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Lutropin-dependent protein phosphorylation and steroidogenesis in rat tumour Leydig cells

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Tumour Leydig cells have been incubated in the presence or absence of lutropin (luteinizing hormone, 'LH'). Stimulation of cells with lutropin (1000 ng/ml) in the presence of 1-methyl-3-isobutylxanthine (0.25 mM) resulted in increased steroid production and increased protein phosphorylation. When pregnenolone metabolism was inhibited, basal pregnenolone production was 26.9 ± 7.4 ng/60 min per 10^6 cells; stimulated production was 156.1 ± 39.5 ng/60 min per 10^6 cells (means ± S.D., $n = 4$). Lutropin-dependent phosphorylated proteins of molecular mass 17000, 22000, 24000, 33000 and 57000 Da were detected. A significant increase of $[^{32}P]P_i$ incorporation into these phosphorylated proteins was observed concomitant with the increased pregnenolone production. The occurrence of the phosphoproteins in nuclei, mitochondria and postmitochondrial-supernatant was investigated. The 17000 Da phosphoprotein was found in the nuclear fraction, whereas the 22000, 24000, 33000 and 57000 Da phosphoproteins were localized in the postmitochondrial-supernatant fraction. Of the cholesterol-side-chain-cleavage activity, $80.3 \pm 6.1\%$ (mean ± S.D., $n = 5$) was present in the mitochondrial fraction isolated from tumour Leydig cells, and this activity was 2.5-fold increased when cells had been preincubated with lutropin/1-methyl-3-isobutylxanthine (basal production: 194.6 ± 28.6 ng/30 min per mg of protein; lutropin-stimulated production: 498.8 ± 91.5 ng/30 min per mg of protein; means ± S.D., $n = 3$). The similarities in the kinetics of the phosphorylation of proteins and the pregnenolone production after addition of lutropin/1-methyl-3-isobutylxanthine indicate that the phosphoproteins could be involved in the lutropin-dependent increase in steroidogenesis in tumour Leydig cells. It remains to be demonstrated, however, to what extent the phosphoproteins outside the mitochondria can influence the cholesterol-side-chain-cleavage activity inside the mitochondria.

The effects of lutropin (luteinizing hormone, 'LH') on testis Leydig cells involve an increase in the cyclic AMP concentration (Moyle & Ramachandran, 1973; Rommerts *et al.*, 1973; Podesta *et al.*, 1978), which is followed by activation of cyclic AMP-dependent protein kinase (Rubin & Rosen, 1975; Cooke *et al.*, 1976) and phosphorylation of proteins (Cooke *et al.*, 1977). It is unknown, however, to what extent phosphorylated proteins are involved in the lutropin-dependent increase in steroidogenesis. It has been shown that: (1) the rate-limiting step of steroidogenesis is the conversion of cholesterol into pregnenolone by the cholesterol-side-chain-cleavage-enzyme system inside mitochondria (Simpson, 1979); (2) Leydig-cell mitochondria, isolated after pretreatment of animals with human choriogonadotropin, show increased steroid production (Van der Vusse *et al.*, 1975); (3) cytosol fractions from lutropin-treated rat testes can stimulate the pregnenolone production by mitochondria from non-stimulated rat testes (Bakker *et al.*, 1978); (4) lutropin (100 ng/ml) causes phosphorylation of proteins in adult rat testis Leydig cells of 17000, 57000 and 76000 Da (Cooke *et al.*, 1977). Hence, it is possible that phosphoproteins are essential and could link the action of lutropin at the plasma membrane to the increased steroid production.

We have studied, in tumour Leydig cells, the

Abbreviation used: SDS, sodium dodecyl sulphate.

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correlation between the kinetics of phosphorylation of proteins and steroid production in the presence of lutropin/1-methyl-3-isobutylxanthine, as well as the subcellular localization of the phosphorylated proteins with particular reference to the mitochondria.

Materials and methods

Sheep lutropin (NIH-LH-S18; 1.03 i.u./mg) was a gift from the Endocrinology Study Section of the National Institute of Health, Bethesda, MD, U.S.A. 1-Methyl-3-isobutylxanthine was purchased from Aldrich Chemical Co., Milwaukee, WI, U.S.A. [^{32}P]Orthophosphate (carrier-free) was purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Cyanoketone (2α-cyano-17β-hydroxy-4,4',17α-trimethylandrost-5-en-3-one), an inhibitor of 3β-hydroxysteroid dehydrogenase activity, and SU-10603 [7-chloro-3,4-dihydro-2-(3-pyridyl)-naphthalen-1(2*H*)-one], an inhibitor of 17α-hydroxylase activity, were kindly donated by Dr. R. Neher from the Friedrich Miescher Institute, Basle, Switzerland. Crude collagenase was purchased from Worthington Biochemical Corp., Freehold, NJ, U.S.A.

