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Effects of Glucocorticoids and Insulin on 3′,5′-AMP Phosphodiesterase Activity in Adrenalectomized Rats*

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Summary. Glucocorticoids stimulate the rate of lipolysis which is reduced in adrenalectomized animals. This hormonal action is antagonized by insulin. The antilipolytic action of insulin appears to be mediated by a reduced intracellular concentration of 3′,5′-AMP. This reduction can partly be attributed to an insulin-induced acceleration of 3′,5′-AMP degradation. — It is shown that the stimulatory influence of glucocorticoids on lipolysis is due to a reduction of 3′,5′-AMP phosphodiesterase (PDE) activity, which is increased by adrenalectomy. PDE activity was also increased in liver, skeletal muscle and kidney of adrenalectomized rats; treatment with a glucocorticoid prevented this increase. *In vitro,* PDE purified from beef heart was inhibited by glucocorticoids in high concentrations ($K_i = 1.1 \cdot 10^{-3}$ M for 6 α-methylprednisolone hemisuccinate, $K_i = 1.6 \cdot 10^{-3}$ M for prednisolone succinate). *In vivo,* the glucocorticoid-induced decrease of PDE activity (with retarded onset as shown in liver), may essentially be attributed to a decreased enzyme synthesis. — Studies on the interaction of insulin and glucocorticoids on PDE activity were performed in the liver. In adrenalectomized, alloxan diabetic rats insulin stimulated PDE activity suppressed by treatment with a glucocorticoid, unsuppressed PDE activity was not increased by insulin. In contrast, the action of glucocorticoids on PDE activity was independent of the presence or the effectiveness of insulin.

Effets des glucocorticoïdes et de l'insuline sur l'activité de la 3′5′-AMP-phosphodiestérase chez des rats surrénalectomisés

Résumé. Les glucocorticoïdes stimulent la lipolyse qui est réduite chez les animaux surrénalectomisés. Cette action hormonale est contrecarrée par l'insuline. L'action antilipolytique de l'insuline semble être due à une réduction de la concentration intracellulaire de 3′5′-AMP. Cette réduction peut être attribuée en partie à une accélération due à l'insuline de la dégradation du 3′5′-AMP. On a montré que l'influence stimulatrice des glucocorticoïdes sur la lipolyse est due à une réduction de l'activité de la 3′5′-AMP-phosphodiestérase (PDE), qui est accrue par la surrénalectomie. L'activité de la phosphodiestérase est également augmentée dans le foie, le muscle strié et les reins des rats surrénalectomisés, le traitement par un glucocorticoïde prévient cette augmentation. *In vitro,* la phosphodiestérase purifiée du coeur de boeuf est inhibée par les glucocorticoïdes à fortes concentrations ($K_i = 1.1 \cdot 10^{-3}$ M pour l'hémisuccinate de 6 α-méthylprednisolone, $K_i = 1.6 \cdot 10^{-3}$ M pour le succinate de prednisolone). *In vivo,* la diminution provoquée par les glucocorticoïdes de l'activité de la phosphodiestérase qui se produit au bout de quelque temps, comme on l'a montré dans le foie, peut essentiellement être attribuée à une synthèse diminuée de l'enzyme. Des études sur l'interaction de l'insuline et des glucocorticoïdes sur l'activité de la phosphodiestérase ont été effectuées sur le foie. Chez les rats surrénalectomisés, rendus diabétiques par l'alloxane, l'insuline stimule l'activité de la phosphodiestérase qui a été supprimée par un traitement avec un glucocorticoïde, l'activité de la phosphodiestérase qui n'a pas été supprimée n'est pas augmentée par l'insuline. Par contre, l'action des glucocorticoïdes sur l'activité de la phosphodiestérase est indépendante de l'action de l'insuline.

