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Dehydroepiandrosterone: Biosynthesis and Metabolism in the Brain

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ABSTRACT

Dehydroepiandrosterone (DHEA) is abundantly found in brain tissues of several species, including human. However, the cellular origin and pathway by which DHEA is synthesized in brain are not yet known. We have, therefore, initiated pilot experiments to explore gene expression of cytochrome P450 17α-hydroxylase (P450c17), the key steroidogenic enzyme for androgen synthesis, and evaluate DHEA production by highly purified astrocytes, oligodendrocytes, and neurons. Using RT-PCR, we have demonstrated for the first time that astrocytes and neurons in the cerebral cortex of neonatal rat brain express P450c17. The presence of P450c17 in astrocytes and neurons was supported by the ability of these cells to metabolize pregnenolone to DHEA in a dose-dependent manner as determined by RIA. These data were further confirmed by production of androstenedione by astrocytes using progesterone as a substrate. However, cortical neurons express a low transcript of P450c17 messenger RNA and produce low levels of DHEA and androstenedione compared with astrocytes. Oligodendrocytes neither express the messenger RNA nor produce DHEA. The production of DHEA by astrocytes is not limited to cerebral cortex, as hypothalamic astrocytes produce DHEA at a level 3 times higher than that produced by cortical astrocytes. Cortical and hypothalamic astrocytes also have the capacity to metabolize DHEA to testosterone and estradiol in a dose-dependent manner. However, hypothalamic astrocytes were 3 times more active than cortical astrocytes in the metabolism of DHEA to estradiol. In conclusion, our data presented evidence that astrocytes and neurons express P450c17 and synthesize DHEA from pregnenolone. Astrocytes also have the capacity to metabolize DHEA into sex steroid hormones. These data suggest that as in gonads and adrenal, DHEA is biosynthesized in the brain by a P450c17-dependent mechanism. (*Endocrinology* **140:** 880–887, 1999)

DEHYDROEPIANDROSTERONE (DHEA) is a multifunctional steroid that is known to be involved in a variety of functional activities in the central nervous system, including an increase of memory and learning, protection of neurons against excitatory amino acid-induced neurotoxicity, and reduction of risk of age-related neurodegenerative disorders (1). DHEA was detected in the extract of brain tissues of monkey, pig, guinea pig, mice, rat, and human at a concentration greater than that in the peripheral circulation (2, 3). In humans, this steroid was also found in the cerebrospinal fluid of both men and women (4). DHEA appears to be produced and accumulated in brain independently of adrenal and gonadal sources, as its concentration in brain maintained for several weeks after adrenalectomy and gonadectomy (3). These data strongly suggest the *de novo* biosynthesis of DHEA in the brain.

In gonads and adrenal gland, P450c17 enzyme is responsible for the conversion of pregnenolone (P_5) into DHEA (5, 6). However, the presence of this enzyme in brain is a matter of controversy. P450c17 messenger RNA (mRNA) was detected in rat and mouse embryonic brain tissues (7) and in whole brain and cerebral cortical tissues of young adult rat brains (8, 9) using RT-PCR. In contrast to these studies, neither P450c17 protein (10), mRNA (11), nor activity (12) was detected in adult rat brain tissues. Moreover, mixed glial cells and astrocytes from rat and mouse embryo brains in cultures did not express P450c17 mRNA, as determined by RT-PCR (11), and were unable to metabolize radioactive P_5 to DHEA, as determined by TLC/HPLC (12). Thus, the cellular origin of DHEA and the mechanism of its biosynthesis in brain remain an open question.

Evidence accumulated during the last years documented that macrophages are involved in the regulation of steroidogenesis in gonads (for review, see Ref. 13). These cells produce abundant quantities of nitric oxide (NO), tumor necrosis factor-α (TNFα), and interleukin-1β (IL-1β), which at low concentrations are able to inhibit androgen production (14–19) in ovary and testis via inhibition of P450c17 gene expression (14–18). Residual macrophages in thecal-interstitial cell cultures have been reported to decrease androgen production by these cells (14–20).