The properties of the tumour Leydig cells were previously described (Cooke *et al.*, 1979*a*). Implantation of tumour tissue was performed by injecting small pieces of tumour subcutaneously into intact male Wistar rats, substrain R_1-Amsterdam, 14–17 weeks of age. Rats were killed 4–7 weeks after injecting the tumour tissue by decapitation after light diethyl ether anaesthesia, and cells were isolated as described previously (Cooke *et al.*, 1979*a*). Cells were incubated under an O_2/CO_2 (19:1) atmosphere in a shaking water-bath (80 cycles/min) at 32°C. Lutropin and 1-methyl-3-isobutylxanthine were added in a concentrated solution and final concentrations were 1000 ng/ml and 0.25 mM respectively.

Kinetic studies of phosphorylation of proteins and pregnenolone production were performed by addition of lutropin/1-methyl-3-isobutylxanthine at different time intervals before the end of the incubation period. For studies of phosphorylation, cells were preincubated for 1 h in Krebs–Ringer buffer (118.3 mM-NaCl/4.75 mM-KCl/25 mM-$NaHCO_3$/1.2 mM-$MgSO_4$/2.5 mM-$CaCl_2$), pH 7.3, without phosphate, containing 0.2% glucose and 0.1% albumin. Incubations were performed with 200 μl suspensions containing 2×10^6 cells without or with lutropin/1-methyl-3-isobutylxanthine. Labelling was started by addition of 50 μCi of [^{32}P]orthophosphate. Incorporation of label was stopped after 1 h by the addition of a cold (4°C) iso-osmotic medium containing 0.125 M-NaH_2PO_4, 82 mM-Tris and 20 mM-NaF, pH 7.3. Cells were centrifuged, resuspended in SDS-containing lysing buffer (0.1 M-glycine/0.1 M-NaCl/10 mM-EDTA/0.1% SDS/10 mM-β-mercaptoethanol/20 mM-NaF) and boiled for 3–5 min. After boiling, the proteins were precipitated by addition of acetone (4:1, v/v), washed with 70% (v/v) ethanol and diethyl ether, and finally dissolved in the SDS-containing sample medium [50 mM-Tris/HCl/10% glycerol (pH 6.8), containing 2% SDS and 1% β-mercaptoethanol]. The amount of protein-bound [^{32}P]orthophosphate was determined after trichloroacetic acid precipitation of a portion of the protein samples on Whatman filters (3 MM Chroma). Precipitates on filters were washed with 70% ethanol and ether, followed by counting of ^{32}P radioactivity on dry filters using methoxytoluene as scintillation fluid. Electrophoresis was performed with SDS/8–15%-polyacrylamide gradient slab gels essentially as described by Laemmli (1970), with a Bio-Rad model 220 dual vertical slab-gel-electrophoresis cell (100 mm × 140 mm × 1.5 mm gels). After electrophoresis the gels were fixed in methanol/water/acetic acid (5:4:1, by vol.) for at least 1 h. Staining of gels was complete in 30–45 min with PAGE Blue 83 (1 g/litre; BDH, Poole, Dorset, U.K.) in ethanol/water/acetic acid (5:11:4m by vol.), containing 1 g of $CuSO_4$/litre. Destaining was achieved by a single wash with methanol/water/acetic acid (30:63:7, by vol.). The gels were dried on a Bio-Rad model-224 gel-slab dryer under continuous heating at 70–80°C.

After being dried, the gels were exposed to Kodak X-ray film SB-5. The autoradiograms obtained were scanned with a Gilford model-2400 spectrophotometer. The correlation between the amount of radioactivity in the gel and the densitogram of the exposed X-ray film was evaluated by using autoradiograms of known increasing amounts of radioactivity. The molecular weights of the proteins were calculated by comparison with the mobilities of the standard proteins present in the Bio-Rad low-molecular-weight-standard mixture.

