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Steroidogenic Fate of the Leydig Cells that Repopulate the Testes of Young and Aged Brown Norway Rats after Elimination of the Preexisting Leydig Cells

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Abstract

The capacity of Brown Norway rat Leydig cells to produce testosterone (T) decreases with aging. In a previous study, we reported that a new generation of Leydig cells can be restored in both young and old rat testes after a single injection of ethane dimethanesulfonate (EDS), and that the abilities of the new Leydig cells in young and old rats to produce T were equivalent. Our objective herein was to compare the steroidogenic fate of the new Leydig cells over time. Young (3 month-old) and old (18 month-old) rats were injected with EDS to eliminate the existing Leydig cells. Ten weeks after EDS, Leydig cells had been restored and T production by the new Leydig cells isolated from young and old rat testes was equivalent. Thirty weeks after EDS treatment of young rats, the ability of the new Leydig cells to produce T had not diminished from 10 weeks post-EDS. In contrast, at 30 weeks post-EDS, T production by new cells in old rat testes was reduced significantly from the 10-week level. Serum T levels at 10 and 30 weeks were consistent with Leydig cell T production. Serum LH levels did not differ in any group. Thus, although the Leydig cells restored to both young and old rats after EDS initially produced T at high, equivalent levels, the cells in the old testes did not maintain this ability. These results suggest that: 1) the cells from which new populations of Leydig cells are derived may differ depending upon the age of the rat; and/or 2) factors extrinsic to the new Leydig cells in young and old testes differ, and it is these differences that are responsible for reductions in T by the newly formed Leydig cells in the testes of old rats.

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Keywords

Leydig cells; aging; steroidogenesis; EDS

1. INTRODUCTION

Aging in men often is associated with decline in serum testosterone (T) levels (Harman et al, 2001; Mohr et al, 2005). In some men, age-related changes in the hypothalamic-pituitary axis result in reduced serum levels of luteinizing hormone (LH), the hormone that stimulates Leydig cell T formation, and thus in reduced serum T levels (Mulligan et al, 1997).

However, in many aging men, serum LH levels either do not change or are increased (Surampudi et al, 2012). Though this does not rule out the involvement of hypothalamic-pituitary deficits, reduced T levels in these men is not the result of reduced LH levels. Rather, studies have shown a reduced ability of Leydig cells to produce T in response to LH stimulation (Veldhuis et al, 2005; 2012). As in men, serum T levels decrease with age in Brown Norway rats, with unchanged or increased LH levels (Gruenewald et al., 1994; Chen et al, 1994, 1996) and a reduced capacity of the Leydig cells to produce testosterone (T) in response to LH (Chen et al., 1994, 2002). Consistent with this, the in vivo administration of exogenous LH to old Brown Norway rats failed to increase T production by the testes to the significantly higher levels produced by young LH-stimulated testes (Grzywacz et al., 1998).

Numerous studies have shown that treatment of young adult rats with the alkylating agent ethane dimethanesulfonate (EDS) eliminates all Leydig cells from the testes, and that new Leydig cells subsequently appear (Kerr et al., 1985; Jackson et al., 1986; Morris et al., 1986). We (Chen et al., 1996) reported that EDS also was able to remove Leydig cells from the testes of aged rats, that new Leydig cells were restored to the testes, and that the new cells produced T at the level of young cells rather than at the low levels of the steroidogenically hypofunctional cells that they replaced. As yet, the steroidogenic fates of the newly formed Leydig cells in the testes of young and old rats have not been determined.

We asked herein whether the post-EDS restored population of Leydig cells in young and old testes would retain the equivalent ability to produce T at high levels over time, or alternatively whether cells restored to the old testes might lose steroidogenic ability earlier than those restored to young testes. We reasoned that if the latter occurred, it could be as a consequence of differences in the stem cells that give rise to the new cells, and/or to differences in the extrinsic factors to which the newly formed Leydig cells are exposed. We show that the new generation of Leydig cells restored to young and old rat testes 10 weeks after EDS treatment produced equivalently high ("young") levels of testosterone, but that the steroidogenic ability of cells restored to old, but not young, testes was reduced significantly by 30 weeks.

