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INTRACELLULAR REDISTRIBUTION OF SCP_2 IN LEYDIG CELLS AFTER HORMONAL STIMULATION MAY CONTRIBUTE TO INCREASED PREGNENOLONE PRODUCTION

Marjolein van Noort, Focko F.G. Rommerts, Aart van Amerongen[1] and Karel W.A. Wirtz[1]

Department of Biochemistry (Division of Chemical Endocrinology), Medical Faculty, Erasmus University Rotterdam, PO Box 1738, 3000 DR Rotterdam and

[1]Department of Biochemistry, State University Utrecht, PO Box 80054, 3508 TB Utrecht, The Netherlands

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Sterol carrier protein$_2$ (SCP_2) also designated non specific lipid transfer protein (nsL-TP), added to tumour Leydig cell mitochondria as a pure compound or in cytosolic preparations, stimulates pregnenolone production two- to three-fold. This stimulation can be abolished by addition of anti rat SCP_2 but not by preimmune IgG-antibodies. SCP_2- levels in the cytosol are increased in less than two minutes after addition of lutropin (LH). This increased SCP_2 level may contribute to stimulation of steroid production in intact cells. After hormonal stimulation the subcellular distribution of SCP_2 changes. A two-fold increase of SCP_2- levels in the supernatant fraction and four-fold decrease in extracts of the particulate fraction was observed 30 min after stimulation of tumour Leydig cells with LH and subsequent fractionation. This apparent shift of SCP_2 can be explained by an altered association with membranes or a true relocation of the protein from the particulate to the supernatant fractions under the influence of the hormone.

Sterol carrier protein$_2$ (SCP_2), also designated non specific lipid transfer protein (nsL-TP), accelerates the transfer of both phospholipid and cholesterol between membranes [1-3]. Addition of SCP_2 to isolated mitochondria of adrenocortical cells results in an enhanced conversion of cholesterol into pregnenolone [4]. In the rat testis SCP_2 is specifically localized in the steroidogenic active Leydig cells [5]. The amount of SCP_2 in soluble fractions of Leydig cells and in adrenocortical cells is regulated by hormones [5-7]. This suggests that SCP_2 is one of the factors involved in the hormonal control of steroidogenesis. After addition of LH to rat tumour Leydig cells the level of SCP_2 in the cytosol is increased two-fold within 2 minutes and this 2-fold higher level is maintained for at least 24 hours (6). This rapid increase in the level of SCP_2 in the supernatant could result from a hormone dependent post-translational processing of a precursor for SCP_2 [7-9]. However maintenance of a constant 2-fold higher SCP_2 level for 24 hours after the very rapid increase while the reported half life of SCP_2 is 32 hours [7], strongly

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indicates that other processes are involved. To investigate if SCP_2 is functionally active in Leydig cells and how the intracellular level may be regulated by hormones , we have determined whether SCP_2 can stimulate steroid production in isolated mitochondria and how SCP_2 is distributed between the soluble and the particulate fraction.

MATERIALS AND METHODS

Leydig cells from the Leydig cell tumour H540 were isolated as described previously [10]. After isolation cells were incubated for 30 minutes with or without lutropin (LH,1000 ng/ml) in a shaking waterbath at 37°C. in modified Eagle's medium with Earle's salts and non-essential amino acids containing 100 µg streptomycin/ml, 0.6 µg fungizone/ml and 100 IU penicillin/ml. Subcellular fractions were prepared and treated as described previously [11]. In this procedure fractions are extracted with high salt to remove SCP_2 from membranes. The amount of SCP_2 released is measured using an enzyme immunoassay (EIA) [11,12]. Protein was determined according to Bradford [13].

For isolation of mitochondria 150×10^6 tumour Leydig cells were suspended in 7 ml buffer containing 10 mM Tris-Cl pH 7.4, 1 mM EDTA and 250 mM D-mannitol [14,15] and homogenized using a Dounce-Wheaton glass homogenizer (clearance 0.025-0.03 mm, 10 strokes). After differential centrifugation mitochondria were suspended in buffer containing 125 mM sucrose, 25 mM Tris-Cl (pH 7.4), 5 mM $MgCl_2$, 60 mM KCl to a final concentration of 1 mg protein/ml.