For studies of pregnenolone production, cells were preincubated for 1 h in Krebs–Ringer buffer, pH 7.3, with 0.2% glucose and 0.1% albumin. Incubations were for 1 h in 500 μl suspensions containing 1×10^6 cells without or with lutropin and 1-methyl-3-isobutylxanthine, in the presence of cyanoketone and SU-10603 at final concentrations of 5 μM and 19 μM respectively. Inhibitors of pregnenolone metabolism and lutropin/1-methyl-3-isobutylxanthine were added simultaneously. Incubations were stopped by the addition 2×2 ml of ethyl acetate, and production of pregnenolone was determined by using a radioimmunoassay for pregnenolone (Van der Vusse *et al.*, 1975).

For studies on the subcellular localization of phosphorylated proteins and cholesterol-side-chain-cleavage activity, cells were preincubated for 1 h and incubated for 1 h (phosphorylation of proteins) or ½h (pregnenolone production) without or with

lutropin/1-methyl-3-isobutylxanthine. Labelled phosphate was present during the whole 60 min incubation period.

Homogenization of (10–15) $\times 10^6$ cells in 1 ml of 0.25 M-sucrose/1 mM-EDTA, pH 7.3, with or without 20 mM-NaF for labelling and pregnenolone incubations respectively, was performed with a Dounce glass homogenizer (clearance 0.025–0.03 mm; ten strokes). Differential centrifugation resulted in isolation of three fractions: nuclei (10 min at 500 *g*), mitochondria (10 min at 15000 *g*) and postmitochondrial-supernatant (15000 *g* supernatant). Nuclear pellets were washed twice with homogenization medium. These fractions were characterized by determination of the DNA content and the cholesterol-side-chain cleavage, monoamine oxidase and lactate dehydrogenase activities in each fraction as described previously (Van der Vusse *et al.*, 1974).

Pregnenolone production was determined by incubation of subcellular fractions (0.015–0.300 mg of protein/500 μl) for 30 min in the presence of inhibitors (Van der Vusse *et al.*, 1974), but without calcium in the incubation medium. Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. Phosphorylated proteins were collected in the same way as described above for cellular proteins, except that postmitochondrial-supernatant proteins were precipitated with trichloroacetic acid [final concn. 10% (w/v)] before addition of SDS-containing sample medium. The subcellular localization of the lutropin-dependent phosphoproteins was determined by comparison of the distributions of marker enzymes and lutropin-dependent phosphoproteins. For this purpose the relative specific activities of both marker enzymes and phosphoproteins were calculated in the three fractions. Calculations of relative specific activity for phosphoproteins were based on densitogram tracings of autoradiograms obtained after electrophoresis, in two ways: (1) equal amounts of the protein-bound radioactivity in the three fractions were applied to the gel; (2) equal portions (e.g. one-third) of the total fractions were applied to the gel. Densitograms were obtained by scanning of the various slots taken from the autoradiograms. A blank section of the film was used to set the baseline and the peak heights of the lutropin-dependent phosphoproteins were measured in the different fractions. The relative-specific-activity values were calculated by dividing the percentage of a lutropin-dependent phosphoprotein (based on peak height) in a fraction by the percentage of protein in that fraction. When necessary, corrections were made for the portion of the fraction that was used for electrophoresis.

The two methods for determination of the relative-specific-activity values ($n = 3$, equal amounts of protein-bound radioactivity; $n = 2$, equal portions; n = number of different cell preparations) resulted in similar distributions of the phosphoproteins. The total amounts of protein-bound radioactivity in the various fractions were determined to calculate relative-specific-activity values for the incorporation of [^{32}P]orthophosphate into proteins by dividing the percentage of protein-bound radioactivity in a fraction by the percentage of protein in that fraction.

Results

The pregnenolone production in tumour Leydig cells was stimulated within 5 min after the addition of lutropin/1-methyl-3-isobutylxanthine (Fig. 1). The kinetics of lutropin-dependent phosphorylation of tumour-Leydig-cell proteins can be derived from the results presented in Fig. 2. Addition of lutropin/1-methyl-3-isobutylxanthine resulted also within 5 min in phosphorylation of proteins of 17000, 22000, 24000, 33000 and 57000 Da. The intensity of the lutropin-dependent phosphoproteins and, for

