Biochemical Pharmacology, Vol. 41, No. 5, pp. 833–835, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

Treatment with anabolic steroids increases the activity of the mitochondrial outer carnitine palmitoyltransferase in rat liver and fast-twitch muscle

(*Received* 22 *May* 1990; *accepted* 24 *September* 1990)

The outer activity of carnitine palmitoyltransferase plays an important role in regulating the flux of long-chain fatty acids to mitochondrial oxidative metabolism in mammalian tissues [1, 2]. In addition, the sensitivity to inhibition by physiological concentrations of malonyl-CoA is an essential property of the enzyme [1, 2]. The activity and the sensitivity to malonyl-CoA of the hepatic enzyme are controlled both on the short [3, 4] and on the long term [5–10] by the nutritional and the hormonal status of the animal. Thus, hormones such as insulin [6, 11], glucagon [11] and oestrogens [9] produce changes in the properties of the mitochondrial outer carnitine palmitoyltransferase in rat liver. Nevertheless, as far as we know, the effects of androgens on the enzyme have not been studied yet.

Anabolic steroids are synthetic derivatives of testosterone widely used by both elite and amateur athletes [12, 13]. Bioconversion of androgens is mainly performed by the liver and yields a series of active metabolites which may mediate the effects of these hormones [14]. For non-clinical purposes, anabolic steroids are mostly used because they are thought to enhance athletic performance and body mass [12], although these effects seem to be achieved only when administration of anabolizing androgens is associated with intensive physical training [15, 16]. However, a number of deleterious effects have been reported in both patients and athletes subjected to anabolic steroid treatment, including a marked decrease in high-density lipoprotein levels, testicular atrophy and severe liver damage [14, 17].

Due to the increasing utilization of anabolizing androgens in endurance exercise, the aim of the present report was the study of the effects of anabolic steroid treatment on the mitochondrial outer carnitine palmitoyltransferase in different tissues of both sedentary and trained rats.

Materials and Methods

Male Wistar rats (110–120 g initial body wt) were subjected to treatment with anabolic steroids and/or to physical training on a motor-driven treadmill. After 4 weeks of adaptation to physical training, animals were running continuously for 45 min at 25 m/min during 8 weeks, 5 days per week [18]. The anabolic steroids fluoxymesterone (11β, 17β-dihydroxy-9α-fluoro-17α-methyl-4-androsten-3-one) or methylandrostanolone (17β-hydroxy-17α-methyl-5α-androstan-3-one) were given orally in aqueous suspension, in a dose of 2 mg/kg body weight, during the last 8 weeks of the experimental period, 5 days per week. Neither food intake nor body weights were affected by anabolic steroid treatment, whereas physical training slightly reduced body weight. Animals were not exercised for 36 hr before the day of the experiment. Rats were killed by decapitation and their livers, hearts and hind-limb skeletal muscles were excised. Both soleus (slow-twitch fibers) and extensor digitorum longus (fast-twitch fibers) were removed from the rear legs. Liver and heart mitochondria were isolated as described in Ref. 8, whereas mitochondria from skeletal muscle were obtained according to Ref. 19. The mitochondrial pellet was resuspended in 0.3 M sucrose, 10 mM Tris–HCl (pH 7.4) and 1 mM EDTA. Mitochondrial preparations were practically devoid of peroxisomes, as judged by the recovery of catalase activity (results not shown).

The outer carnitine palmitoyltransferase was assayed essentially as described before [10]. Each assay contained, in a total volume of 1 mL, 150 mM sucrose, 25 mM Tris–HCl (pH 7.4), 60 mM KCl, 1 mM EDTA, 1 mM dithioerythritol, 50 μM palmitoyl-CoA, 1.5 mg of bovine serum albumin (defatted and dialysed) and 0.5 mM L-[methyl-^{3}H]carnitine (1 Ci/mol). [^{3}H]Acylcarnitine product was extracted with *n*-butanol [8]. Carnitine palmitoyltransferase activity which was insensitive to 100 μM malonyl-CoA, representing the latent form of carnitine palmitoyltransferase, was always discounted from the enzyme activity experimentally measured. This malonyl-CoA-insensitive carnitine palmitoyltransferase routinely accounted for 5–10% of the total enzyme activity experimentally determined.

Results shown represent the means ± SD of five to six animals of each group, with incubations carried out in triplicate. Statistical comparison was performed using a two-way analysis of variance. A *post hoc* analysis was made by the Student–Neuman–Keuls test.