Wirkungen von Glucocorticoiden und Insulin auf die 3′,5′-AMP-Phosphodiesterase-Aktivität bei adrenalektomierten Tieren

Zusammenfassung. Die Lipolyse, die bei adrenalektomierten Tieren vermindert ist, wird durch Glucocorticoide verstärkt. Die gegenüber Glucocorticoiden antagonistische Wirkung von Insulin, das die Lipolyse vermindert, kann durch eine Abnahme der intracellulären 3′,5′-AMP-Konzentration erklärt werden. Diese ist teilweise auf einen beschleunigten Abbau des Nucleotids zurückzuführen. — Die Lipolysesteigerung durch Glucocorticoide ist durch eine Verminderung der 3′,5′-AMP-Phosphodiesterase (PDE)-Aktivität bedingt, die bei adrenalektomierten Ratten erhöht ist. Die PDE-Aktivität adrenalektomierter Tiere ist auch in Leber, Skeletmuskulatur und Niere erhöht, die Gabe eines Glucocorticoids verhindert diesen Anstieg. Glucocorticoide hemmen aus Rinderherz isolierte PDE *in vitro,* jedoch sind hohe Steroidkonzentrationen erforderlich ($K_i = 1{,}1 \cdot 10^{-3}$ M für 6 α-Methylprednisolon-Hemisuccinat, $K_i = 1{,}6 \cdot 10^{-3}$ M für Prednisolon-Succinat). Die einige Stunden nach Gabe eines Glucocorticoids einsetzende Abnahme der PDE-Aktivität kann im wesentlichen auf eine verminderte Enzymsynthese zurückgeführt werden. — Insulin steigert bei adrenalektomierten, alloxandiabetischen Ratten die PDE-Aktivität in der Leber nur, wenn die Tiere mit einem Glucocorticoid behandelt sind, nicht jedoch die erhöhte PDE-Aktivität bei Fehlen von Glucocorticoiden. Der Einfluß von Glucocorticoiden auf die PDE-Aktivität ist dagegen nicht an die Wirkung von Insulin gebunden.

Key-words: Glucocorticoids, 3′,5′-AMP phosphodiesterase, lipolysis, glycogen metabolism, insulin, lipolytic action of glucocorticoids, glucocorticoid-interaction with insulin, cyclic adenosine 3′,5′-monophosphate.

Non-Standard Abbreviations

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

Enzymes

Adenylate kinase, ATP: AMP phosphotransferase (E.C. 2.7.4.3); pyruvate kinase, ATP: pyruvate phosphotransferase (E.C. 2.7.1.40); lactate dehydrogenase, L-lactate NAD oxidoreductase (E.C. 1.1.1.27); glycogen synthetase, UDP-glucose: α-1,4-glucan α-4-glucosyltransferase (E.C. 2.4.1.11).

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In adipose tissue the rate of triglyceride breakdown depends on triglyceride lipase activity. This enzyme is activated by 3′,5′-AMP. The lipolytic action of several hormones, e.g. catecholamines, is mediated by an increased formation of this nucleotide [29, 5]. Insulin injected or added to the incubation medium, reduces the rate of lipolysis [16, 19, 21, 30, 11] which is increased in insulin deficiency, i.e. after starvation or in alloxan diabetes [15, 10, 22]. The antilipolytic action of insulin is due to a decreased intracellular concentration of 3′,5′-AMP [5, 6]. This may be explained by a hormonally-induced decrease of the 3′,5′-AMP formation [20], and an accelerated degradation of the nucleotide [32].

In contrast, glucocorticoids have been shown to exert a lipolytic action. In adrenalectomized animals, release of FFA from adipose tissue is reduced [15, 28]. Treatment with glucocorticoids leads to an elevation of plasma FFA concentration in adrenalectomized and intact animals [15, 41, 13, 3]. The release of FFA and glycerol from adipose tissue incubated with these hormones is also increased [19, 10, 9, 24]. The increased FFA release caused by glucocorticoids is reduced by insulin [10, 41].

These findings have raised the question whether the stimulatory action of glucocorticoids on lipolysis, which is opposite to the action of insulin, is also mediated by an influence on 3′,5′-AMP con centration. In the present paper it is shown that glucocorticoids suppressed 3′,5′-AMP phosphodiesterase activity which was elevated in adrenalectomized animals. These enzymic alterations not only occurred in adipose tissue, but also in liver, skeletal muscle and kidney. Insulin increases liver PDE activity provided that it is not heightened by glucocorticoid deficiency. The suppression of PDE activity by steroids appears to enable insulin to regulate the activity of this enzyme.

