PROTEIN KINASE AND CYCLIC AMP-BINDING ACTIVITIES IN LIVER AND ADIPOSE TISSUE OF NORMAL, STREPTOZOTOCIN-DIABETIC AND ADRENALECTOMIZED RATS

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Received 30 August 1973

1. Introduction

Glycogen breakdown and synthesis as well as lipolysis are subject to regulation by cyclic AMP–protein kinase-mediated phosphorylation of phosphorylase b kinase, [1, 2], glycogen synthase a [2] and hormone-sensitive triglyceride lipase [3, 4]. The implications of the cyclic AMP–protein kinase system in the hormonal control of these metabolic pathways might be of relevance in diabetes and adrenocortical failure, since in the absence of insulin, glycogen and lipid metabolism are severely impaired, and since adrenalectomy considerably reduces the response of the liver [5, 6] and adipose tissue to hormonal stimuli [6, 7, 10]. The latter has been attributed to the loss of the so-called permissive effect of glucocorticoids. It has been shown by Schaeffer et al. [5] in liver and by Exton et al. [6] in adipose tissue that the metabolic lesion lies beyond adenylcyclase.

Therefore, it seemed of interest to examine whether protein kinase and cyclic AMP-binding undergo any changes in liver and adipose tissue of diabetic and adrenalectomized animals.

2. Materials and methods

2.1. *Animals*

Male Osborne–Mendel rats weighing between 140 and 160 g were used throughout. They were fed normal chow. Diabetes was induced by intravenous administration of streptozotocin (Hoffmann-La Roche, Basel), 70 mg/kg. Adrenalectomy was carried out in ether-aneasthetised animals by paravertebral incisions. Diabetic animals were sacrificed 5 days after the streptozotocin injection (blood sugar 400–500 mg%) and adrenalectomized animals 5 days after the operation.

2.2. *Tissue preparation*

After decapitation of the animals, tissues were rapidly excised and frozen in liquid nitrogen. Livers were perfused with cold 4 mM EDTA, pH 7.0 prior to freezing. The tissues were stored at −20° C until homogenization which was performed at 4° C in 5 vol of 4 mM EDTA, pH 7.0.

Homogenates were centrifuged at 30 000 *g* and 4° C for 30 min and the clear supernatants were used for protein kinase and cyclic AMP-binding assays after appropriate dilution.

2.3. *Protein kinase assay*

Protein kinase assays were performed by the method of Walsh et al. [11] with slight modifications. The assay medium contained in a volume of 0.25 ml: sodium glycerol phosphate buffer, pH 6.0, 12.5 μmoles; theophylline 0.75 μmoles; NaF 2.5 μmoles; EDTA 0.2 μmoles; casein (Serva, Heidelberg) 1.5 mg; between 100 and 200 μg of tissue protein; cyclic AMP, as added, 2.5 μmoles; and [γ-^{32}P]ATP (Amersham, Radiochemical Centre) 0.25 μmoles (1.5–3 × 10^6 cpm). After 5 min of incubation at 30° C the reaction was stopped by addition of 0.5 ml of 0.625% serum albumin and 2.5 ml of 10% trichloroacetic acid. ^{32}P incorporation into casein was determined according to De Lange et al. [12].