Results and Discussion

The effects of anabolic steroid administration on the activity of the mitochondrial outer carnitine palmitoyltransferase from different rat tissues were studied in sedentary as well as in physically-exercised rats. Treatment with fluoxymesterone or methylandrostanolone markedly increased the activity of the mitochondrial outer carnitine palmitoyltransferase in extensor digitorum longus and liver, whereas no effect was observed in heart and soleus, both in sedentary and trained animals (Table 1).

It is important to point out that the effects of anabolic steroids on the enzyme from extensor digitorum longus were not potentiated by endurance physical exercise, despite the stimulatory effect of physical training on the activity of the mitochondrial outer carnitine palmitoyltransferase in this muscle (Table 1). However, although physical exercise enhances enzyme activity in cardiac, slow-twitch and fast-twitch muscle fibers (Table 1 and Ref. 18), anabolic steroids seem to exert their effects selectively upon fast-twitch muscle. Similarly, anabolic steroid administration to rats enhances succinate dehydrogenase activity in mitochondria from fast-twitch muscle but not from slow-twitch muscle [19]. This is in line with the higher sensitivity of fast-twitch fibers to the catabolic action of corticosteroid hormones [20–22].

The increase in the activity of the outer carnitine palmitoyltransferase in liver mitochondria induced by anabolic steroids (Table 1) contrasts with the oestrogen-mediated decrease of enzyme activity reported by Weinstein *et al.* [9]. These results are in agreement with the observation that isolated perfused livers from male rats oxidize more fatty acids and esterify less fatty acids into triacylglycerols as compared with livers from female rats [23]. Nevertheless, although oestrogens also enhance the sensitivity of the mitochondrial outer carnitine palmitoyltransferase to inhibition by malonyl-CoA [9], treatment with anabolic steroids did not affect enzyme sensitivity to this inhibitor (Table 2). It is noteworthy that adaptive changes in both the activity and the response to malonyl-CoA of the mitochondrial outer carnitine palmitoyltransferase under different physio-pathological situations, such as starvation, diabetes and hypo- and hyperthyroidism, seem to be

Table 1. Effects of anabolic steroids on the activity of the mitochondrial outer carnitine palmitoyltransferase in rat heart, skeletal muscle and liver

Tissue	Training	Outer carnitine palmitoyltransferase activity (nmol/min/mg protein)		
		No hormone	Fluoxymesterone	Methylandrostanolone
Heart	No	9.66 ± 1.76	10.89 ± 1.81	9.67 ± 1.91
	Yes	16.71 ± 1.02*	17.45 ± 1.28*	15.47 ± 1.28*
Soleus	No	8.69 ± 0.81	8.88 ± 0.75	9.30 ± 0.68
	Yes	15.03 ± 1.90*	14.01 ± 2.11*	15.62 ± 1.58*
Extensor	No	5.36 ± 0.59	7.13 ± 1.21†	7.50 ± 0.33†
	Yes	7.92 ± 0.81*	8.86 ± 0.73	8.12 ± 0.75
Liver	No	4.71 ± 0.70	7.35 ± 1.28‡	8.40 ± 1.64‡
	Yes	4.94 ± 1.03	7.31 ± 0.19§	7.27 ± 0.90§

The outer carnitine palmitoyltransferase was assayed as described in Materials and Methods.
* $P < 0.01$ vs the corresponding sedentary group.
† $P < 0.05$ vs sedentary group not treated with steroids.
‡ $P < 0.01$ vs sedentary group not treated with steroids.
§ $P < 0.01$ vs trained group not treated with steroids.

Table 2. Effects of anabolic steroids on the sensitivity of the outer carnitine palmitoyltransferase to inhibition by malonyl-CoA in rat liver mitochondria

Anabolic steroid	Training	IC_{50} (μM)
No	No	8.0 ± 0.7
	Yes	7.8 ± 1.0
Fluoxymesterone	No	8.5 ± 2.7
	Yes	8.3 ± 1.4
Methylandrostanolone	No	7.2 ± 1.4
	Yes	6.8 ± 1.9

The outer carnitine palmitoyltransferase was assayed as described in Materials and Methods. The values of IC_{50} (concentration of malonyl-CoA causing 50% of the maximal inhibition of the outer carnitine palmitoyltransferase activity) were directly calculated from the plots of activity vs logarithm of malonyl-CoA concentration. These were corrected for malonyl-CoA-insensitive carnitine palmitoyltransferase activity. The concentrations of malonyl-CoA used to calculate the values of IC_{50} were 0, 0.5, 1.0, 2.0, 5.0, 8.0, 10, 20 and 100 μM.

