ACTH ACTIVATION OF CYTOSOL TRIGLYCERIDE HYDROLASE IN THE ADRENAL OF THE RAT

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1. Introduction

The administration of ACTH in vivo to certain mammals results in an increase in the adrenal cytosolic cholesterol ester hydrolase activity [1–3]. It has been shown that in vivo, the adrenal cortex responds to ACTH by activation of adenyl cyclase, the intracellular cAMP concentration increases, which in turn activates a cytosolic protein kinase [4,5]. The activation of cholesterol ester hydrolase in the cytosol of these cells requires the intervention of such a cAMP-dependent protein kinase. Hydrolysis of cholesterol esters provides free cholesterol for utilisation by the adrenal mitochondria for pregnenolone production [6–8].

Adipose tissue responds to a variety of endocrine signals in vivo and in vitro [9–15], it contains a substantial amount of esterified cholesterol [11] as well as a high triglyceride concentration. Adipose tissue contains an activatable triglyceride lipase 'hormone-sensitive lipase' and it has been shown that adipose tissue also contains activatable cholesterol ester hydrolase [11,12,16]. The adrenal cortex, on the other hand, is very rich in cholesterol esters but also contains triglyceride [1,4,17]. Since adipose tissue responds to some hormones by activation of a hormone sensitive triglyceride lipase and a hormone sensitive cholesterol ester hydrolase, while the adrenal cortex has been shown to respond to ACTH by activation of cholesterol ester hydrolase we decided to study whether the adrenal cortex would respond to ACTH by activation of a triglyceride lipase.

In this study we have investigated the effects of ACTH in vivo on the activity of the cytosol cholesterol ester hydrolase and cytosol triglyceride lipase in the adrenal cortex and in adipose tissue in the rat.

2. Materials and methods

2.1. *Chemicals and radiochemicals*

Porcine pituitary's adrenocorticotrophic hormone (ACTH) (Acthar Corticotropic (Sterile)) 40 IU, was the product of Armour Pharmaceutical Co. Ltd (Eastbourne). ATP (di-sodium salt), cyclic AMP acid, bovine albumin (Fraction V essentially fatty acid free) and cholesteryl oleate (99% pure) were the products of Sigma Chemicals Co. Ltd (St Louis). Glycerol-tri-oleate (Triolein), oleic acid and all other chemicals and solvents were obtained from British Drug Houses (Poole) and were of analytical grade.

Cholesteryl[1-^{14}C]oleate (21.5 mCi/mmol), 99% pure and glycerol-tri-[1-^{14}C]oleate (60 mCi/mmol), more than 96% pure, were obtained from the Radiochemical Centre (Amersham). They were used without further purification.

2.2. *Animal treatment*

Female Wistar rats (150–220 g) were obtained from the Small Animal Breeding Centre (University of Edinburgh). Twelve rats were used for each experiment, and they were divided randomly into two groups. One group was injected subcutaneously with 0.2 ml saline solution, while the other group received 4 IU ACTH dissolved in water. The animals from both groups were killed by decapitation 12–15 min after receiving the injection. The adrenal glands and perirenal adipose tissue were quickly excised and immersed in ice-cold 0.25 M sucrose solution, pH 7.0.

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2.3. *Preparation of the 105 000 × g supernatant*

The adrenal glands were trimmed of adhering fat and connective tissue, and were homogenised in 12 ml 0.25 M sucrose, pH 7.0, while the adipose tissue from six rats was homogenised in 30 ml 0.25 M sucrose. The homogenates of both tissues were centrifuged at 10 000 × *g* for 10 min. The fat cake present in the homogenate of adipose tissue was discarded. The clear infranatant of adipose tissue and adrenals was centrifuged at 105 000 × *g* for 1 h. The clear delipidated supernatant from both tissues was used as a source of cholesterol ester hydrolase and hormone-sensitive triglyceride lipase.