Materials and Methods

Male Wistar rats were used in this study whose body weight was between 150 and 200 g. The animals were fed a standard pellet diet (Altromin®) *ad libitum* unless otherwise stated. Studies in diabetic rats were performed 48 h after i.v. injection of 80 mg/kg alloxan tetrahydrate. Only those animals were used whose blood glucose concentration exceeded 200 mg/100 ml. Crystalline, glucagon-free insulin from cattle (Farbwerke Hoechst AG[1]) diluted in 0.9% NaCl solution was injected i.v.

Adrenalectomy was performed under diethylether narcosis by paravertebral incisions. 0.9% NaCl solution served these animals as drinking fluid. If glucocorticoids were given, 6 α-methylprednisolone was injected s.c. as acetate or i.v. as hemisuccinate (Farbwerke Hoechst AG[1]). For experiments *in vitro* the latter substance, as well as prednisolone-21-succinate (Merck AG, Darmstadt[1]), were used. Solvents were injected into respective controls.

Blood glucose and tissue protein determinations as well as measurement of tissue PDE activity were performed as previously described [32]. PDE purified from beef heart according to BUTCHER and SUTHERLAND [4] was used for studies *in vitro*. In these experiments the enzymatic activity was determined by continuous measurement of 5′-AMP formed under the influence of PDE in a 0.15 M glycylglycine buffer, pH 7.5, containing 6mM $MgCl_2$, by aid of adenylate kinase, pyruvate kinase, and lactate dehydrogenase [1, 31].

Data are given as mean ± S.E.M. and compared using Student's *t*-test and the *t*-tables of PÄTAU [27].

Results

In liver, adipose tissue and kidney of fed rats adrenalectomized 5 days before the experiments, PDE activity was increased over the values obtained from intact animals. Daily s.c. injections of 1 mg/kg 6α-methylprednisolone prevented this increase. In skeletal muscle, similar changes of PDE activity were measured, but the increase in PDE activity caused by adrenalectomy was smaller than that in the other tissues. The changes in PDE activities were independent of enzyme activities being referred to wet weight or tissue protein (Tab. 1).

These findings prompted us to investigate whether purified PDE is inhibited by glucocorticoids *in vitro*. The activity of PDE isolated from beef heart, was reduced by 6 α-methylprednisolone added as hemisuccinate. The type of inhibition was non-competitive with respect to 3′,5′-AMP, the K_i value was approx.

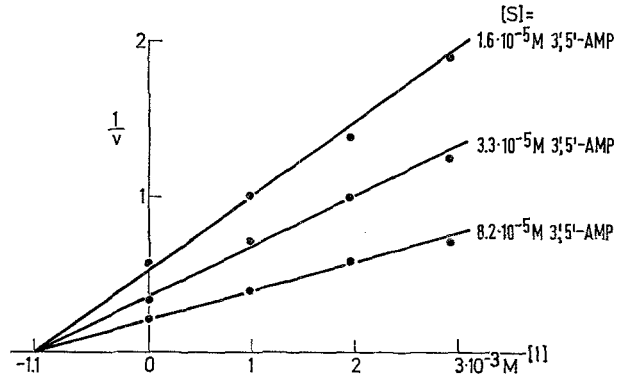

Fig. 1. Inhibition of 3′,5′-AMP phosphodiesterase (purified from beef heart) by 6-methylprednislone (hemisuccinate)

$1.1 \cdot 10^{-3}$ M (Fig. 1). Prednisolone, added as succinate, was also found to inhibit PDE non-competetively, the K_i value was approx. $1.6 \cdot 10^{-3}$M (Fig. 2). Addition of succinate ($3 \cdot 10^{-3}$M) did not influence PDE activity.

[1] The authors wish to thank Dr. A. FLAMME, Farbwerke Hoechst AG, and the Merck AG, Darmstadt, who kindly supplied these substances.

