 3′,5′-AMP degradation is evident after injection of a low dose of insulin or after stimulation of endogenous insulin secretion by injection of glucose. Not only in adipose tissue but also in liver, PDE activity can be shown to depend on the plasma insulin concentration, i.e. PDE activity is reduced in insulin-deficient states and is increased by exogenous or endogenous elevation of plasma insulin[3].

Activation of PDE by insulin can be regarded as unimportant for the hormonally-induced augmentation of enzyme activity *in vivo*. Insulin concentrations which stimulate PDE activity *in vitro*, are much higher than those occurring *in vivo*. The inhibition of the insulin-induced increase of liver PDE activity by actinomycin D may also be regarded as indicative of an influence of insulin on PDE synthesis. This corresponds with observations that application of substances which inhibit RNA or protein synthesis, prevents the antilipolytic effect of insulin [26] and the hormonal influence on glycogen synthesis [50, 49]. The early onset of increased PDE activity after insulin injection is not contradictory to the assumption that insulin increases PDE synthesis, since it has been shown that insulin can stimulate ribosomal protein synthesis within 5 min [58].

The insulin-induced rapid augmentation of 3′,5′-AMP degradation is revealed by altered enzymic reactions of glycogen and lipid metabolism which depend on the intracellular 3′,5′-AMP concentration. 10—30 min after application of insulin, glycogen synthetase has been found to be activated in liver [2, 46], adipose tissue [24] and skeletal muscle [55, 8, 9, 48, 56, 14]. This activation results from an increase of the more active form (I), the activity of which is independent of G6P concentration, without change of the total activity of the enzyme (I + D) at this time. Glycogen transferase-I-kinase, the enzyme which catalyzes the conversion of the I-form to the less active form (D) of glycogen synthetase [2, 12], is stimulated by 3′,5′-AMP [23, 1] (for schematic demonstration of the role of 3′,5′-AMP in the regulation of glycogen metabolism see Fig. 5). Thereby, a decreased intracellular 3′,5′-AMP concentration leads to a reduced inactivation of glycogen synthetase. In contrast, an increased 3′,5′-AMP concentration caused e.g. by epinephrine, leads to a decrease of the active form of glycogen synthetase [8, 9].

An insulin-induced decrease of intracellular 3′,5′-AMP not only affects the synthesis, but also the degradation

[3] Added in proof. Adenyl cyclase activity was not decreased by insulin either in "ghosts" [40a] or in homogenates [3a, 24] of fat cells. — In relatively small groups of animals, insulin-induced alterations in the PDE activity of adipose tissue [2a. 34a] and liver [34a] could not be detected by some other investigators. The use of much higher tissue concentrations for the determination of enzyme activity by one of these groups [34a] may account for this difference. In agreement with present results, insulin reduced the lipolysis [7a] and the hepatic glucose output [12a] induced by 3′,5′-AMP.

of glycogen. 3′,5′-AMP stimulates the kinases that catalyze the conversion of the less active form of glycogen phosphorylase to the more active form in several tissues [4, 28]. Thereby, it can be explained that 10 min after application of insulin more glycogen phosphorylase has been found in the inactive form in liver [2], adipose tissue [24] and skeletal muscle [53].

tase I to the less active form (D), but insulin also promotes an augmentation of the total activity (D + I) of liver glycogen synthetase [51, 50]. This is brought about by an increased enzyme synthesis, which can be prevented by application of puromycin or actinomycin D [50]. The increased synthesis of this enzyme, which is not yet demonstrable within the first half hour after