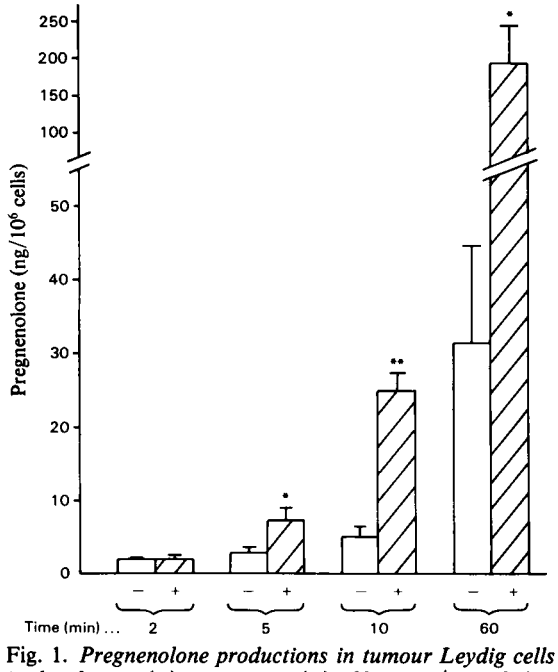

Fig. 1. *Pregnenolone productions in tumour Leydig cells in the absence (−) or presence (+) of lutropin/1-methyl-3-isobutylxanthine*

Incubations were performed as described in the text. Values are means ± S.D. for duplicate incubations of three different cell preparations. The significance of the lutropin-dependent increased pregnenolone production was determined by Student's paired *t* test with the corresponding incubations without lutropin/1-methyl-3-isobutylxanthine: $*P < 0.025$; $**P < 0.005$.

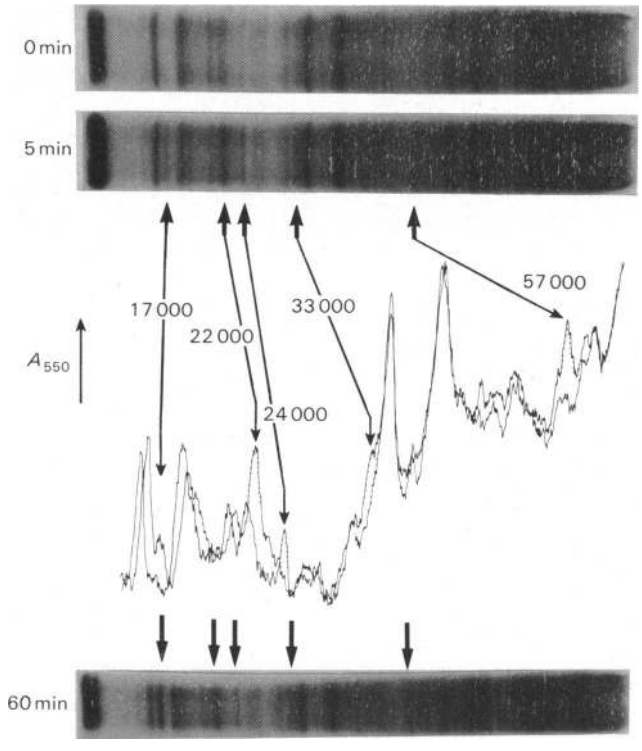

Fig. 2. *Kinetics of lutropin-dependent incorporation of [^{32}P]orthophosphate into proteins of tumour Leydig cells*
The Figure shows autoradiograms of [^{32}P]orthophosphate-labelled proteins isolated from tumour Leydig cells incubated for 0, 5 and 60 min with lutropin/1-methyl-3-isobutylxanthine (see the text) and separated by SDS/polyacrylamide-gel electrophoresis. Included above are densitograms obtained with the 0 and 5 min incubations. Arrows indicate lutropin-dependent phosphoproteins (numerical values are molecular masses in daltons).

Table 1. *Kinetics of lutropin-dependent incorporation of [^{32}P]orthophosphate into proteins of tumour Leydig cells*
Cells were incubated for 1 h in the presence of [^{32}P]orthophosphate, and lutropin/1-methyl-3-isobutylxanthine was added for the times indicated before the end of the incubation. Peak heights of the indicated proteins measured from the densitograms were expressed as the percentage of the peak height of a standard protein of molecular mass 40000 Da. Statistical analysis of the lutropin-dependent increments was done by Student's paired *t* test with the 0-min incubation. Results are means ± S.D. ($n = 3$–4); *$P < 0.05$; **$P < 0.01$.