2.4. *Preparation of labelled substrate mixtures*

The procedure for preparing a mixture of labelled substrate with buffer, is based upon the method of Khoo et al. [12] with some modification. Cholesteryl-[1-^{14}C]oleate or glycerol-tri-[1-^{14}C]oleate (1 μCi) was added to unlabelled cholesteryl oleate or glycerol-tri-oleate (14.399 μmol) in benzene. This stock labelled substrate of either material was then taken to dryness under N_2 gas. The pellet was dissolved in 1 ml absolute ethanol. The solubilised substrate was added slowly to 19 ml of a mixture containing bovine albumin, phosphate buffer, pH 7.2 and EDTA, pH 7.2. Thus in the final volume of 20 ml the concentration of cholesteryl[1-^{14}C]oleate or glycerol-tri-[1-^{14}C]oleate was 720 μM; bovine albumin 10% (w/v), phosphate buffer, 50 mM, EDTA 20 mM and ethanol 5% (v/v).

2.5. *Assay of cholesterol ester hydrolase and hormone-sensitive triglyceride lipase*

Cholesterol ester hydrolase and hormone-sensitive lipase were assayed in the 105 000 × *g* supernatant of rat adrenal and adipose tissue as follows. 200 μl supernatant in 50 mM Tris–HCl buffer, pH 7.2, was incubated with 200 μl of the respective substrate buffer mixture for 30 min at 37°C. The hydrolysis of the oleic acid ester was terminated by adding 1.5 ml of the fatty acid extraction mixture [16] containing 0.288 mM unlabelled oleic acid as a carrier. NaOH (50 μl 1 M) was then added and the tubes were shaken vigorously for 15 s, followed by 10 min centrifugation at 2300 × *g*. Aliquots from the upper aqueous phase were taken into 10 ml Triton X-100–toluene scintillation cocktail. The release of [1-^{14}C]oleic acid was measured in a Packard Tri-Carb Scintillation Spectrometer. Blank tubes containing boiled enzyme were included as controls.

2.6. *Activation of hormone-sensitive triglyceride lipase and cholesterol ester hydrolase in vitro*

The activation of both enzymes was carried out in a final volume of 0.2 ml, and contained 80–200 μg protein, 2 mM magnesium acetate, 2 mM ATP, 10 μM cAMP and 50 mM Tris–HCl buffer, pH 7.2. The activation reaction was allowed to proceed for 10 min at 37°C, after which it was terminated by the addition of 0.2 ml of the respective substrate buffer mixture, which contained 20 mM EDTA. Control tubes contained no ATP or cAMP.

2.7. *Protein determination*

The protein concentration in the 105 000 × *g* supernatant was measured by the method of Lowry et al. [18], using bovine serum albumin as a standard.

3. Results

3.1. *Effect of ACTH treatment*

Both cholesterol ester hydrolase and hormone-sensitive triglyceride lipase, from the adrenals of ACTH injected rats, were stimulated significantly in response to ACTH administration. The increase in the activity of both enzymes was comparable to the activation observed when the supernatant was preincubated with ATP and cAMP. The similarity in the magnitude of activation suggests that both enzymes might be activated in vivo and in vitro by a similar mechanism involving a cAMP-dependent protein kinase.

Similarly, both cholesterol ester hydrolase and triglyceride lipase from adipose tissue were stimulated in vivo in response to ACTH administration, although the elevation of the activities were not as great as that seen in the adrenal gland. Pittman et al. [11] reported similar results for rat adipose tissue hydrolase, when adipocytes were preincubated with 10 μM epinephrine.

3.2. *In vitro activation of hormone-sensitive lipase and cholesterol ester hydrolase*

When the 105 000 × *g* supernatant, of adipose tissue or adrenal glands, was pre-incubated with cAMP and ATP both cholesterol ester hydrolase and hormone-sensitive triglyceride lipase were stimulated 30–70%