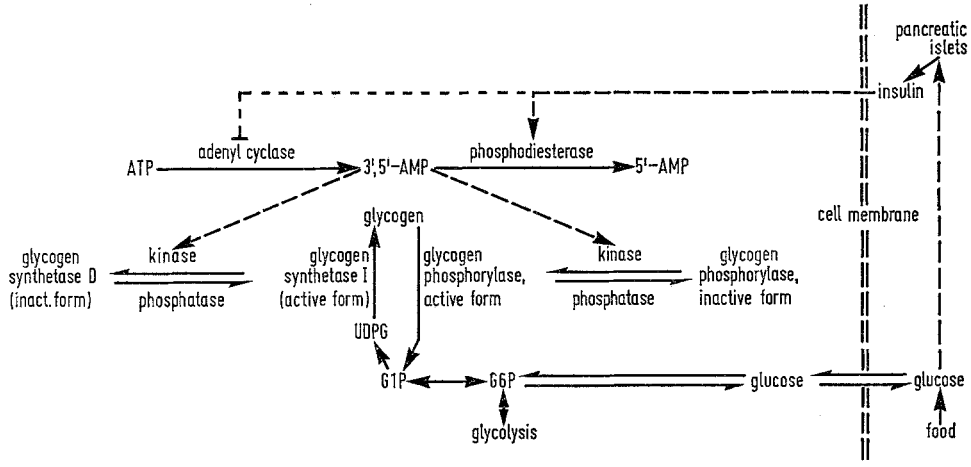

Fig. 5. Influence of insulin on 3′,5′-AMP concentration regulating glycogen metabolism (schematically for liver)

In addition to the insulin-induced alterations in glycogen metabolism of adipose tissue, the antilipolytic action of this hormone is an indication that insulin lowers intracellular 3′,5′-AMP concentration. The rate of lipolysis is controlled by 3′,5′-AMP, which nucleotide stimulates triglyceride lipase activity [5, 38, 54]. Free fatty acid and glycerol release, which are increased in insulin deficiency, i. e. in starvation or alloxan diabetes [15, 39, 27], are decreased by injection or addition *in vitro* of insulin [40, 26, 25, 16].

Relative to food intake, insulin is secreted from pancreatic islets. Plasma insulin is reduced in the fasting state with low blood glucose concentration [17, 47]. This results in an increased 3′,5′-AMP concentration. Thereby, lipolysis, liver glycogenolysis and glucose output are increased. An increased intake of carbohydrates, accompanied by an elevated blood glucose concentration, leads to an increased insulin secretion [44, 45, 7]. Glycogen synthesis is favoured; glycogen degradation, glucose output from the liver, and the rate of lipolysis are reduced; all mediated by an insulin-induced decrease in 3′,5′-AMP concentration (see Fig. 5).

The activities of glycogen synthetase and phosphorylase are not only regulated by 3′,5′-AMP concentration, but also by many other factors. Therefore, the possibility that insulin alters the activities of these enzymes by other means, must be taken into consideration.

Insulin not only leads to an activation of glycogen synthetase by lowered conversion of glycogen synthe-

insulin injection, may contribute to the insulin-induced increase in glycogen formation. The early activation of glycogen synthetase, however, cannot be explained by this hormonal action. An insulin-induced increase of G6P concentration may contribute to glycogen synthetase activation, but could not always be measured [51, 55, 10]. Furthermore, the increase in the I-form of glycogen synthetase is not achieved by a direct influence of insulin on transferase phosphatase activity. This enzyme converting the more active form (I) into the less active form (D) of glycogen synthetase, has been shown to remain unaffected by the hormone [56, 20].

UDPG has been found to inhibit glycogen-phosphorylase [32, 33]. Therefore, an insulin-induced increase of UDPG concentration may represent an additional factor influencing glycogen-phosphorylase activity. A lack of increase of liver UDPG concentration, however, has been shown in the presence of an increased glycogen formation that was caused by glucose application stimulating insulin secretion [10].

In skeletal muscle, diabetes-caused decrease and insulin-induced increase of PDE activity are smaller than in liver and adipose tissue. Injection of insulin or addition *in vitro* produces an increase in activity of glycogen synthetase I without a change of total enzyme activity [55, 8, 9, 48, 56, 14]. A simultaneous decrease of the active form of muscle glycogen-phosphorylase could, however, not be measured by all investigators [8]. A decreased 3′,5′-AMP concentration could not be detected [14, 37] either after injection of insulin or

after insulin addition to incubated diaphragms. Therefore, it has been postulated by LARNER and collaborators [2, 56, 14, 29] that insulin could directly alter the sensitivity of transferase-I-kinase to 3′,5′-AMP, i.e. that insulin could promote a change in enzyme activity which is relatively independent of tissue 3′,5′-AMP concentration. Otherwise, the lack of influence of insulin on muscle phosphorylase and on muscle 3′,5′-AMP concentration could be explained by the involvement of another cyclic nucleotide in the action of insulin in muscle. This nucleotide must differ from 3′,5′-AMP in the influence on transferase-I-kinase and phosphorylase-b-kinase. It has been shown that cyclic 3′,5′-GMP and probably 3′,5′-UMP occur in mammals [52, 18], but their significance is as yet unknown.