Protein molecular mass (Da)	Incubation-time with lutropin/1-methyl-3-isobutylxanthine (min) ...	Incorporation (% peak height)			
		0	5	10	60
17000		14.3 ± 1.5	34.0 ± 5.2*	32.0 ± 8.0*	56.5 ± 8.7**
22000		37.3 ± 3.5	52.7 ± 1.5**	52.7 ± 4.7*	50.5 ± 4.0*
24000		17.2 ± 6.6	26.7 ± 4.9*	28.0 ± 9.0*	28.8 ± 6.2*
33000		47.0 ± 4.7	60.7 ± 7.6*	66.0 ± 10.4*	65.2 ± 7.9**
57000		72.5 ± 5.2	93.3 ± 5.0**	95.0 ± 3.6*	90.0 ± 5.9**
35000		89.0 ± 1.8	91.3 ± 3.2	90.3 ± 0.6	89.8 ± 2.2
50000		78.0 ± 5.3	82.7 ± 9.6	86.0 ± 9.2	82.8 ± 7.4
73000		106.3 ± 3.8	113.0 ± 3.6	112.7 ± 2.9	106.5 ± 6.4

comparison, of three other lutropin-independent phosphoproteins of 35000, 50000 and 73000 Da was expressed relative to a lutropin-independent phosphoprotein of 40000 Da (Table 1). The results showed that phosphorylation of the 22000, 24000, 33000 and 57000 Da proteins was almost maximal after 5 min of incubation with lutropin/1-methyl-3-isobutylxanthine, whereas phosphorylation of the

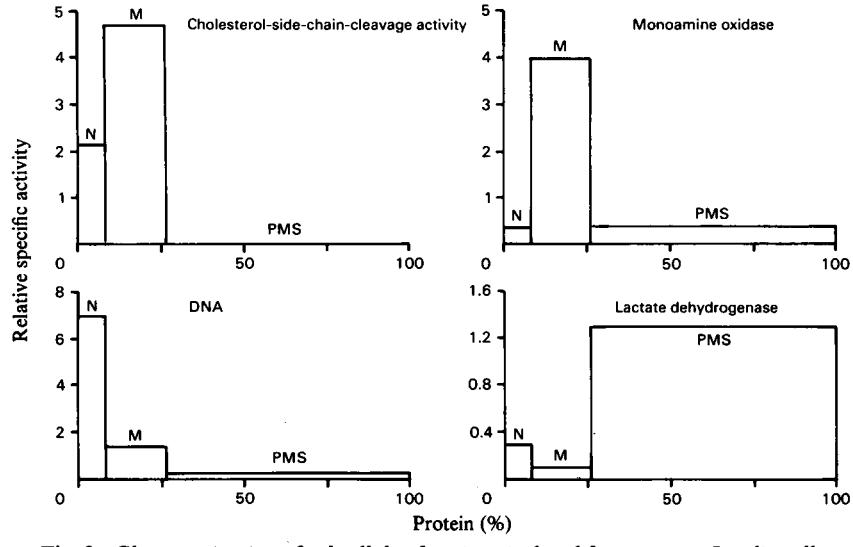

Fig. 3. *Characterization of subcellular fractions isolated from tumour Leydig cells*
Subcellular distribution of cholesterol-side-chain-cleavage activity, monoamine oxidase, DNA and lactate dehydrogenase is shown. Abscissae: the percentage of the total protein content in each fraction is presented as cumulative values. Ordinates: relative specific activity (percentage of total enzyme activity or amount of DNA per percentage of total protein content). Abbreviations used: N, nuclear fraction; M, mitochondrial fraction; PMS, post-mitochondrial-supernatant fraction. Results shown are mean values for three to five different cell preparations.

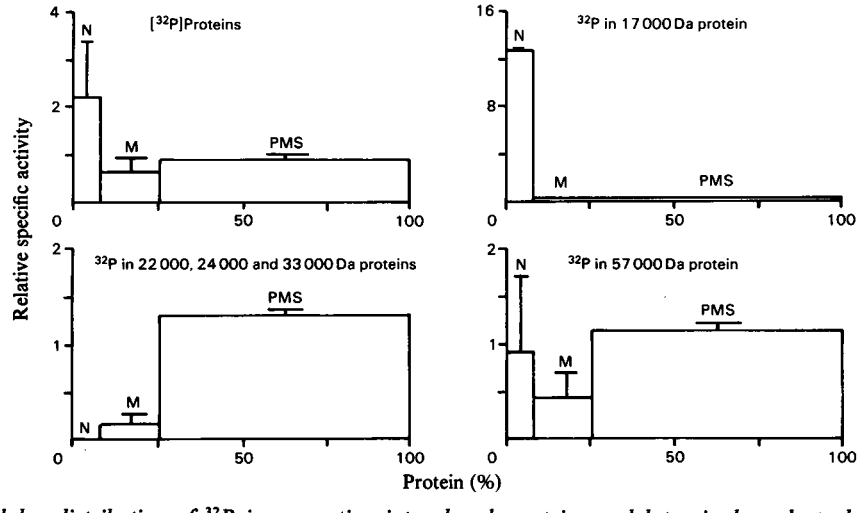

Fig. 4. *Subcellular distribution of ^{32}P incorporation into phosphoproteins and lutropin-dependent phosphoproteins in fractions of tumour Leydig cells*
Relative specific activities of the incorporation of [^{32}P]orthophosphate into proteins of subcellular fractions and relative-specific-activity values for the lutropin-dependent phosphoproteins, calculated as described in the text, are shown. Results are means ± S.D. for twelve (^{32}P incorporation) and three (phosphoproteins) different cell preparations. For details, see the Materials and methods section.