In contrast to the high glucocorticoid concentration required for reducing PDE activity *in vitro*, discontinuation of adrenal steroid secretion and substitution with exogenous glucocorticoid influenced PDE activity markedly. In adrenalectomized, fasted rats PDE activity was slightly reduced 2 and 4 h after glucocorticoid application, a further reduction was measured 15 and 27 h after steroid injection (Fig. 3).

In order to elucidate the relationship between the opposite actions of insulin and glucocorticoids on PDE

Table 1. *Influence of adrenalectomy and of application of 6-methylprednisolone (1 mg · kg^{-1} · d^{-1} s.c. for 5 days) on 3′,5′-AMP phosphodiesterase activity. Animals were fed ad libitum*

	intact rats	adrenalectomized rats (5 days post op.)	
			+ 6-methylprednisolone
Liver			
$\frac{n \text{ moles}}{\text{min} \cdot \text{g w.w.}}$	645 ±20	734 ±16	596 ±26
	$n=18$ $p<0.002$	$n=24$ $p<0.0002$	$n=19$
Adipose tissue			
$\frac{n \text{ moles}}{\text{min} \cdot \text{g w.w.}}$	32.8 ±2.1	84.7 ±12.6	49.1 ±4.1
	$p<0.0002$	$p<0.02$	
$\frac{n \text{ moles}}{\text{min} \cdot \text{mg prot.}}$	1.62 ±0.08	2.78 ±0.22	1.82 ±0.17
	$p<0.0002$	$p<0.002$	
	$n=25$	$n=17$	$n=20$
Kidney			
$\frac{n \text{ moles}}{\text{min} \cdot \text{g w.w.}}$	484 ±19	649 ±21	460 ±15
	$p<0.0002$	$p<0.0002$	
$\frac{n \text{ moles}}{\text{min} \cdot \text{mg prot.}}$	8.51 ±0.33	11.95±0.44	7.61 ±0.28
	$p<0.0002$	$p<0.0002$	
	$n=19$	$n=26$	$n=19$
Skeletal muscle			
$\frac{n \text{ moles}}{\text{min} \cdot \text{g w.w.}}$	67.0 ±3.2	71.6 ±3.1	57.2 ±3.3
		$p<0.003$	
$\frac{n \text{ moles}}{\text{min} \cdot \text{mg prot.}}$	0.839±0.041	0.902±0.042	0.707±0.043
		$p<0.003$	
	$n=21$	$n=25$	$n=21$

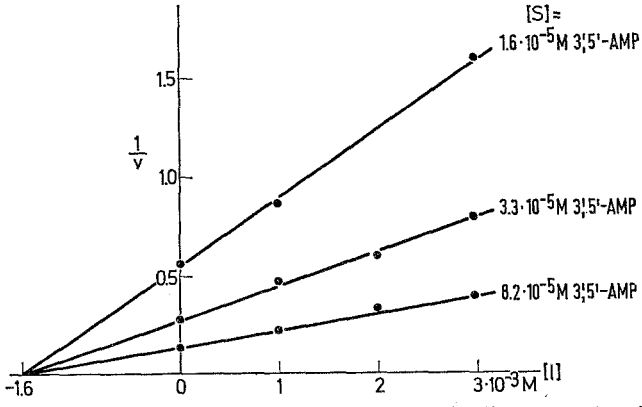

Fig. 2. Inhibition of 3′,5′-AMP phosphodiesterase (purified from beef heart) by prednisolone (-21-succinate)