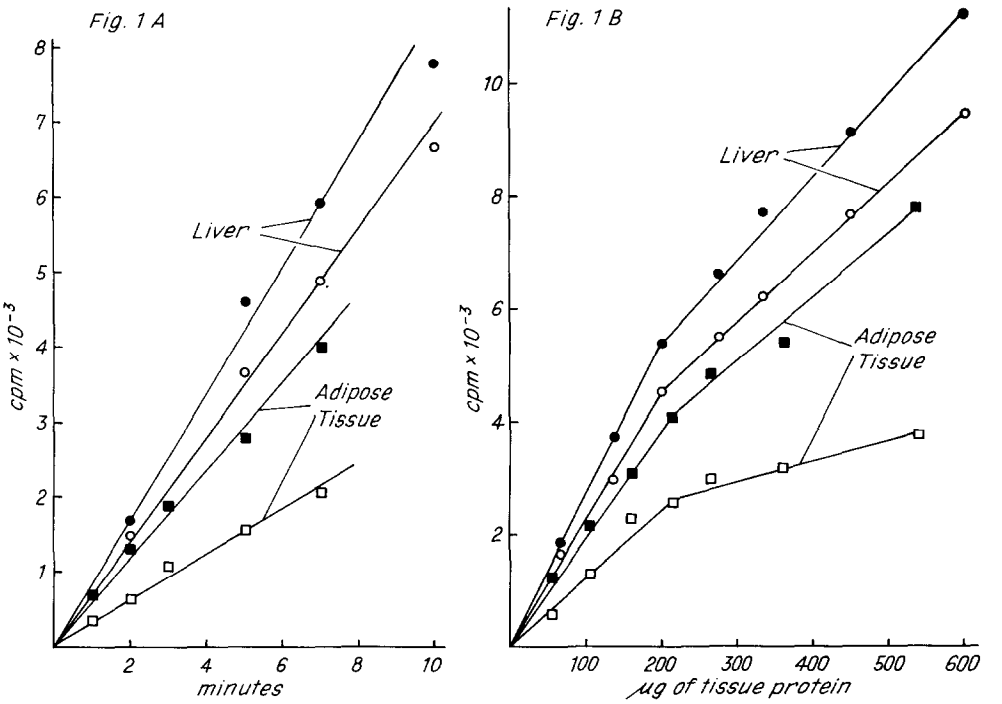

Fig. 1: A) Time course of phosphorylation of casein by crude tissue extracts of adipose tissue (180 µg of tissue protein) and liver (168 µg of tissue protein) in the absence (open symbols) and presence (closed symbols) of 10^{-5}M cyclic AMP. Assay as described in Materials and methods; B) ^{32}P incorporation into casein by various amounts of protein from adipose tissue and liver in the absence (open symbols) and presence (closed symbols) of 10^{-5}M cyclic AMP. Incubations were carried out for 5 min at 30°C as described in the text.

All values were corrected for endogenous ^{32}P incorporation in the absence of casein.

2.4. *Determination of cyclic AMP-binding activity*

This was carried out according to the procedure of Walton and Garren [13]. The reaction mixture contained in a total volume of 0.2 ml: Tris–HCl buffer, pH 7.5, 10 µmoles; $MgCl_2$ 2 µmoles; theophylline 3.2 µmoles; between 100 and 200 µg of tissue protein and 13.3 pmoles of [^{3}H]cyclic AMP (50 nCi; Amersham. Radiochemical Centre). Incubations lasted 30 min at 25° C. A similar procedure has lately been used in our laboratory for measuring cyclic AMP levels in liver perfusates [14].

3. Results and discussion

Since many factors may influence the kinase reaction in crude tissue preparations, assay conditions were used in which the reaction kinetics were linear. Fig. 1A shows that ^{32}P incorporation into casein in the absence and presence of 10^{-5}M cyclic AMP is linear over 7–10 min with both tissue extracts. With ATP concentrations lower than 1 mM (0.2 and 0.05 mM) the reaction rate significantly decreases after 2–3 min (not shown). As demonstrated in fig. 1B the reaction is linear only at tissue concentrations below 200 µg per 0.25 ml of assay medium. One may see from fig. 1A and 1B that the ratio of cyclic AMP-stimulated to unstimulated protein kinase activities remains constant during the incubations. All these criteria indicate that neither ATP- nor cyclic AMP-degradation affect the kinase reaction to a detectable extent under the conditions applied.

A similar linear relationship is observed for cyclic AMP-binding (fig. 2) if the amount of tissue protein in the assay does not exceed 200 µg.

Figs. 3A and 3B show that in the diabetic liver basal (unstimulated) and cyclic AMP-stimulated (total)

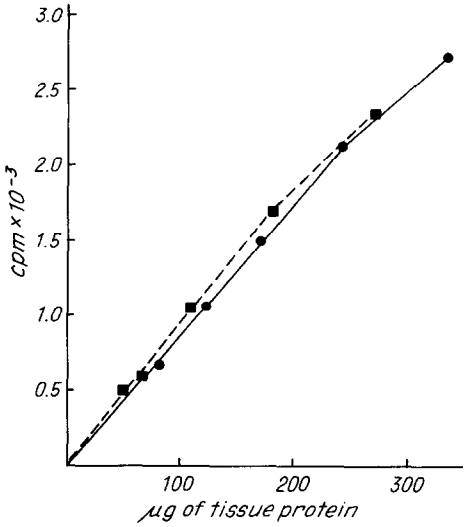

Fig. 2. Cyclic AMP-binding by various amounts of protein from adipose tissue (■-■-■) and liver (●-●-●). Assay conditions are given in the text.

protein kinase activities are reduced by 35%, and cyclic AMP-binding by 30%, probably reflecting decreased protein kinase de novo synthesis during insulin deficiency. Changes in cyclic AMP levels in vivo which might cause a shift of the equilibrium between the holoenzyme RC and the catalytic sub-unit C [15] would only influence unstimulated but not total kinase activity. Besides, the state of this equilibrium within the intact cell is not very likely to persist throughout the killing of the animals and the subsequent homogenization of the tissue. However, in contrast to the findings of Corbin et al. [16] reconstitution of the regulatory and catalytic sub-units does not take place in our assay system since the ratio between cyclic AMP-stimulated and unstimulated kinase activities does not increase but remains constant upon dilution (fig. 1B).