17000 Da protein increased during the whole 60 min incubation period. Finally, lutropin appeared to decrease the phosphorylation of a protein of 20000 Da.

The correlation between steroid production and phosphorylation of proteins was further investigated at the subcellular level. Three subcellular fractions were used: nuclei, mitochondria and postmito-

chondrial-supernatant. Particular attention was given to the mitochondrial fraction, because of the mitochondrial localization of the cholesterol-side-chain-cleavage activity (Simpson, 1979). The fractions were characterized by determining, in addition to the cholesterol-side-chain-cleavage activity, the DNA content and the activity of monoamine oxidase and lactate dehydrogenase.

The results showed that the nuclear fraction contained most of the DNA (55%) and that 96% of the lactate dehydrogenase activity was recovered in the postmitochondrial-supernatant fraction. Most of the activities of the mitochondrial marker enzymes (70–80%) were present in the mitochondrial fraction, but significant nuclear contamination and some postmitochondrial-supernatant activities were also present (Fig. 3).

Preincubation of cells with lutropin/1-methyl-3-isobutylxanthine for 30 min resulted in a 2.5-fold-increased pregnenolone production in the isolated mitochondria [194.6 ± 28.6 ng/30 min per mg of protein for the controls and 498.8 ± 91.5 ng/30 min per mg of protein for the mitochondria from lutropin/1-methyl-3-isobutylxanthine-stimulated cells (means $\pm$ S.D.; $n = 3$; $P < 0.01$)]. Relative specific activities were calculated for the incorporation of [^{32}P]orthophosphate into proteins of the fractions isolated (Fig. 4). In spite of the 4–5-fold purification of mitochondria in the mitochondrial fraction, the relative-specific-activity value of this fraction for ^{32}P incorporation into proteins was rather low [0.65 ± 0.35 (mean $\pm$ S.D., $n = 12$)].

The distribution of the separate lutropin-dependent phosphoproteins is also shown in Fig. 4. The 17000 Da protein could only be detected in the nuclear fraction, whereas the 22000, 24000, 33000 and 57000 Da proteins were concentrated in the postmitochondrial-supernatant fraction. A small amount of the 57000 Da protein was present in the nuclear and mitochondrial fraction. Qualitative analyses of the subcellular distribution of phosphoproteins showed that the phosphoproteins that had been concentrated in the nuclear and postmitochondrial-superantant fractions were present in the mitochondrial fraction (electrophoresis with equal amounts of protein-bound radioactivity) and that the mitochondrial fraction hardly contained phosphoproteins (electrophoresis with equal portions; Fig. 5).

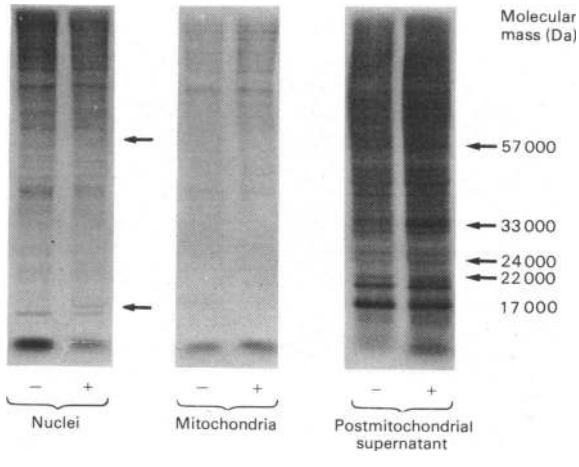

Fig. 5. *Subcellular distribution of phosphorylated proteins isolated from tumour Leydig cells incubated without* (−) *or with* (+) *lutropin/1-methyl-3-isobutylxanthine*

Autoradiograms of [^{32}P]orthophosphate-labelled proteins in subcellular fractions of tumour Leydig cells separated by SDS/polyacrylamide-gel electrophoresis are shown. Equal portions of the total amount of protein isolated in the fractions were applied.