2. MATERIALS AND METHODS

2.1 Chemicals

EDS (99% purity) was obtained from Winthrop Sterling (Rensselaer, NY). Ovine LH (NIADDK-oLH 26) was a gift from the National Hormone and Pituitary Program (NIDDK Bethesda, MD). [1,2,6,7,16,17-³H(N)]Testosterone (SA, 140.9 Ci/mmol) and 25-[26,27-³H]hydroxycholesterol (SA, 75 Ci/mmol) were obtained from New England Nuclear (Wilmington, DE). 2-[1-¹⁴C]-Ketoisocaproic acid (SA, 50-62 mCi/mmol) and a cyclic AMP [³H] assay system were obtained from Amersham Corp (Arlington Heights, IL). 5-Cholesten-3 β , 25-diol (25-hydroxycholesterol) was purchased from Sigma (St. Louis, MO). All other unlabeled steroids, including pregnenolone, progesterone, 17 α -hydroxyprogesterone, androstenedione and testosterone were obtained from Steraloids Inc. (Wilton, NY) and were crystallized to constant melting points before used as enzyme substrates or as HPLC or RIA standards. Organic solvents for HPLC, including methanol, acetonitrile and tetrahydrofuran, were of HPLC grade and were purchased from J.T. Baker (Phillipsburg, NJ).

2.2 Animals

Male Brown Norway rats of 3 and 18 months of age were obtained through the National Institute on Aging, supplied by Harlan Sprague Dawley, Inc. (Frederick, MD). Rats were housed in a virus-free facility at the Johns Hopkins Bloomberg School of Public Health under controlled light (14h light: 10h dark) and temperature (22C) and with free access to rat chow and water. Experiments were conducted in accordance with the standards established by the United States Animal Welfare Act, set forth in NIH guidelines and the Policy and Procedures Manual of the Johns Hopkins University Animal Care and Use Committee.

2.3 Experimental design

EDS was dissolved in dimethylsulfoxide (DMSO):PBS (1:3) at a concentration of 85 mg/ml. Rats of 3 (N=21) and 18 (N=21) months of age were injected with EDS (ip, 85 mg/kg BW), and control rats of the two ages (N=18 for each) were injected with the vehicle only (DMSO/PBS, 1:3). To confirm that EDS treatment eliminated Leydig cells, 3 animals from each age were killed one week after EDS treatment. Testes were fixed with 5% glutaraldehyde in 0.05 M cacodylate buffer, embedded in Epon, and sectioned for morphological analysis (see below). Ten and 30 weeks after EDS or vehicle injections, half the rats in each group (N=9) were killed by decapitation. Protocols for EDS administration and animal sacrifice were approved by the Johns Hopkins Institutional Animal Care and Use Committee. Trunk blood was collected, allowed to clot at 4C for 24 hours, and then centrifuged to obtain serum. The serum samples were stored at -20C until used for determination of testosterone concentration by radioimmunoassay (RIA). Leydig cells were isolated (see Section 2.4) from one testis of each animal. Cells were isolated from a total of 9 animals of each treatment group. The capacity of the cells to produce testosterone and cAMP in response to LH, and the activity of the steroidogenic enzymes that convert cholesterol to testosterone, were determined in vitro (see Section 2.4). The contralateral testis from each animal was perfusion-fixed in 5% glutaraldehyde and embedded in Epon for

morphological analysis. Sections of 2 μm were stained with toluidine blue and examined using a Nikon Eclipse 800 microscope.

2.4 Leydig cell isolation and measurement of testosterone and cAMP productions

Leydig cells were isolated according to previously described procedures, with minor modification (Klinefelter et al., 1987). In brief, the testicular artery was cannulated and perfused with collagenase IV (1 mg/ml) in dissociation buffer (M199 with 2.2 g/L Hepes, 0.1% BSA, 25 mg/L trypsin inhibitor, 0.7 g/L sodium bicarbonate, pH 7.4) to testis blood. After decapsulation, the testes from 3 animals were pooled and digested in collagenase (0.25 mg/ml dissociation buffer) with shaking (90 cycles/min, 30 min). Seminiferous tubules were removed by filtration through 100 μm pore nylon mesh. The dissociated cells in the filtrate were washed once in dissociation buffer, and were subjected to centrifugal elutriation (16 ml/min, 2000 rpm). The enriched Leydig cell fraction remaining in the chamber was collected and centrifuged (27,000g, 1h) on a 50% Percoll gradient formed *in situ*. Leydig cells were harvested for subsequent assessment of their ability to produce testosterone and cAMP, and for steroidogenic enzyme activity measurements. The cells, identified by their staining for 3 β -hydroxysteroid dehydrogenase (3 β -HSD), were about 95% pure for all groups.