confined to the liver enzyme [2, 8]. In addition, under these situations alterations in the kinetic and regulatory properties of the liver enzyme occur in concert, i.e. increases in the activity of the outer carnitine palmitoyltransferase are accompanied by decreases in enzyme sensitivity to inhibition by malonyl-CoA, and vice versa [5–10]. Hence the effects of anabolic steroids on the mitochondrial outer carnitine palmitoyltransferase described herein are of special interest since (i) they are not exclusive of the liver enzyme and (ii) only the activity of the enzyme in liver mitochondria is affected and not its sensitivity to malonyl-CoA. Changes in the activity of extrahepatic outer carnitine palmitoyltransferase also occur upon endurance exercise (Table 1 and Ref. 18), whereas changes in the activity of the hepatic outer carnitine palmitoyltransferase without variations in enzyme sensitivity have also been observed after feeding of lovastatin to rats [24]. It is thus possible that the mechanism of anabolizing androgen action on the control of the enzyme is different from that (those) exerted by diet and other hormones. The possibility that the effects of anabolic steroids are mediated by a metabolite arising from their bioconversion by target tissues [14] remains to be tested.

In summary, treatment of male rats with the anabolic steroids fluoxymesterone or methylandrostanolone increased the activity of the outer carnitine palmitoyltransferase in liver and fast-twitch muscle mitochondria. This effect was not potentiated by physical exercise and was not observed in heart and slow-twitch muscle mitochondria. Anabolic steroids did not affect the sensitivity of the liver enzyme to inhibition by malonyl-CoA. The data presented herein suggest that androgens may have an important physiological role in the regulation of fatty acid oxidation in liver and fast-twitch muscle mitochondria. In addition, our results are at odds with the notion that (most of) the metabolic effects of anabolic steroids on muscle are only evident when physical training is parallely performed [15, 16].

Acknowledgements—This study was supported by grants from the Consejo Superior de Deportes and the Universidad Complutense de Madrid, Spain.

Department of Biochemistry and Molecular Biology
Faculty of Chemistry
Complutense University
28040 Madrid, Spain

MANUEL GUZMÁN
ANA SABORIDO
JOSÉ CASTRO*
FULGENCIO MOLANO
ALICIA MEGIAS

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* To whom correspondence should be addressed.

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Biochemical Pharmacology, Vol. 41, No. 5, pp. 835–838, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

The diacylglycerol kinase inhibitor, R59949, potentiates secretion but not increased phosphorylation of a 47 kDalton protein in human platelets

(*Received* 2 *August* 1990; *accepted* 22 *October* 1990)

Receptor-stimulated hydrolysis of inositol phospholipids is now established as a major transmembrane signalling pathway generating two second messengers, inositol 1,4,5-trisphosphate (IP3), which releases intracellular Ca^{2+}, and 1,2-diacylglycerol (DG), which activates protein kinase C [1]. Activation of this pathway in human platelets e.g. following stimulation of thrombin, platelet activating factor, vasopressin or collagen receptors, leads to secretion of the contents of intracellular storage granules including 5-hydroxytryptamine (5-HT) and ATP [2, 3].

Nishizuka and others have shown that phorbol esters, which are potent activators of protein kinase C, produce a slow secretion of ATP or 5-HT in human platelets but that this response is markedly potentiated in the presence of raised intracellular Ca^{2+} [4, 5]. This suggests that receptor-stimulated phosphoinositide hydrolysis induces secretion by a synergistic interaction between PKC and Ca^{2+}. However, the relative importance of the role of PKC in secretion is uncertain since the activation of PKC is also associated with inhibition of certain platelet responses e.g. inhibition of phospholipase C [6] or stimulation of Ca^{2+} extrusion [7].

Recently, de Chaffoy de Courcelles *et al.* [8] have described a novel inhibitor of DG-kinase R59949. They have shown that R59949 inhibits DG-kinase in human platelets and potentiates 5-HT secretion induced by vasopressin. In the present study we have used this agent to investigate the role of PKC during 5-HT secretion induced by thrombin in human platelets and show that R59949 partially inhibits DG-kinase and potentiates secretion but that this is not associated with increased phosphorylation of a 47 kDa protein (identified as a protein of relative molecular mass 40–47 kDa and the major substrate for PKC [13]).

Methods

Platelets were obtained either from aspirin-free volunteers or from platelet concentrates, prepared from blood donated within the previous 24 hr to the Blood Transfusion Unit, John Radcliffe Hospital. Platelet-rich plasma was