Discussion

The aim of the present study was to investigate the kinetics of pregnenolone production and phosphorylation of proteins in tumour Leydig cells after addition of lutropin/1-methyl-3-isobutylxanthine. Both steroid production and phosphorylation of five proteins of 17000, 22000, 24000, 33000 and 57000 Da were significantly increased within 5 min after addition of lutropin/1-methyl-3-isobutylxanthine.

The possible role of the lutropin-dependent phosphoproteins was further investigated by determination of their subcellular localization. Characterization of subcellular fractions with marker enzymes showed that the distributions of the mitochondrial marker enzymes (cholesterol-side-chain-cleavage activity and monoamine oxidase) were similar and reflected an approx. 4-fold purification of mitochondria. Marker-enzyme activities for the nuclear and postmitochondrial-supernatant fractions were also present in the mitochondrial fraction. The incorporation of ^{32}P into proteins showed a low incorporation into proteins of the mitochondrial fraction. After electrophoresis of equal amounts of protein-bound radioactivity of subcellular fractions, the autoradiograms showed that the mitochondrial fraction contained mainly nuclear and postmitochondrial-supernatant phosphoproteins. Thus, in spite of the 4-fold purification of mitochondria, there appeared to be a low incorporation of [^{32}P]orthophosphate into proteins of mitochondria. Moreover, part of this activity originated from nuclear and postmitochondrial-supernatant contamination. On the basis of these observations it is concluded that activity of protein phosphorylation

in mitochondria is low. This conclusion is supported by the results in Fig. 5, and so far there have been reports on just a few mitochondrial proteins that become phosphorylated. Among the latter, succinyl-CoA synthetase and pyruvate dehydrogenase are the only phosphoproteins that have been identified (Weller, 1979). In fact, none of the lutropin-dependent phosphoproteins could be demonstrated in the mitochondrial fraction. The 17000Da phosphoprotein was found in the nuclei and the 22000, 24000, 33000 and 57000Da phosphoproteins were found in the postmitochondrial-supernatant fraction.

The lutropin-dependent phosphoprotein of 57000Da appears to be the regulatory subunit of the type-II cyclic AMP-dependent protein kinase (Cooke *et al.*, 1979*b*). A partially membrane-bound localization of this regulatory subunit (cf. Potter & Taylor, 1979) could explain the presence of this protein in all fractions isolated. Nothing is known about the nature of the phosphoproteins of 22000, 24000 and 33000Da, although there have been reports on cyclic AMP-binding proteins of similar molecular mass that may have originated from the 57000Da protein after proteolytic cleavage (Weber & Hilz, 1978, 1979; Wallace & Frazier, 1979; Jaynes *et al.*, 1980). The 17000Da protein could be involved in lutropin-induced protein synthesis, both in adult Leydig cells (Cooke *et al.*, 1977; Janszen *et al.*, 1977) and in tumour Leydig cells (Janszen *et al.*, 1978).

Hormone-dependent phosphorylation of proteins has also been reported for other steroidogenic cell types, such as rat adrenocortical cells (Podesta *et al.*, 1979), porcine granulosa cells (Halpren-Ruder *et al.*, 1980) and bovine luteal cells (Darbon *et al.*, 1980). In those studies a large number of phosphoproteins with molecular masses ranging from 43000 to 150000Da was observed in cytosol fractions, but none of these phosphoproteins were functionally characterized. More recently, increased phosphorylation of phosphoproteins of 22000, 24000, 54000 and 210000Da was demonstrated in rat adrenal quarters under the influence of corticotropin (Koroscil & Gallant, 1980). The 210000Da phosphoprotein was reported to be localized in the mitochondrial fraction, but the characterization of the mitochondrial fraction was not supported by the distribution of proper marker enzymes. The rather low incorporation of labelled phosphate into mitochondrial proteins demonstrated in the present study may rule out involvement of phosphoproteins with enzymic processes occurring inside mitochondria. Studies with reconstituted cholesterol-side-chain-cleavage enzymes of bovine corpus luteum (Caron *et al.*, 1975) and bovine adrenal (Defaye *et al.*, 1981) gave conflicting results with regard to phosphorylation of the enzyme. The mitochondrial membranes may even prevent the entry of phosphoproteins into mitochondria. However, recent observations on the possible importance of polyphosphoinositides for stimulation of cholesterol-side-chain-cleavage activity in the adrenal (Farese *et al.*, 1979, 1980; Farese & Sabir, 1980) may suggest some role for hormone-dependent phosphoproteins in the post-mitochondrial-supernatant or cytosol. These phosphoproteins might stimulate synthesis of (poly)-phosphoinositides at the endoplasmic reticulum or facilitate in some way their transport to the mitochondria, where increased amounts of mitochondrial phosphoinositides could be involved in stimulating steroid production. It remains to be demonstrated to what extent the postmitochondrial-supernatant proteins demonstrated in the present study with tumour Leydig cells are involved in such a process.