Incubation was started by addition of 100 µl of the mitochondrial suspension to 400 µl buffer at 37°C, containing 5 mM DL-isocitrate. Incubation was stopped after 10 minutes by cooling on ice and by addition of 2 volumes ethylacetate (p.a.). Pregnenolone production was determined as described previously [16]. Where indicated cytosolic preparations were preincubated with anti rat SCP_2 (50 µg) [17] or preimmune IgG antibody (150 µg) in a total volume of 200 µl for 18 h at 4°C. The mixtures were centrifuged at 10.000xg for 20 min and aliquots of the supernatant were used for experiments.

RESULTS

The effect of SCP_2, pure or in cytosolic preparations, on pregnenolone synthesis by mitochondria isolated from tumour Leydig cells was investigated first. Addition of SCP_2 (0.7 µM) to a suspension of mitochondria from tumour Leydig cells resulted in a 2- fold stimulation of pregnenolone production from endogenous cholesterol (Table 1). The steroidogenic activity increased 1.5- to 3- fold depending on SCP_2-concentrations between 10^{-7} M and 10^{-5} M (data not shown). The stimulatory effect of SCP_2 was neutralized by pretreatment with anti rat SCP_2 IgG (anti-SCP_2). The addition of this antibody to the mitochondria also inhibited pregnenolone production within the incubation period of 10 minutes. Addition of cytosolic preparations from Leydig tumour cells incubated with LH also stimulated the pregnenolone production 2-fold. This stimulatory effect was abolished by pretreatment with anti-SCP_2 whereas pretreatment with rat serum IgG (IgG) had no effect.

Hormonal regulation of intracellular SCP_2 levels was studied in intact rat tumour (H540) Leydig cells [18]. After incubation for 30 minutes, 1000 ng/ml LH stimulated pregnenolone production approximately 3-fold (basal: 90 ± 13

Table 1. Steroid production by isolated mitochondria

Additions to mitochondria	pmol pregnenolone/ 100 µg protein/min
none	22.4 ± 2.7
cytosol	36.8 ± 1.4
cytosol + IgG	38.4 ± 1.4
cytosol + anti-SCP_2	21.6 ± 1.1
SCP_2	39.6 ± 1.6
SCP_2 + IgG	40.3 ± 2.3
SCP_2 + anti-SCP_2	12.8 ± 0.8
IgG	20.3 ± 1.6
anti-SCP_2	15.0 ± 2.1

Results are mean ± SD (6) of 3 different mitochondrial preparations. For further details see Methods.

pmol/10^6 cells/30 min; stimulated: 275 ± 43 pmol/10^6 cells/30 min; mean ± SD, n=4). The subcellular distribution of SCP_2 in rat tumour Leydig cells was studied after fractionation of homogenates of cells by differential centrifugation in a 105,000xg particulate membranous fraction (P) and a membrane-free supernatant (S) fraction. After exposure of cells to LH the amount of SCP_2 was decreased 4-fold in extracts of the particulate fraction (P) and increased 2-fold in the membrane-free supernatant fractions (S) (Fig.1). After incubation of cells the SCP_2 levels in the cytosol from control cells : 238 ± 51 ng/mg 105,000xg protein and from cells incubated with LH : 406 ± 41 ng/mg 105,000xg protein (mean ± SD, n=4), were similar to those earlier reported by us [5,6]. The total cellular amount of protein and SCP_2 in extracts were not affected by hormonal stimulation.

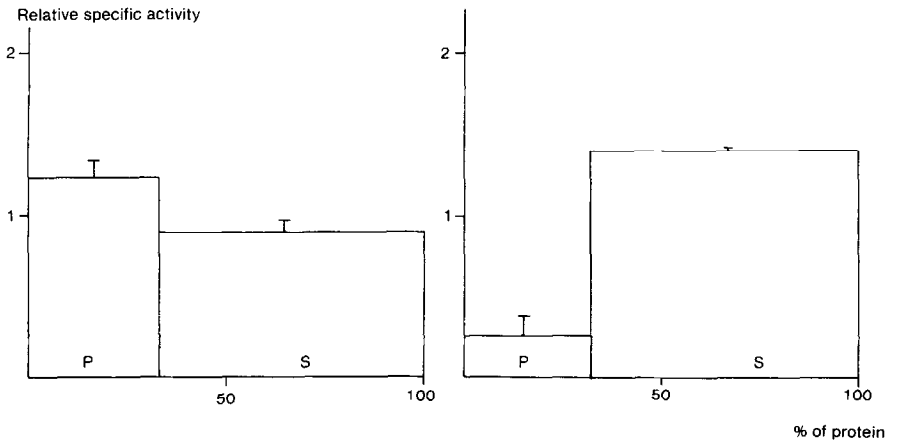

Figure 1
Effect of LH on the distribution of SCP_2 between the 105,000xg particulate (P) and supernatant (S) fractions of isolated rat tumour cells. Cells were incubated for 30 min without LH (left panel) or with 1000 ng/ml LH (right panel). The total amount of SCP_2 was not changed after hormonal stimulation.