To determine the ability of the Leydig cells to produce cAMP and testosterone, aliquots of purified cells (2×10^5) were incubated in 0.25 ml of M199 medium containing 0.1% BSA, 2.2g/L Hepes, and 1.2g/L sodium bicarbonate for 2h at 34C. To assay testosterone, LH (100 ng/ml) was included in the medium during the 2h incubation, at the end of which the medium was collected and frozen at -80°C . To assay cAMP, the cells were pre-incubated in M199 medium for 2 hours and then incubated for an additional 30 min in 0.05 ml fresh medium containing LH (100 ng/ml) and the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX, 0.1mM). At the end of the incubation, an equal volume of Tris-HCl buffer (0.05 ml; 0.05M, pH7.5) containing 4mM EDTA and 2g/L theophylline) was added, and the cells and the medium were frozen together at -80°C . cAMP levels were measured using the [^3H]cAMP assay system from GE Healthcare Bio-Sciences Corp (Piscataway, NJ), according to the manufacturer's instructions. The sensitivity of the assay was 0.05 pmol per assay tube.

2.5 Steroidogenic enzyme activity assays

Cholesterol side-chain cleavage (P450_{scc}) activity was determined by measuring the conversion of 25-[26,27- ^3H] hydroxycholesterol to [^3H]4-hydroxyl-4-methyl-pentanoic acid (Georgiou et al., 1987). Briefly, 2×10^5 purified cells were incubated in 0.25 ml M199 medium containing 25-[26,27- ^3H] hydroxylcholesterol (0.2 uCi/2uM) at 34C for 1h. At the end of the incubation, 0.025 ml of NaOH (1N) was added to stop the reaction. After the medium was transferred to an extraction tube, the incubation wells were washed with 0.275 ml alkalized medium and combined with the original medium. [^{14}C]-isocaproic acid was added to monitor recovery. To remove non-metabolized substrate, the mixture was extracted with chloroform and vortexed with alumina, and a 0.5 ml aliquot was counted in a liquid scintillation counter. In a preliminary experiment, the production of [^3H]4-hydroxyl-4-methyl-pentanoic acid was linear during 1 hour incubation.

The activities of 3 β -HSD, 17 α -hydroxylase/C17-20 lyase (P45017 α or CYP17A1) and 17 β -hydroxysteroid dehydrogenase (17 β -HSD) were assayed by incubating Leydig cells (2×10^5 cells/0.25ml M199 medium containing 1g/L BSA) with pregnenolone (P5, 50 μ M), progesterone (P4, 12.5 μ M), 17-hydroxyprogesterone (17P4, 25 μ M), or androstenedione (A, 12.5 μ M) in vitro for 1h, and then measuring and summing all 4 -3-ketosteroid products (Wing et al., 1984; Luo et al., 1996). For example, to assess the activity of 3 β -HSD, P5 was added as substrate and P4 plus 17P4 plus A plus T were measured as products. To assay 17-hydroxylase activity, P4 was added as substrate and 17P4 plus A plus T were measured. To assay C17-C20 lyase activity, 17P4 was added as substrate, and A plus T were measured. To assay 17 β -HSD activity, A was added as substrate and T was measured. P4, 17P4, A and T were measured with a reverse phase HPLC equipped with a flow-through UV spectrophotometer. As determined in preliminary experiments, the substrate concentrations used for each enzyme were saturating, and the reactions were linear for 1 hour. When the cells were incubated without substrate, there were undetectable amounts of the four 4 -3-ketosteroids, and over 95% of the added substrates were recovered as products. This indicates that little or no substrate was converted to 5 pathway steroids.

2.6 Serum testosterone and LH assays

Serum testosterone concentration was assayed by RIA. In brief, 100 μ l of serum was extracted with ether and dried in a 50 °C water bath. The extracts were dissolved in PBS and assayed for testosterone by RIA. The sensitivity of the assay was 10 pg/tube. The intra- and inter-assay coefficients of variation were 8.9% and 13.6%. Serum LH concentrations were determined using the enzyme-linked immunosorbent assay (ELISA) kit from MyBioSource (San Diego, CA). The minimum detectable dose of rat LH was less than 0.15 mIU/ml. The detection range was 0.3 mIU/ml –60 mIU/ml. All samples were run in one assay, with intra-assay coefficient of variation below 15%.

2.7 Statistical analysis

Data are expressed as the mean $\pm$ SEM. Group means were evaluated by one-way ANOVA. If group differences were revealed by ANOVA ($P \leq 0.05$), differences between individual groups were determined with the Student-Neuman-Kuels test, using SigmaStat software (Systat Software Inc., Richmond, CA). Values were considered significant at $P \leq 0.05$.