References

Bakker, C. P., van der Plank-van Winsen, M. P. I. & van der Molen, H. J. (1978) *Biochim. Biophys. Acta* **543**, 235–242

Caron, M. G., Goldstein, S., Savard, K. & Marsh, J. M. (1975) *J. Biol. Chem.* **250**, 5137–5143

Cooke, B. A., Lindh, L. M. & Janszen, F. H. A. (1976) *Biochem. J.* **160**, 439–446

Cooke, B. A., Lindh, L. M. & Janszen, F. H. A. (1977) *Biochem. J.* **168**, 43–48

Cooke, B. A., Lindh, L. M., Janszen, F. H. A., van Driel, M. J. A., Bakker, C. P., van der Plank, M. P. I. & van der Molen, H. J. (1979*a*) *Biochim. Biophys. Acta* **583**, 320–331

Cooke, B. A., Lindh, L. M. & van der Molen, H. J. (1979*b*) *J. Endocrinol.* **83**, 32P–33P

Darbon, J. M, Ursely, J., Mangue, N. & Leymarie, P. (1980) *FEBS Lett.* **113**, 120–124

Defaye, G., Monnier, N., Guidicelli, C. & Chambaz, E. M. (1981) *Adv. Cyclic Nucleotide Res.* in the press

Farese, R. V. & Sabir, A. M. (1980) *Endocrinology* **106**, 1869–1879

Farese, R. V., Sabir, A. M. & Vandor, S. L. (1979) *J. Biol. Chem.* **254**, 6842–6844

Farese, R. V., Sabir, A. M., Vandor, S. L. & Larson, R. E. (1980) *J. Biol. Chem.* **255**, 5728–5734

Halpren-Ruder, D. H., Jungmann, R. A., George, W. J. & Jeter, J. (1980) *J. Endocrinol.* **84**, 49–63

Janszen, F. H. A., Cooke, B. A. & van der Molen, H. J. (1977) *Biochem. J.* **162**, 341–346

Janszen, F. H. A., Cooke, B. A., van Driel, M. J. A. & van der Molen, H. J. (1978) *Biochem. J.* **172**, 147–153

Jaynes, P. K., McDonough, J. P. & Mahler, H. R. (1980) *Biochem. Biophys. Res. Commun.* **94**, 16–22

Koroscil, T. M. & Gallant, S. (1980) *J. Biol. Chem.* **255**, 6276–6283

Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685

Lowry O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Moyle, W. R. & Ramachandran, J. (1973) *Endocrinology* **93**, 127–134

Podesta, E. J., Dufau, M. L., Solano, A. R. & Catt, K. J. (1978) *J. Biol. Chem.* **253**, 8994–9001

Podesta, E. J., Milani, A., Steffen, H. & Neher, R. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5187–5191

Potter, R. L. & Taylor, S. S. (1979) *J. Biol. Chem.* **254**, 9000–9005
Rommerts, F. F. G., Cooke, B. A., van der Kemp, J. W. C. M. & van der Molen, H. J. (1973) *FEBS Lett.* **33**, 114–118
Rubin, C. S. & Rosen, O. M. (1975) *Annu. Rev. Biochem.* **44**, 831–887
Simpson, E. R. (1979) *Mol. Cell. Endocrinol.* **13**, 213–227
Van der Vusse, G. J., Kalkman, M. L. & van der Molen, H. J. (1974) *Biochim. Biophys. Acta* **348**, 404–414
Van der Vusse, G. J., Kalkman, M. L. & van der Molen, H. J. (1975) *Biochim. Biophys. Acta* **380**, 473–485
Wallace, L. J. & Frazier, W. A. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4250–4254
Weber, W. & Hilz, H. (1978) *Eur. J. Biochem.* **83**, 215–225
Weber, W. & Hilz, H. (1979) *Biochem. Biophys. Res. Commun.* **90**, 1073–1081
Weller, M. (1979) *Protein Phosphorylation*, 1st edn., pp. 306–311, Pion Limited, London