Table 1

Tissue	No. rats	Value	Cholesterol ester hydrolase				Hormone-sensitive triglyceride lipase			
			Saline injected rats		ACTH injected rats		Saline injected rats		ACTH injected rats	
			Control Mg^{2+} only	Incubated with ATP and cAMP	Control Mg^{2+} only	Incubated with ATP and cAMP	Control Mg^{2+} only	Incubated with ATP and cAMP	Control Mg^{2+} only	Incubated with ATP and cAMP
Adrenal gland	24	Percentage	100	153	139	181	100	146	131	160
		Absolute rate oleic acid produced (pmol/min/mg protein)	1054	1613	1465	1908	405	591	531	648
Adipose tissue	24	Percentage	100	141	121	147	100	152	110	138
		Absolute rate oleic acid produced (pmol/min/mg protein)	3090	4357	3793	4542	1305	1984	1436	1801

over the control values. Exogenous protein kinase was not added since Trzeciak and Boyd [1] and Pittman et al. [11] found protein kinase activity in the 105 000 × *g* supernatant of the adrenal and adipose tissue enough to stimulate cholesterol ester hydrolase and hormone-sensitive triglyceride lipase.

The activation by ACTH of cholesterol ester hydrolase and hormone-sensitive triglyceride lipase in adrenal and adipose tissue was greater when the supernatant was preincubated with ATP and cAMP. This suggests that the enzymes were not fully activated by ACTH treatment, or, the active form of both enzymes lost activity during processing the tissues.

Table 1 summarises the results of four experiments, in which the mean control value from the experiments was considered as 100%, and the mean percentage increase in different experiments was referred to that figure in absolute values.

4. Discussion

This report shows the presence of a hormone-sensitive triglyceride lipase in rat adrenal cytosol. This enzyme activity can be activated in vivo by exposing the animal to ACTH, while ATP and cAMP stimulate the enzyme in vitro, when the adrenal supernatant is pre-incubated with these cofactors. ACTH is reported to cause a marked decrease of triglycerides in the adrenals of hypophysectomised rats [17] and it has been postulated that a 'hormone-sensitive' triglyceride lipase might be involved. It has been reported that there is a dose-dependent in vitro lipolytic response to ACTH in rat adrenal homogenates [19]. Rat adrenal cholesterol ester hydrolase can be activated in vivo by subjecting the animals to ether anaesthesia, a condition known to elevate ACTH concentration in the blood [1,20] while ATP and cAMP activated the enzyme in vitro. The finding of ACTH stimulation of cholesterol ester hydrolase reported here is in agreement with the previous report.

The average in vivo activation by ACTH of cholesterol ester hydrolase and hormone-sensitive triglyceride lipase, of rat adipose tissue is not very striking, although in two experiments out of four reported here, a marked stimulation was observed. The reasons for the varying results are not known, but it might be that cholesterol ester hydrolase and hormone-sensitive triglyceride lipase in adipose tissue of saline injected rats are activated by release of endogenous ACTH, so that the basal activities of both enzymes are at a somewhat higher level, thus obscuring the difference in response to ACTH administration. It is also possible that the system is very labile for activation–deactivation of cholesterol ester hydrolase and hormone-sensitive lipase in rat adipose tissue [10,11,15,21]. Recently, the dramatic effect of phospho-protein phosphatase from different sources on the protein kinase-activated cholesterol ester hydrolase and triglyceride lipase of chicken adipose tissue has been demonstrated [22]. It is possible that fluctuations of a phosphoprotein phosphatase activity may regulate the cholesterol ester hydrolase and triglyceride lipase activities in these tissues.

Activation of bovine adrenal cholesterol ester hydrolase by phosphorylation of the inactive enzyme in a process involving a cAMP-dependent protein kinase is now fully substantiated [1,3,23–25]. It has also been shown that a 100-fold purified hormone-sensitive triglyceride lipase from adipose tissue cytosol is activated by the transfer of a terminal phosphate group from ATP, in the presence of a cAMP-dependent protein kinase [26]. The finding that cholesterol ester hydrolase in rat adipose tissue cytosol cofractionated with hormone-sensitive lipase led to the suggestion that the two activities might reside in one single enzyme protein or in a single enzyme complex [11,12]. Hence, the presence of a hormone-sensitive triglyceride lipase in rat adrenal will pose the question about its nature, and its relationship to cholesterol ester hydrolase. Hormone-sensitive triglyceride lipase may be under a similar activation-deactivation control as the cholesterol ester hydrolase in the adrenal. The two enzymic hydrolytic activities may reside in a single protein or in separate but related proteins. This problem is under active investigation.

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