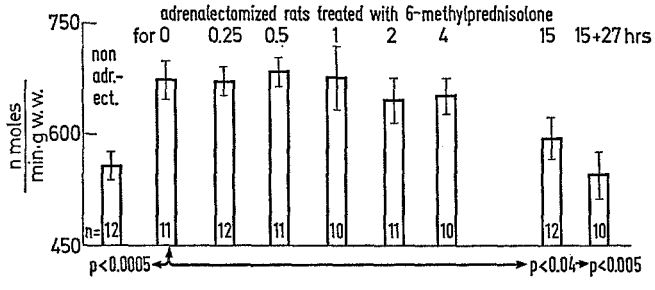

Fig. 3. Influence of 6-methylprednisolone on liver 3′,5′-AMP phosphodiesterase activity. Adrenalectomy was performed 3 days before experiment. All rats were fasted for 2 days. 1 mg/kg 6-methylprednisolone was injected as hemisuccinate Na^+ i.v. 0.25, 0.5, 1, and 2 h, s.c. 4 h, as acetate s.c. 15 and 27 h prior to decapitation

with low insulin plasma concentration [2], liver PDE activity was increased compared with intact rats. After a single injection of 6 α-methylprednisolone there was no decrease in enzyme activity within the first hour. activity, insulin was injected into alloxan diabetic rats that were adrenalectomized and treated with various doses of a glucocorticoid. In adrenalectomized, diabetic animals not substituted with glucocorticoids,

liver-PDE activity was not increased by injection of 0.5 U/kg insulin (Fig. 4). This dose has been shown to stimulate enzyme activity in diabetic rats whose adrenals had not been removed [32]. Even the application of an insulin dose 10 times higher did not influence liver PDE activity in adrenalectomized rats. If, however, in adrenalectomized rats PDE activity was normalized by treatment with 6α-methylprednisolone, insulin induced a dose-dependent increase of PDE activity. The glucocorticoid-induced suppression of enzyme activity was nearly prevented by 5 U/kg insulin. In rats treated with 6α-methylprednisolone 3 mg/kg, the decrease of PDE activity was insignificantly greater than after application of 1 mg/kg. Injection of 0.5 U insulin/kg resulted in a higher enzymic activity in adrenalectomized animals treated with the lower dose of the glucocorticoid than in those treated with the higher dose (Fig. 4).

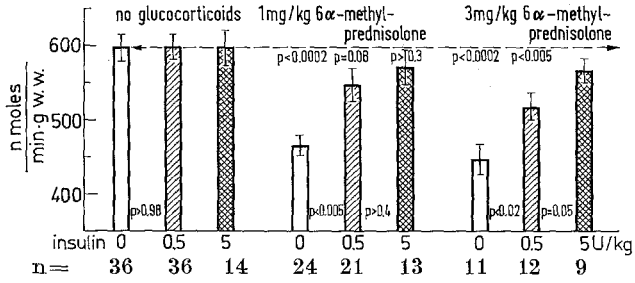

Fig. 4. Influence of insulin on liver 3′,5′-AMP phosphodiesterase activity in diabetic rats adrenalectomized 24 h prior to decapitation. Alloxan (80 mg/kg) was injected i.v. 48 h, 6-methylprednisolone acetate s.c. 12 h, insulin i.v. 45 min before decapitation

Discussion

Glucocorticoids are known to be inducers of the key enzymes of hepatic gluconeogenesis, which are oppositely influenced by insulin [40, 39, 23]. In contrast to the inductive action of glucocorticoids on other enzymes, there is a suppressive action of these hormones on PDE in several tissues.

It has been shown by FAIN, SCOW and CHERNICK [10] that the stimulation of lipolysis caused by glucocorticoids is delayed. More than 2 h is required for an acceleration of triglyceride breakdown. These findings correspond to the glucocorticoid-induced decrease in enzymic hydrolysis of 3′,5′-AMP, and the delayed hormonal action on PDE activity shown in the liver. Owing to this fact and to the lack of PDE inhibition by low glucocorticoid concentrations occurring physiologically, an inhibitory action of glucocorticoids on PDE may be regarded as unimportant for the hormonal-induced reduction of enzymic activity. *In vivo*, the decrease of PDE activity must mainly be referred to a suppressed enzyme synthesis. The inhibitory action of glucocorticoids on PDE can, however, be taken into consideration for explaining the stimulation of lipolysis within the first few hours after application of high doses of a steroid.