In kidney no influence of insulin on carbohydrate or lipid metabolisms is known apart from the suppressing action of insulin on the activity of some gluconeogenic enzymes [57, 19]. In addition, the PDE activity was not influenced by insulin in kidney. In contrast, glucocorticoids modify PDE activity in liver, skeletal muscle, adipose tissue as well as in kidney [43]. The interaction of these hormones and of insulin on PDE activity will be the subject of a following paper [43].

References

1. APPLEMAN, M.M., L. BIRNBAUMER, and H.N. TORRES: Factors affecting the activity of muscle glycogen synthetase. III. The reaction with adenosine triphosphate, Mg^{++}, and cyclic 3′,5′-adenosine monophosphate. Arch. biochem. **116**, 39 – 43 (1966).
2. BISHOP, J.S., and J. LARNER: Rapid activation-inactivation of liver uridine diphosphate glucose-glycogen transferase and phosphorylase by insulin and glucagon in vivo. J. biol. Chem. **242**, 1354—1356 (1967).

2a. BLECHER, M., N. S. MERLINO, and J. T. RO'ANE: Control of metabolism and lipolytic effects of cyclic 3′,5′-adenosine monophosphate in adipose tissue by insulin, methyl xanthines, and nicotinic acid. J. biol. Chem. **243**, 3973 — 3977 (1968).

3. BUTCHER, R.W., J.G.T. SNEYD, C.R. PARK, and E.W. SUTHERLAND, jr.: Effect of insulin on adenosine 3′,5′-monophosphate in the rat epididymal fat pad. J. biol. Chem. **241**, 1651—1653 (1966).

3a. — C. E. BAIRD, and E. W. SUTHERLAND: Effects of lipolytic and antilipolytic substances on adenosine 3′,5′-monophosphate levels in isolated fat cells. J. biol. Chem. **243**, 1705—1712 (1968).

4. — R.J. HO, H.C. MENG, and E.W. SUTHERLAND: Adenosine 3′,5′-monophosphate in biological materials. II. The measurement of adenosine 3′,5′-monophosphate in tissues and the role of the cyclic nucleotide in the lipolytic response of fat to epinephrine. J. biol. Chem. **240**, 4515—4523 (1965).
5. BUTCHER, R.W., and E.W. SUTHERLAND: The effects of the catecholamines, adrenergic blocking agents, prostaglandin E_1, and insulin on cyclic AMP levels in the rat epididymal fat pad in vitro. Ann. N. Y. Acad. Sci. **139**, 849—859 (1967).
6. BUTCHER, R.W., and E.W. SUTHERLAND: Adenosine 3′,5′-phosphate in biological materials. I. Purification and properties of cyclic 3′,5′-nucleotide phosphodiesterase and use of this enzyme to characterize adenosine 3′,5′-phosphate in human urine. J. biol. Chem. **237**, 1244—1250 (1962).
7. CERASI, E., and R. LUFT: The plasma insulin response to glucose infusion in healthy subjects and in diabetes mellitus. Acta Endocrin. (Kbh.) **55**, 278—304 (1967).

7a. CORBIN, J. D., J. G. T. SNEYD, and R. W. BUTCHER: Control of lipolysis in fat tissne by cyclic adenylate. Fed. Proc. **27**, 649 (1968).