3. RESULTS

3.1 Leydig cell morphology in EDS treated rats

One week after EDS, Leydig cells were absent from both young (Fig. 1A) and aged (Fig. 1B) testes. At 10 weeks post-EDS, Leydig cells had been restored to the testes of young (Fig. 1C) and aged (Fig. 1D) rats, and were similar in appearance and size to each other. At 30 weeks post-EDS, when the EDS-injected young rats were 10 months of age, the Leydig cells (Fig. 1E) were indistinguishable from those in the testes of young rats at 10 weeks post-EDS (Fig. 1C). In contrast, Leydig cells in the testes of old rats at 30 weeks post-EDS, when the rats had reached 25 months of age, were reduced the size (Fig. 1F) compared to cells in the old rats 10 weeks after EDS (Fig. 1D) and to cells in the young rats 30 weeks after EDS (Fig. 1E).

3.2 Serum testosterone and LH concentrations

Serum testosterone concentrations are shown at 10 (Fig 2A) and 30 (Fig 2B) weeks after vehicle administration (C, control) and EDS injection (10E, 30E). At both 10 and 30 weeks, serum testosterone concentrations were significantly lower in the old than the young controls. Ten weeks after EDS administration, when Leydig cells had been restored (Fig. 1), serum testosterone concentrations in both young and old rats were at the level of young controls, a level that was significantly higher than that in the old controls (Fig. 2A). Thirty weeks after EDS administration, however, serum T concentration in aged rats was reduced to the aged control level, while the concentration in young rats was maintained at the young control level (Fig. 2B).

To examine the possibility that the changes in serum T might be due to differences in pituitary LH secretion, serum LH was assayed in rats at 10 (Fig. 2C) and 30 (Fig. 2D) weeks after vehicle or EDS administration. Neither age nor EDS treatment affected serum LH concentrations significantly.

3.3 Testosterone and cAMP productions

Figure 3 shows T production by Leydig cells isolated from rats at 10 (Fig. 3A) and 30 (Fig. 3B) weeks after vehicle or EDS administration, and cultured with maximally stimulating LH. Consistent with serum T concentration, Leydig cells from the control aged rats produced significantly less T than the cells from young controls at both 10 and 30 weeks after treatment. At 10 weeks after EDS, the Leydig cells that were restored to the testes of young and aged rats produced comparable T, at levels even higher than those of the young controls (Fig 3A). At 30 weeks after EDS administration, however, Leydig cells restored to the aged testes produced significantly less testosterone than the cells that had been restored to the young testes, with the latter unchanged from the 10 week level (Fig. 3B).

Also shown in Figure 3 is cAMP production by Leydig cells isolated from rats at 10 (Fig. 3A) and 30 (Fig. 3B) weeks after vehicle or EDS administration and cultured with LH. As with testosterone production, Leydig cells from young control rats produced significantly more cAMP than cells from aged control rats at 10 and 30 weeks after treatment (Fig. 3C, D). At 10 weeks after EDS the Leydig cells restored to the testes of young and aged rats produced comparable cAMP, at levels higher than those of the young controls (Fig 3C). By 30 weeks after EDS administration, however, Leydig cells that were restored to aged testes produced significantly less cAMP than the cells that had been restored to the young testes, while the latter cells did not change from the 10 week level (Fig. 3D).

3.4 Steroidogenic enzyme activities

The activity of P450scc (CYP11A1) was significantly higher in Leydig cells isolated from the testes of young than aged control rats (Figs. 4A, 4B). Ten weeks after EDS, the P450scc activity of Leydig cells restored to both young and aged testes was equivalent, and significantly higher than that of the controls (Fig. 4A). At 30 weeks after EDS, the P450scc activity of cells from old rats was reduced almost to the low level of aged controls, but the P450scc activity of cells from young rats remained high (Fig 4B).

Figure 5 shows the activities of the steroidogenic enzymes of the smooth endoplasmic reticulum, namely 3 β -HSD (Figs. 5A, 5B), 17 α -hydroxylase/C17-20 lyase (CYP17A1) (Figs. 5C, 5D), and 17 β -HSD (Figs. 5E, 5F). As with testosterone, cAMP and P450scc, each of these enzyme activities was significantly lower in aged control than young control cells at both 10 and 30 weeks after vehicle administration. At 10 weeks post-EDS, the activities of these enzymes in the Leydig cells restored to the testes were similar between the two ages. In contrast to testosterone and cAMP, the levels remained equivalent at 30 weeks.