Microglia cells (macrophages of brain) are also known as the main sources for NO, TNFα, and IL-1β in the brain (21–23). These cells were found to be the major contaminant cell type (5%) in primary glial cell cultures (24) and could persist more than 4 months in culture (25). It, therefore, is possible that the presence of contaminant microglia cells in glial cell cultures could result in inhibition of gene expression and biosynthesis of P450c17 enzyme and make its detection difficult. This may explain the unsuccessful attempts (11, 12) to identify P450c17 mRNA and its activity *in vitro*. We, therefore, attempted to determine the gene expression of P450c17 and biosynthesis of DHEA in the brain using highly purified microglia-free astrocytes, oligodendrocytes, and neurons. We demonstrated that astrocytes express P450c17, produce DHEA, and are able to metabolize this hormone to testosterone (T) and estradiol (E_2). Neurons express low levels of

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P450c17 mRNA and produce low concentrations of DHEA, whereas oligodendrocytes neither express P450c17 nor produce DHEA.

Materials and Methods

Isolation and culture of astrocytes and oligodendrocytes

Mixed glial cells were isolated from cerebral cortex of neonatal rat brains by mechanical or mild enzymatic dispersion as previously described by Zwain *et al.* (26, 27), based on method described by McCarthy and de Vellis (28). Glial cells were cultured for 10 days in a T75 flask in 10% serum-supplemented Ham's F-12-DMEM at 37 C in a humidified atmosphere. Oligodendrocytes are normally layered on top of astrocytes. To isolate oligodendrocytes from astrocytes, flasks were shaken at 200 cycles/min for 18 h at 37 C in an orbit shaker. Floating cells (oligodendrocytes) were collected, washed, and seeded in culture flasks in 10% serum-supplemented Ham's F-12-DMEM. The attached cells (mainly astrocytes) were washed with fresh medium and shaken again for an additional 15 h to remove any possible contaminant oligodendrocytes. The contaminant microglia cells were eliminated from glial cell cultures by treating cells with L-leucine methyl-ester (LME), which is known to be a potent macrophage-cytotoxic agent (24, 29). Astrocytes and oligodendrocytes were removed from culture flasks by mild trypsinization and treated in suspension with 10 mM LME for 1 h at room temperature with shaking, as previously described (24, 25). As microglia are very adhesive cells and will adhere to cell culture substrata within a half-hour, glial cells were successively plated in a flask three times (30 min each time) with systematic changing of culture flasks, as described by Devon (30). This additional purification step was performed to remove any possible remaining contaminant microglia cells. Glial cells were finally plated at various densities in different culture plates. Astrocytes and oligodendrocytes isolated by this method were more than 99% pure as determined by immunocytochemical analysis of glial fibrillary acid protein and galactocerebroside, which are specific protein markers for astrocytes and oligodendrocytes, respectively. The immunocytochemical analysis of Leu M5 protein, microglia/macrophage-specific protein marker, was also performed to determine whether microglia cells are eradicated from glial cell cultures. No microglia cells were detected in glial cell cultures after LME treatment and successive plating of glial cells in culture flasks. Before treatment of glial cells with LME, astrocyte and oligodendrocyte cell cultures contained 5% and 3% Leu M5-immunostained cells, respectively.

The microglia-free astrocytes and oligodendrocytes were extensively washed with serum-free medium (SFM) and cultured for 48 h in SFM with various treatments.

Isolation and culture of neurons

Neuronal culture was performed as previously described by Hertz *et al.* (31) with modification. Briefly, cerebral cortical and hypothalamic tissues from neonatal rat brains was mechanically dispersed, and cells were cultured in medium containing 5% horse serum for 3 days at 37 C in culture plates precoated with poly-L-lysine substrates. Cell cultures were then exposed for 24 h to cytosine arabinoside (40 μM) to eliminate the nonneuronal cells, including microglia and macroglia cells (astrocytes and oligodendrocytes). To remove any possible remaining contaminant microglia, neurons were successively plated three times with systematic changing of culture plates as described above. Neurons were then cultured in precoated plates in SFM. After 48 h in culture, neurons were washed and cultured for an additional 48 h in SFM with various treatments. The purity of the neurons was 99%, as judged by immunocytochemical analysis of neurofilment protein (Sigma Chemical Co., St. Louis, MO), a specific protein marker for neurons. No microglia cells were detected in neuronal cell cultures, as determined by immunocytochemical analysis of Leu M5.