8. CRAIG, J.W., and J. LARNER: Influence of epinephrine and insulin on uridine diphosphate glucose-α-glucan transferase and phosphorylase in muscle. Nature (Lond.) **202**, 971—973 (1964).
9. DANFORTH, W. H.: Glycogen synthetase activity in skeletal muscle. Interconversion of two forms and control of glycogen synthesis. J. biol. Chem. **240**, 588—593 (1965).
10. DE WULF, H., and H.G. HERS: The stimulation of glycogen synthesis and of glycogen synthetase in the liver by the administration of glucose. Europ. J. Biochem. **2**, 50—56 (1967).
11. EXTON, J.H., L.S. JEFFERSON, jr., R.W. BUTCHER, and C.R. PARK: Gluconeogenesis in the perfused liver. The effects of fasting, alloxan diabetes, glucagon, epinephrine, adenosine 3′,5′-monophosphate and insulin. Amer. J. Med. **40**, 709—715 (1966).
12. FRIEDMAN, D.L., and J. LARNER: Studies on uridine diphosphate glucose: α 1,4-glucan α-4-glucosyltransferase. VIII. Catalysis of the phosphorylation of muscle phosphorylase and transferase by separate enzymes. Biochemistry **4**, 2261—2264 (1965).

12a. GUINSMANN, W. H., and G. E. MORTIMORE: Influence of glucagon and 3′,5′-AMP on insulin responsiveness of the perfused rat liver. J. biol. Chem. **215**, 553—559 (1968).

13. GOLDBERG, I.H., M. RABINOWITZ, and E. REICH: Basis of actinomycin action. I. DNA binding and inhibition of RNA-polymerase synthetic reactions by actinomycin. Proc. nat. Acad. Sci (Wash.) **48**, 2094—2101 (1962).
14. GOLDBERG, N.D., C. VILLAR-PALASI, H. SASKO, and J. LARNER: Effects of insulin treatment on muscle 3′,5′-cyclic adenylate levels in vivo and in vitro. Biochim. biophys. Acta **148**, 665—672 (1967).
15. GOODMANN, H.M., and E. KNOBIL: Some endocrine factors in regulation of fatty acid mobilization during fasting. Amer. J. Physiol. **201**, 1—3 (1961).
16. GORDON, R.S., jr.: Unesterified fatty acid in human blood plasma. II. The transport function of unesterified fatty acid. J. clin. Invest. **36**, 810—815 (1957).
17. HALES, C.N., and P.J. RANDLE: Immunoassay of insulin with insulin-antibody precipitate. Biochem. J. **88**, 137—146 (1963).
18. HARDMAN, J.G., J.W. DAVIS, and E.W. SUTHERLAND: Measurement of guanosine 3′,5′-monophosphate and other cyclic nucleotides. Variations in urinary excretion with hormonal state of the rat. J. biol. Chem. **241**, 4812—4815 (1966).
19. HENNING, H.V., B. STUMPF, B. OHLY, and W. SEUBERT: On the mechanism of gluconeogenesis and its regulation. III. The gluconeogenic capacity and the activities of pyruvate carboxylase and PEP-carboxylase of rat kidney and rat liver after cortisol treatment and starvation. Biochem. Z. **344**, 274—288 (1966).
20. HIZUKURI, S., and J. LARNER: Studies on UDPG: α-1,4-glucan α-4-glucosyltransferase. VII. Conversion of the enzyme from glucose-6-phosphate-dependent to independent form in liver. Biochemistry **3**, 1783—1788 (1964).

21. HOFFMANN, M.: Einfluß von Hormonen und Pharmaka auf die Bildung und den Abbau von cyclischem Adenosin-3′,5′-Monophosphat in der Rattenniere. Inaug.-Diss. Math.-nat. Fak. Freie Universität Berlin, 1967.

22. HUGGETT, A.S.G., and D.A. NIXON: Use of glucose oxidase, peroxidase, and o-dianisine in determination of blood and urinary glucose. Lancet **1957 II**, 368—370.

23. HUIJING, F., and J. LARNER: On the mechanism of action of adenosine 3′,5′-cyclophosphate. Proc. nat. Acad. Sci. (Wash.) **56**, 647—653 (1966).

23a. JEFFERSON, L. S., J. H. EXTON, R. W. BUTCHER, E. W. SUTHERLAND, and C. R. PARK: Role of adenosine 3′,5′-monophosphate in the effects of insulin and anti-insulin serum on liver metabolism. J. biol. Chem. **243**, 1031—1038 (1068).