4. DISCUSSION

It is well established that Leydig cells become steroidogenically hypofunctional with age in Brown Norway rats; Leydig cells of aged rats produce about 50% less testosterone than those from young adults (Chen et al., 1994; 1996). Leydig cells synthesize testosterone from cholesterol through steps that include LH binding to the LH receptor, production of cAMP, cholesterol movement from intracellular stores into mitochondria, conversion of cholesterol to pregnenolone by the CYP11A1 enzyme at the inner mitochondrial membrane, and subsequent transformation of pregnenolone through a series of steps to testosterone in the smooth endoplasmic reticulum, catalyzed by 3 β -HSD, CYP17A1, and 17 β -HSD (Haider, 2004). The rate-determining step in this process is cholesterol transport into the mitochondria to the site of CYP11A1 (Papadopoulos and Miller, 2012). Aged Leydig cells have deficits in LH receptor number, cAMP production, cholesterol transport, and the steroidogenic enzymes of the mitochondria and smooth endoplasmic reticulum (Chen et al., 1994; 2002; Luo et al., 2001; Culty et al., 2002). Though serum levels of LH do not differ between young and aged rats, aged Leydig cells are relatively non-responsive to LH, perhaps as a consequence of altered LH receptor-G protein coupling (Chen et al., 2004).

Redox imbalance and thus increased oxidative stress within the Leydig cells appear to play important roles in the reductions in testosterone production that occur in aging Leydig cells (Midzak et al., 2009). Thus, increases in reactive oxygen species (ROS) production by the mitochondrial electron transport chain and by P450 enzyme activities within Leydig cells occur with aging (Chen et al., 2001; Cao et al., 2004; Hanukoglu, 2006). There is age-related down-regulation of a number of Leydig cell genes and their protein products that are involved in free radical scavenging and repair of ROS-induced damage, including superoxide dismutase 1 and 2, glutathione peroxidase 1, and glutathione (Cao et al., 2004; Chen et al., 2005; Luo et al., 2006). Although LH stimulates Leydig cell testosterone production, significant increases in the expression of genes related to stress response as well as increased generation of ROS and DNA damage were reported in response to LH stimulation (Beattie et al., 2013). These LH effects were suppressed by incubating the cells with an antioxidant (Beattie et al., 2013). Additionally, the long-term in vivo suppression of LH in rats was shown to prevent the reductions in testosterone production that characterize aged rat Leydig cells (Chen and Zirkin, 1999). These results, taken together, suggest that redox imbalance and thus increased oxidative stress within aging Leydig cells result at least in part from changes that are intrinsic to the Leydig cells. It also has been suggested that there may be exogenous influences on Leydig cell steroidogenic function over time (Hales, 2002), including factors from within the seminiferous tubular compartment of the testis, from macrophages, and from outside the testes (Saez, 1994; Huhtaniemi and Toppari, 1995;

Habert et al., 2001; Haider, 2004). Whether or how these extrinsic factors influence steroid production during Leydig cell aging are unknown.

Numerous studies have shown that Leydig cells can be eliminated from the young adult testis with EDS, and that a new population of testosterone-producing Leydig cells arises thereafter (Kerr et al., 1985; Jackson et al., 1986; Morris et al., 1986). We have shown that the steroidogenically hypofunctional Leydig cells in old testes also can be eliminated with EDS, and that, as in the young adult testis, new Leydig cells arise (Chen et al., 1996). At 10 weeks after EDS, the new Leydig cells restored to old testes were equivalent to cells restored to young testes in their ability to produce testosterone. Adult Leydig cells originally differentiate from stem cells of the neonatal testis (Yao et al., 2002; Ge et al., 2006; Chen et al., 2009, 2010). The results showing that new Leydig cells arise in young and aged testes after EDS suggest that there may be stem cells in adult and aged testes as well as in the neonatal testis (Chen et al., 2009, 2010; Davidoff et al., 2009). Indeed, we and others isolated cells from neonatal and adult testes which, depending upon in vitro conditions, proliferated indefinitely or differentiated to produce testosterone (Ge et al., 2006; Stanley et al., 2012; Landreh et al., 2013). These results suggest that the new populations of Leydig cells that arise after EDS treatment of young or aged rats derive from stem cells. The observation that the newly formed Leydig cells in the testes of young and aged EDS-treated rats produce testosterone at equivalent levels suggests that although the adult Leydig cells age (Chen et al., 1994; Chen et al., 2009), the stem cells in the interstitial compartment from which the Leydig cells arise are equivalent.