The purity of isolated neurons, glial cells, astrocytes, and oligodendrocytes was analyzed using cells from three different experiments. Before treatment of cells with steroids, six-well culture plates containing astrocytes, oligodendrocytes, and neurons (one plate of each cell type) from each experiment were randomly selected for assessment of cell purity by immunocytochemical analysis. The purity of the isolated cells in the three experiments was 99%.

Preparation of samples for quantitative analysis of steroids and P450c17 mRNA

At the conclusion of culture times, astrocytes, oligodendrocytes, and neurons were removed by mild trypsinization, and cell viability was determined by trypan blue staining. Media from astrocyte, oligodendrocyte, and neuron cultures were collected, centrifuged, and stored at −20 C until analysis of steroid concentrations using a RIA kit (Diagnostic System Laboratories, Inc., Webster, TX). The minimum detection limit of the DHEA assay was 9 pg/ml. The intra- and interassay variations of the assay were 3.2% and 6.4%, respectively. The cross-reactivity of the DHEA antibody with other steroids was determined by the manufacturer of the RIA kit. This antibody was mainly shown to cross-react with isoandrosterone (0.733%), androstenedione (A_4; 0.460%), 5α-androstane-3,17-dione (0.240%), 11-deoxycortisol (0.061%), progesterone (P_4; 0.045%), androsterone (0.035%), and T (0.028%).

Total RNA from cultured cells was isolated using the guanidine phenol-chloroform extraction method, treated with deoxyribonuclease to remove contaminant DNA, and stored at −70 C until analysis of steroidogenic enzyme gene expression by RT-PCR. The integrity of the RNA was demonstrated by analysis of ribosomal RNAs using ethidium bromide staining.

RT-PCR

RT-PCR analysis was performed as previously described by Zwain *et al.* (27). Briefly, total RNA was reverse transcribed using Moloney murine leukemia virus reverse transcriptase (Perkin-Elmer, Branchburg, NJ) in the presence of the oligo(deoxythymidine) primer. One tenth of the RT reaction was used as a template for amplification by PCR using AmpliTaq Gold DNA polymerase (Perkin-Elmer). The specific sense and antisense oligonucleotide primers used in amplification of P450c17 complementary DNA (cDNA) were prepared as previously described by Stromstedt and Waterman (14). PCR products were resolved on 2% agarose gel, stained with ethidium bromide, and visualized under UV light. The identities of the PCR products were further confirmed by restriction digestion and Southern blot analyses. RNA from rat ovary and testis was also extracted and used as experimental positive controls for RT-PCR analysis. A negative control was included where reverse transcriptase was omitted in the RT reaction.

Southern analysis

Southern blot analysis of P450c17 was performed as previously described by Zwain *et al.* (27). Briefly, PCR products were fractionated on 2% agarose gel, denatured, transferred onto Nytran nylon membranes (Schleicher & Schuell, Inc., Keene, NH), and immobilized by UV cross-linking. Membranes (blots) were prehybridized for 4 h at 37 C in the prehybridization buffer, which consisted of 6 × SSC (standard saline citrate) containing 50% deionized formamide, 10% dextran sulfate, 1% SDS, and 100 μg/ml sheared and denatured salmon sperm DNA. Blots were then hybridized for 18 h at 37 C in the prehybridization buffer containing 2×10^6 cpm/ml of α-^{32}P-labeled specific P450c17 internal oligonucleotide probe, prepared as previously described by Stromstedt and Waterman (9). Membranes were washed and exposed to x-ray film at −70 C for 3–10 h.