The influence of glucocorticoids on the degradation of 3′,5′-AMP, the formation of which is increased by several hormones, can help to explain the following findings. The capacity of adipose tissue to respond *in vivo* or *in vitro* to catecholamine-stimulation by an increased FFA release, is strongly reduced in glucocorticoid deficiency [28, 33, 34, 25] accompanied by an accelerated hydrolysis of 3′,5′-AMP. The responsiveness to epinephrine is restored after the animals have been treated with a glucocorticoid. It can be assumed that this treatment normalizes the increased 3′,5′-AMP degradation, and thereby allows a higher catecholamine-induced increase of tissue 3′,5′-AMP concentration.

Insulin may influence glycogen metabolism and lipolysis by accelerating 3′,5′-AMP degradation [32]. Suppression of PDE activity by glucocorticoids is required for the regulation of enzyme activity by insulin. This can be concluded from the following findings. 1. Glucocorticoids suppress renal PDE activity, although, even in high doses, there is no influence of insulin on the activity of this enzyme in the kidney [32]. 2. Glucocorticoids suppress PDE activity not only if insulin is ineffective, i.e. in kidney, but also if it is absent, i.e. in alloxan diabetes. 3. Insulin does not increase PDE activity unless it has been suppressed by glucocorticoids. The dose-dependent increase of enzyme activity by insulin is limited to the range of glucocorticoid-induced suppression. Actinomycin D interfering with DNA-dependent synthesis of messenger RNA [14], has been shown to block the insulin-induced increase of PDE activity [32]. Therefore, it can be assumed that the regulation of enzymic activity by insulin, which reduces glucocorticoid-caused suppression of PDE activity, is on the level of transcription.

That the action of glucocorticoids on PDE is necessary for the action of insulin on the activity of this enzyme, can also be seen from the following findings. The early stimulation of liver glycogen synthetase activity by insulin can be explained by a decreased concentration of 3′,5′-AMP, which nucleotide activates transferase-I-kinase [18]. This enzyme catalyzes the conversion of the more active form of glycogen synthetase (I) to the less active form (D). This activation of liver glycogen synthetase measured 30 min after stimulation of insulin secretion by glucose administration, has only been found in intact animals [7]. In adrenalectomized rats whose liver PDE activity is not increased by insulin, glycogen synthetase I activity remained unchanged[2].

Liver and muscle glycogen concentrations are reduced in adrenalectomized animals, and are slowly

[2] Added in proof. In adrenalectomized animals, however, increased liver glycogen synthetase I activity caused by stimulation of insulin secretion or by injection of insulin (6 U/kg i.p., 75 min) into diabetic rats was measured by other authors [22a]. This indicates that insulin may also influence this enzymatic activity through another, glucocorticoid-independent action.

increased by glucocorticoid application [13, 12, 38, 17, 8]. There are difficulties in correlating these findings with an increased PDE activity in glucocorticoid deficiency, and a reduction of PDE activity by glucocorticoids. Increases of gluconeogenesis [40, 39, 23] and of G6P concentration [38, 17], caused by application of a glucocorticoid, may be regarded as factors that favour glycogen formation. An increased concentration of G6P, which stimulates glycogen synthetase activity [37], has, however, not always been found after application of glucocorticoids [8]. Increased glycogen formation by glucocorticoids must mainly be referred to an increased synthesis of the enzyme glycogen synthetase [38, 17, 35]. This hormonal action, which can be prevented by application of actinomycin D [35], may be regarded as more important than a possible stimulation of transferase-I-kinase mediated by 3′,5′-AMP. This enzyme, which catalyzes the inactivation of glycogen synthetase, could be activated in the case of a glucocorticoid-caused reduction of 3′,5′-AMP degradation in insulin deficiency. Plasma insulin concentrations of glucocorticoid-treated animals can, however, be assumed to be high [2, 26]. There is even a possible participation of insulin in the increased formation of the enzyme glycogen-synthetase that is observed after application of glucocorticoids. Insulin is known to promote the formation of liver glycogen-synthetase [36]. Since glucocorticoids increase the secretion of insulin [2, 26], this hormone could possibly be involved in the action of adrenal steroids on glycogen synthetase activity.

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