24. JUNGAS, R.L.: Role of cyclic-3′,5′-AMP in the response of adipose tissue to insulin. Proc. nat. Acad. Sci. (Wash.) **56**, 757—763 (1966).

25. JUNGAS, R.L., and E.G. BALL: Studies on the metabolism of adipose tissue. XII. The effects of insulin and epinephrine on free fatty acid and glycerol production in the presence and absence of glucose. Biochemistry **2**, 383—388 (1963).

26. KORNER, A., and M.S. RABEN: Effect of aminonucleoside and puromycin on insulin and epinephrine control of fatty acid release from adipose tissue. Nature (Lond.) **203**, 1287—1289 (1964).

27. KOVACEV, V.P., and R.O. SCOW: Effect of hormones on fatty acid release by rat adipose tissue in vivo. Amer. J. Physiol. **210**, 1199—1208 (1966).

28. KREBS, E.G., D.S. LOVE, G.E. BRATVOLD, K.A. TRAYSER, W.L. MEYER, and E.H. FISCHER: Purification and properties of rabbit skeletal muscle phosphorylase b kinase. Biochemistry **3**, 1022—1033 (1964).

29. LARNER, J.: Hormonal and nonhormonal control of glycogen metabolism. Trans. N.Y. Acad. Sci. **29**, 192—209 (1967).

30. LINEWEAVER, H., and D. BURK: The determination of enzyme dissociation constants. J. Amer. chem. Soc. **56**, 658—666 (1934).

31. LOWRY, O.H., N.J. ROSENBROUGH, A. L. RARR, and R.J. RANDALL: Protein measurement with the Folin phenol reagent. J. biol. Chem. **193**, 265—275 (1951).

32. MADDAIAH, V.T., and N.B. MADSEN: Kinetics of purified liver phosphorylase. J. biol. Chem. **241**, 3873—3881 (1966).

33. MADSEN, N.B.: The inhibition of glycogen phosphorylase by uridine diphosphate glucose. Biochem. biophys. Res. Comm. **6**, 310—315 (1961).

34. MÖLLERING, H., and H.U. BERGMEYER: In Bergmeyer, H.U.: Methoden der enzymatischen Analyse. Weinheim: Verlag Chemie 1962.

34a. MÜLLER-OERLINGHAUSEN, B., U. SCHWABE, A. HASSELBLATT, and F. H. SCHMIDT: Activity of 3′,5′-AMP phosphodiesterase in liver and adipose tissue of normal and diabetic rats. Life Sciences **7**, part II, 593—598 (1968).

35. NEUBERT, D., H. HELGE, and R. BASS: Einbau von Thymidin in die Deoxyribonucleinsäure von Mitochondrien. Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak. **252**, 258—268 (1965).

36. PÄTAU, K.: Zur statistischen Beurteilung von Messungsreihen. (Eine *t*-Tafel). Biol. Zbl. **63**, 152—168 (1943).

37. RALL, T.W., J.W. CRAIG, and J. LARNER: unpublished observations, cited by (29).

38. RIZACK, M.A.: Activation of an epinephrine-sensitive lipolytic activity from adipose tissue by adenosine 3′,5′-phosphate. J. biol. Chem. **239**, 392—395 (1964).

39. — An epinephrine-sensitive lipolytic activity in adipose tissue. J. biol. Chem. **236**, 657—662 (1961).

40. RODBELL, M., and A.B. JONES: Metabolism of isolated fat cells. III. The similar inhibitory action of phospholipase C (clostridium perfringens α toxin) and of insulin on lipolysis stimulated by lipolytic hormones and theophylline. J. biol. Chem. **241**, 140—142 (1966).

40a. — The actions of insulin and catabolic hormones on the plasma membrane of fat cells. Biochem. J. **105**, 2 P (1967).

41. SCHULTZ, G., G. SENFT, and K. MUNSKE: Der Einfluß von Insulin auf die enzymatische Regulation der Glycogenolyse. Naturwissenschaften **53**, 529 (1966).

42. — — — Die Abhängigkeit glykogenauf- und -abbauender enzymatischer Reaktionen von der Insulininkretion. Naunyn-Schmiedebergs Arch. Pharmak. exp. Path **257**, 62—63 (1967).