Treatment of astrocytes with antisense oligonucleotide

To determine the specificity of metabolism of DHEA by astrocytes, the biosynthesis of cytochrome P450 aromatase enzymes (P450arom) was inhibited by treatment of astrocytes with antisense oligonucleotide to P450arom cDNA using a transfection kit from Life Technologies (Gaithersburg, MD). A 20-mer phosphorthioate antisense and a sense oligonucleotide overlapping the initiation codon of P450arom cDNA were designed and HPLC purified as previously described by Ackermann *et al.* (32). The sense oligonucleotide was used as a negative experimental control. Astrocytes were seeded in 12-well plates and cultured for 48 h at 37 C in the presence and absence of the antisense (1 and 10 μM) or sense (10 μM) oligonucleotides. At the conclusion of culture time, media were harvested, centrifuged, and stored at −20 C until analysis of the E_2 concentration by RIA. To determine whether the antisense oligonucleotide has real impact on P450arom, RNA from

treated and untreated astrocytes was extracted and analyzed for P450arom gene expression by RT-PCR as described above. The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide cytotoxcity assay was used to determine the viability of astrocytes treated with the antisense and sense oligonucleotides as previously described by Schlingeniepen and Klinger (33). The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide assay kit was purchased from Sigma Chemical Co. (St. Louis, MO). Oligonucleotides had no effect on the viability of astrocytes compared with the controls where cells were cultured alone.

Statistical analysis

Student's unpaired *t* test was used when only two individual groups were compared. For multiple comparison, data were analyzed by one-way ANOVA with *post-hoc* Bonferroni test using the Statistical Analysis System (SAS Institute, Cary, NC; StatView software). Results are expressed as the mean ± SD of three replicate cultures. The statistical analysis was performed for three independent experiments and gave the same results. $P < 0.05$ was considered statistically significant.

Results

Biosynthesis of DHEA in the brain

Using RT-PCR, we demonstrated that cerebral cortical astrocytes (Fig. 1A, lane 1) and neurons (Fig. 1A, lane 3), but not oligodendrocytes (Fig. 1A, lane 2), isolated from neonatal rat brains express P450c17 mRNA. The mRNA level of P450c17 in the cortical neurons was extremely low compared with that in astrocytes (Fig. 1A, lane 3 *vs.* lane 1). These data were further confirmed by RT-PCR/Southern blot analysis using a specific P450c17 oligonucleotide probe (Fig. 1B, lanes 1–3). The restriction digestion enzyme analysis of PCR products has been used to confirm the identity of P450c17 in astrocytes and neurons. The PCR products from astrocytes and neurons were reamplified and digested with the restriction enzyme, NCO1. Two fragments of the expected sizes were generated in samples from astrocytes and neurons (data not shown). These DNA fragments were identical in size to fragments from adult rat ovary and testis.

The presence of P450c17 in astrocytes and neurons was

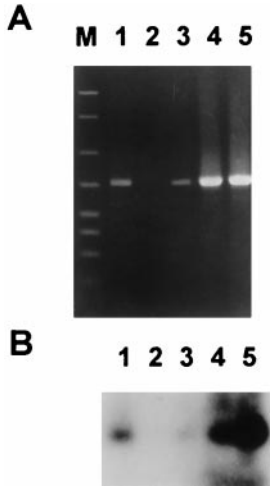

FIG. 1. RT-PCR (A) and Southern blot (B) analyses of P450c17 in cerebral cortical astrocytes (lane 1), oligodendrocytes (lane 2), and neurons (lane 3) of neonatal rat brains. RNA from ovarian (lane 4) and testicular (lane 5) tissues of adult rats was extracted and used as a positive control for PCR amplification. These experiments were repeated twice, and each experiment yielded similar results. M, DNA size marker.