Adrenalectomy is without effect on protein kinase and cyclic AMP-binding activities in liver (figs. 3A and 3B) suggesting that in this organ insulin rather than glucocorticoids is necessary to maintain normal levels of these proteins. Furthermore, this finding ren-

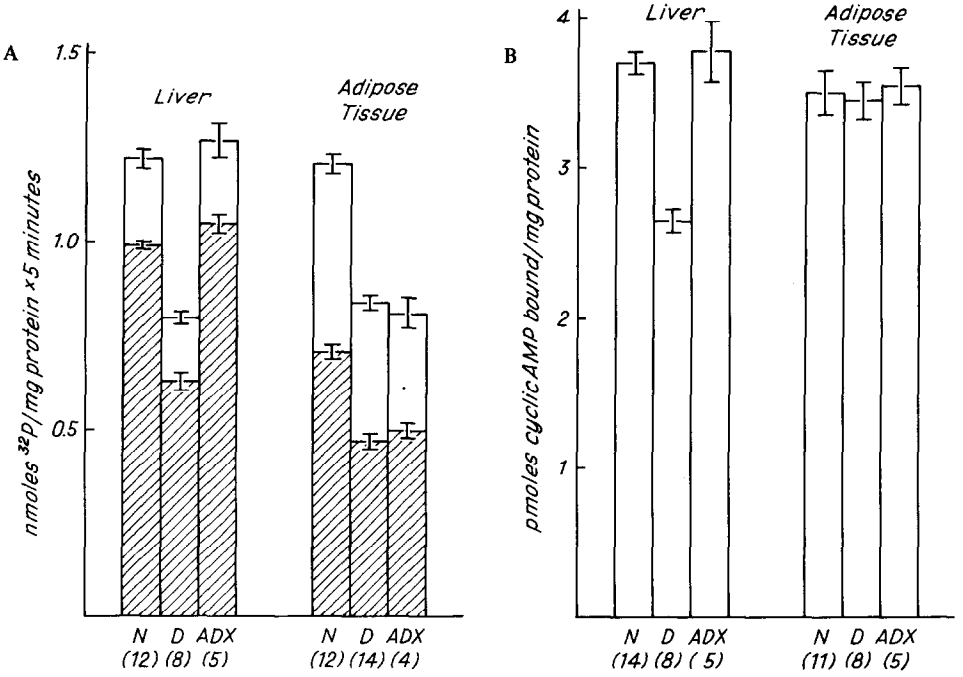

Fig. 3. Specific activities of cyclic AMP-stimulated (10^{-5} M cyclic AMP; whole columns) and unstimulated (hatched columns) protein kinase (3A) and specific cyclic AMP-binding activities (3B) in crude liver and adipose tissue preparations (30 000 *g* supernatants) from normal (N), diabetic (D) and adrenalectomized (ADX) rats. In parentheses, number of animals tested; the bars give the standard error of the mean. The assays are described in the text.

ders it unlikely that the failure of the liver of adrenalectomized animals to respond adequately to hormones which stimulate glucose mobilization is quantitatively related to impaired de novo synthesis of protein kinase or cyclic AMP-binding protein.

In adipose tissue the absence of glucocorticoids as well as insulin deficiency cause a 30% decrease of protein kinase activity, whereas cyclic AMP-binding is the same as in normal adipose tissue (figs. 3A and 3B). These results are not only compatible with decreased de novo synthesis of protein kinase, but, in addition, with an increase of the protein kinase inhibitor. The latter has been shown by Walsh et al. to form an inactive complex with the enzyme but to cause increased cyclic AMP-binding at nucleotide concentrations below 10^{-7}M [17, 18]. At normal protein kinase levels an increase of the kinase inhibitor alone could also account for lower enzyme activities, but cyclic AMP-binding would be increased above normal. Therefore, decreased de novo synthesis of kinase must, in addition, be inferred to explain normal cyclic AMP-binding and decreased kinase activities. Since the ratio of cyclic AMP-binding to protein kinase activity is increased (as calculated from figs. 3A and 3B) the same cyclic AMP levels would be less effective in activating protein kinase than in normal adipose tissue. This could, at least in part, account for the insufficient response of adipose tissue to lipolytic hormones after adrenalectomy, and it would provide a means of attenuating the bombardment by lipolytic hormones of adipose tissue in the absence of insulin, which in normal adipose tissue counteracts their effects.

The decrease of both cyclic AMP-stimulated and unstimulated kinase activities is in contrast to the elevated levels of inactive (phosphorylated) glycogen synthase in livers of diabetic rats [19, 20], and to the increased rate of lipolysis in diabetic fat pads pointing to a fairly active (phosphorylated) triglyceride lipase. Maybe elevated cyclic AMP levels (as observed in livers of alloxan-diabetic rats, [21], overcompensate for the decrease of kinase activity. A more attractive explanation would be that the imbalance of the phosphorylation–dephosphorylation cycle in diabetes might result in decreased dephosphorylation of these enzymes.

Acknowledgement

The work was supported by the Swiss National Science Foundation grant No 38569.

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