However, though the regenerated Leydig cells after 10 week EDS treatment were similar in young and aged rat testes, the cells became different 20 weeks later with respect to their steroidogenic ability. Thus, whereas the restored cells in young rat testes maintained their high levels of testosterone production, the ability of the restored cells in the testes of old rats to produce testosterone, as well as cAMP and P450_{scc}, were reduced. Reductions in serum T and changes in the morphology of the cells were consistent with the reductions in steroid formation by the cells. Age-related changes in pituitary function are unlikely to be the primary cause of the changes in the newly formed Leydig cells in old testes because serum LH concentrations did not change significantly. That said, the fact that serum LH levels failed to increase in aged animals despite reductions in serum testosterone levels suggests that aging also affected hypothalamic-pituitary function. Taken together, these results suggest strongly that factors within or outside the testis, perhaps including changes in LH pulsatility, play roles in Leydig cell aging.

In summary, there is clear evidence to suggest that oxidative stress resulting from changes in the redox environment of the Leydig cells is an important component of the reduced steroid formation that occurs with Leydig cell aging (Chen et al., 2001, 2015; Cao et al., 2004; Luo et al., 2006; Midzak et al., 2009). The present results suggest that extrinsic factors also may be associated with aging. Leydig cell function is known to be regulated by factors in the circulatory system, including those from the pituitary (gonadotropins), liver (IGF-1), thyroid (T3) (Maran, 2003), pancreas β -cells (insulin), bone marrow (osteocalcin), and immune system (Bornstein et al., 2004; Ferlin et al., 2013). Within the testis, Sertoli cells and macrophages produce some of these factors (Meinhardt, et al., 2000; Hutson, 2006). In

addition to these regulatory factors, hydrogen peroxide produced by macrophages (Hales 2002) may negatively impact neighboring Leydig cells over time. Changes in LH pulsatility also may be involved. Whether and how these extrinsic factors affect Leydig cells remain uncertain.

5. ACKNOWLEDGEMENTS

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Highlights

- T production by Leydig cells restored to young and old rat testes is equivalent.
- However, in time, T production was reduced in old but not young testes.
- Serum LH levels were equivalent in young and old rats.
- Conclude: Factors extrinsic to the new Leydig cells in young and old testes differ.

Figure 1.

Morphology of Leydig cells in the testes of young (A, C, E) and aged (B, D, F) rats at weeks 1 (A, B), 10 (C, D) and 30 (E, F) after EDS treatment of the rats.

Figure 2. Serum testosterone (A, B) and LH (C, D) concentrations in young and aged rats at 10 weeks (A, C) and 30 weeks (B, D) after EDS treatment. C: age-matched controls; 10E, 30 E: 10 and 30 weeks post-EDS, respectively. ^aSignificantly different from young control or young EDS-treated ($P < 0.05$). ^bSignificantly different from the age-matched control (no-EDS) ($P < 0.05$).

Figure 3.

Testosterone (A, B) and cAMP (C, D) productions by Leydig cells isolated from the testes of young and aged rats at 10 weeks (A, C) and 30 weeks (B, D) after EDS treatment. C: age-matched controls; 10E, 30E: 10 and 30 weeks post-EDS, respectively. ^aSignificantly different from young control or young EDS-treated ($P < 0.05$). ^bSignificantly different from the age-matched control (no-EDS) ($P < 0.05$).

Figure 4.

CYP11A1 activity in Leydig cells isolated from the testes of young and aged rats at 10 weeks (top) and 30 weeks (bottom) after EDS treatment. C, age-matched controls; 10E, 30E: 10 and 30 weeks post-EDS, respectively. ^aSignificantly different from young control or young EDS-treated rats ($P < 0.05$). ^bSignificantly different from the age-matched controls (no-EDS) ($P < 0.05$).

Figure 5.

3 β -HSD (A, B), CYP17A1 (C, D) and 17 β -HSD (E, F) activities in Leydig cells isolated from the testes of young and aged rats at 10 weeks (A, C, E) and 30 weeks (B, D, F) after EDS treatment. C: age-matched controls; 10E, 30E: 10 and 30 weeks post-EDS, respectively. ^aSignificantly different from young control or young EDS-treated rats (P<0.05). ^bSignificantly different from the age-matched controls (no-EDS) (P<0.05).