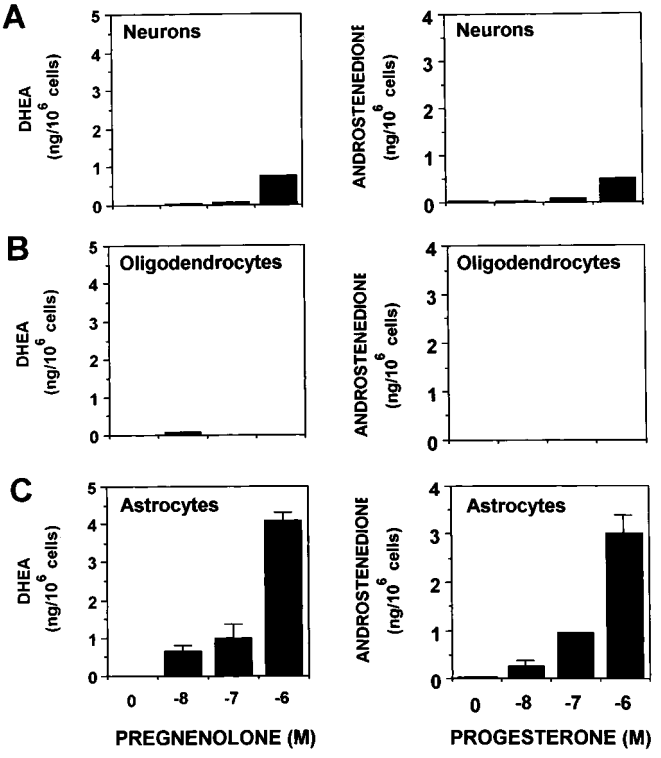

FIG. 2. Conversion of P_5 to DHEA (*left panel*) and P_4 to A_4 (*right panel*) by cortical neurons (A), oligodendrocytes (B), and astrocytes (C) of neonatal rat brains, as determined by RIA. ns, No significant difference from the control (cells cultured without any treatment). *, $P < 0.05$; **, $P < 0.001$ (significantly different from the control). Significant differences of at least $P < 0.05$ in DHEA or A_4 production were found among the different groups of neurons and astrocytes treated with various doses of P_5 or P_4, except with P_5 doses between 10^{-8}-10^{-7} M, the production of DHEA by astrocytes was not significantly different.

further evaluated by the ability of these cells to convert P_5 into DHEA using highly specific and sensitive RIA. As shown in Fig. 2 (A and C, *left panel*), culture of cerebral cortical astrocytes and neurons for 48 h in the presence of increasing concentrations of P_5 resulted in a dose-dependent increase in DHEA production. At a concentration of 10^{-6} M P_5, DHEA production by cortical astrocytes was 10 times higher than that by cortical neurons. Oligodendrocytes were not able to convert P_5 into DHEA (Fig. 2B, *left panel*). These data were further confirmed by the ability of astrocytes and neurons, but not oligodendrocytes, to convert P_4 into A_4 in a dose-dependent fashion (Fig. 2, A–C, *right panel*).

The specificity of DHEA production by cortical astrocytes was evaluated by treating astrocytes with increasing concentrations of ketoconazole, a potent inhibitor of cytochrome P450 steroidogenic enzymes including P450c17. Ketoconazole inhibited the conversion of P_5 to DHEA by astrocytes in a dose-dependent manner (Fig. 3). At a concentration of 10 μM ketoconazole, DHEA production by cortical astrocytes was inhibited by 84% compared with that in the controls where cells were cultured with only P_5.

The production of DHEA is not limited to cerebral cortical astrocytes, as hypothalamic astrocytes were also able to convert P_5 into DHEA in a dose-dependent manner (Fig. 4). At