DISCUSSION

Sterol carrier protein$_2$ (SCP_2) also designated nonspecific lipid transfer protein (nsL-TP), has been purified from rat, bovine and human liver [1,9,19-22]. In vitro the protein stimulates the transfer and biosynthesis of cholesterol [1,23] and accelerates the transfer of both phospholipid and cholesterol between membranes.

When added to mitochondria from adrenocortical cells [24] and tumour Leydig cells (Table 1), SCP_2 enhanced the conversion of cholesterol into pregnenolone. Antibodies to SCP_2 blocked the stimulatory effect of both pure rat liver SCP_2 and SCP_2 in cytosolic preparations. Anti-SCP_2 could even lower the basal production of pregnenolone in isolated mitochondria from Leydig cells (Table 1), presumably by binding of the antibody to SCP_2 located at the outer mitochondrial membrane. The basal production of pregnenolone production was not lowered when an antibody to SCP_2 was added together with cytosolic preparations. This may be caused by the presence of other stimulatory proteins or peptides in the cytosolic preparations. A 2kDa peptide, sterol activator protein (SAP), has been isolated and this peptide can stimulate steroid production in isolated mitochondria from Leydig-and adrenocortical cells [25,26].

The rapid increase in SCP_2-levels in the cytosol after exposure of cells to LH may contribute to the stimulation of steroid production in intact cells because in isolated mitochondria, pregnenolone production can be regulated by SCP_2. Also, fusion of adrenocortical cells with liposomes containing anti-SCP_2 antibody reduced ACTH stimulated steroidogenesis [27]. It is unlikely that SCP_2 is the labile intracellular protein postulated to mediate hormone action [18,28] since the half life of this protein in adrenal cells is 32 hours [7]. The half life in Leydig cells seems to be high also since pretreatment of cells with cycloheximide for 18 hours did not significantly lower the amount of SCP_2 detected in the supernatant fraction of the cells [6].

In addition, it seems unlikely that the rapid 2-fold increase in the level of soluble SCP_2 between 1 and 2 min and the maintenance of this level for at least 24 hours results from de novo synthesis or precursor proteolysis [6]. Since the half time of SCP_2 is 32 hours [7] this rapid increase can only be explained by assuming that 200 ng SCP_2/mg protein/min is produced within 2 min by fast processing of a precursor, immediately followed by a 1000-fold decrease in the production rate. Our data on SCP_2-levels in fractions of tumour Leydig cells after incubation with LH show that the rise of SCP_2 in supernatant fractions can better be explained by a relocation of extractable (loosly bound) protein from the particulate to the supernatant fractions (Fig. 1).

It is not certain if hormonal stimulation results in true redistribution of SCP_2 or in diminished binding of the protein to membranes to such extent that

it can be released during homogenization. Whatever explanation is correct, the data show that LH can modify the interaction between SCP_2 and membranes.

SCP_2 bound to rat liver mitochondria could be removed by washing with a high ionic strength buffer [12]. This suggests that the interaction between SCP_2 and membranes is of an electrostatic nature. We have observed earlier that calcium-ions are important for increasing SCP_2-levels in Leydig cell supernatant fractions [6]. Rapid changes in the cellular Ca^{2+} concentrations that occur in Leydig cells after incubation with LH [29] could play an important role in regulating the interaction between SCP_2 and membranes.

Hormone effects on subcellular redistribution of other proteins also have been demonstrated. For instance phorbol esters cause redistribution of protein kinase C [30,31] and it has been shown recently that translocation of this protein is coupled with activation [31]. Hormones may thus influence the subcellular redistribution of various proteins or aggregates of proteins such as multi-enzyme complexes. More attention should be paid to this "topodynamic regulation" of proteins when studying mechanisms for regulation of cell function [32].

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