43. SENFT, G., G. SCHULTZ, K. MUNSKE, and M. HOFFMANN: Effects of glucocorticoids and insulin on 3′,5′-AMP phosphodiesterase activity in adrenalectomized rats. Diabetologia **4**, 330–335 (1968)

44. —, W. LOSERT, G. SCHULTZ, R. SITT, and H.K. BARTELHEIMER: Ursachen der Störungen im Kohlenhydratstoffwechsel unter dem Einfluß sulfonamidierter Diuretica. Naunyn-Schmiedebergs Arch. Pharmak. exp. Path. **255**, 369—382 (1966).

45. SELTZER, H.S., E.W. ALLEN, A.L. HERRON, jr., and M.T. BRENNAN: Insulin secretion in response to glycemic stimulus: Relation of delayed initial release to carbohydrate intolerance in mild diabetes mellitus. J. clin. Invest. **46**, 323—335 (1967).

46. SHIMAZU, T.: Glycogen synthetase activity in liver: Regulation by the autonomic nerves. Science **156**, 1256—1257 (1967).

47. SITT, R., W. LOSERT, G. SCHULTZ, H. KAESS, and G. SENFT: Der Einfluß von Insulin auf den Kaliumtransport in der Skeletmuskulatur. Naunyn-Schmiedebergs Arch. Pharmak. exp. Path. **255**, 398—406 (1966).

48. SØVIK, O.: The action of insulin on glycogen synthesis in rat diaphragm. Acta physiol. scand. **68**, 246—254 (1966).

49. —, and O. WALAAS: Insulin stimulation of glycogen synthesis in the isolated rat diaphragm in the absence and in the presence of puromycin and actinomycin D. Nature (Lond.) **202**, 396—397 (1964).

50. STEINER, D.F., and J. KING: Induced synthesis of hepatic uridine diphosphate glucose-glycogen glucosyltransferase after administration of insulin to alloxan-diabetic rats. J. biol. Chem. **239**, 1292—1298 (1964).

51. — V. RAUDA, and R.H. WILLIAMS: Effects of insulin, glucagon, and glucocorticoids upon hepatic glycogen synthesis from uridine diphosphate glucose. J. biol. Chem. **236**, 299—304 (1961).

52. SUTHERLAND, E.W., R.W. BUTCHER, G.A. ROBISON, and J.G. HARDMAN: The role of adenosine 3′,5′-monophosphate in hormone action. In: Wirkungsmechanismen der Hormone, 18. Colloqu. Ges. Physiol. Chem., Mosbach 1967, p. 1, ed. by P. KARLSON. Berlin-Heidelberg-New York: Springer 1967.

53. TORRES, H.N., L.R. MARECHAL, E. BERNARD, and E. BELOCOPITOW: Control of muscle glycogen phosphorylase activity by insulin. Biochim. biophys. Acta **156**, 206—209 (1968).

54. VAUGHAN, M., J.E. BERGER, and D. STEINBERG: Hormone-sensitive lipase and monoglyceride lipase activities in adipose tissue. J. biol. Chem. **239**, 401—409 (1964).
55. VILLAR-PALASI, C., and J. LARNER: Levels of activity of the enzymes of the glycogen cycle in rat tissues. Arch. biochem. **86**, 270—273 (1960).
56. —, and J.I. WENGER: In vivo effect of insulin on muscle glycogen synthetase. Identification of the action pathway. Fed. Proc. **26**, 563 (1967).
57. WEBER, G.: Study and evaluation of regulation of enzyme activity and synthesis in mammalian liver. In: Advances in Enzyme Regulation, Vol. I, p. 1, ed. by G. Weber. Oxford, London, New York, Paris: Pergamon Press 1963.
58. WOOL, I.G., and PH. CAVICCHI: Protein synthesis by skeletal muscle ribosomes. Effect of diabetes and insulin. Biochemistry **6**, 1231—1242 (1967).

Dr. G. SCHULTZ
Pharmakologisches Institut
der Universität Heidelberg
6900 Heidelberg
Hauptstr. 47—51