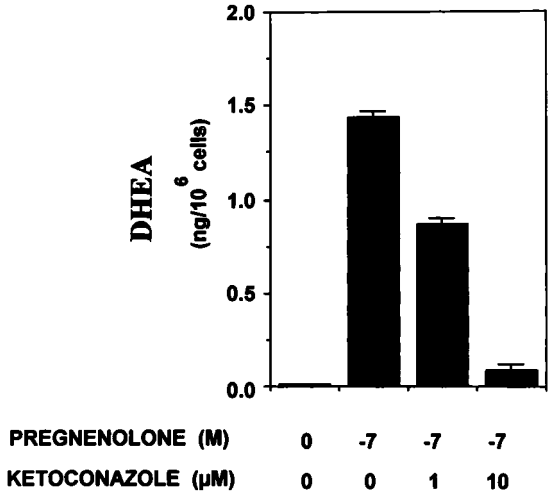

FIG. 3. Effect of ketoconazole on the conversion of P_5 to DHEA by cortical astrocytes of neonatal rat brains, as determined by RIA. **, $P < 0.001$ (significant difference). Significant differences of $P < 0.001$ in DHEA production were found among the different groups of astrocytes cultured alone, with P_5, or with P_5 plus ketoconazole.

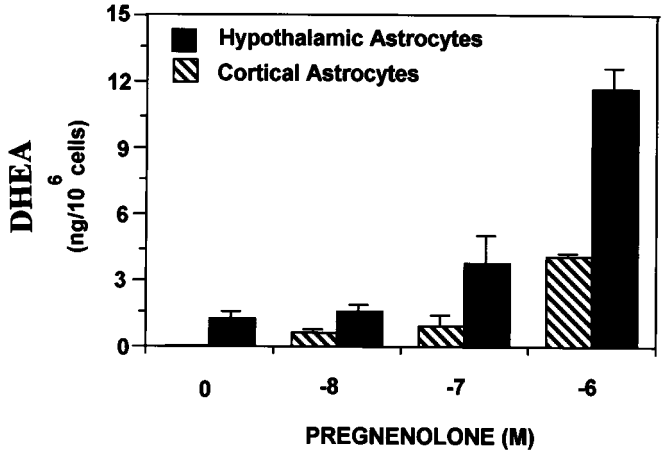

FIG. 4. Comparison between DHEA production by hypothalamic and cortical astrocytes of neonatal rat brains as determined by RIA. *, $P < 0.05$; **, $P < 0.01$ (significant differences). Significant differences of at least $P < 0.05$ in DHEA production were found among the different groups of cortical or hypothalamic astrocytes treated with various doses of P_5, except between P_5 doses of 10^{-8}–10^{-7} M, the production of DHEA by cortical astrocytes was not significantly different.

a concentration of 10^{-6} M P_5, DHEA production by hypothalamic astrocytes was 3 times higher than that by cortical astrocytes (Fig. 4).

To determine whether microglia cells could affect the ability of astrocytes to produce DHEA, purified (without residual microglia cells) and unpurified (with residual microglia cells) astrocytes were cultured for 48 h in the presence of P_5 as a substrate. The DHEA level in the medium was determined by RIA. As shown in Fig. 5, purified and unpurified astrocytes converted P_5 into DHEA in a dose-dependent manner. However, DHEA production by astrocytes containing contaminant microglia cells was extremely low compared with that by microglia-free astrocytes.

Metabolism of DHEA by astrocytes

We investigated whether astrocytes, the major source of DHEA in the brain, have the ability to metabolize DHEA to T and E_2. Culture of astrocytes from hypothalamus or cerebral cortex of neonatal rat brains for 48 h with increasing concentrations of DHEA resulted in a dose-dependent increase in T (Fig. 6A) and E_2 (Fig. 6B) levels in the cultured medium. However, the production of E_2 by hypothalamic astrocytes was higher than that by cortical astrocytes, indicating that hypothalamic astrocytes are more active than cortical astrocytes in aromatization of androgen to estrogen.

We further investigated whether the metabolism of DHEA to androgen and subsequently to estrogen is specific and not an artifact by evaluating the effects of trilostane and antisense oligonucleotide to P450arom cDNA on the metabolism of DHEA by astrocytes. Trilostane is known to inhibit the conversion of DHEA to androgen by inhibiting 3β-hydroxysteroid dehydrogenase (3βHSD) enzymatic activity (34–36). The antisense oligonucleotide to P450arom cDNA was effectively used to inhibit the conversion of androgen to estrogen by blocking the biosynthesis of P450arom (32, 37). Astrocytes were cultured for 48 h in the presence and absence of trilostane or the antisense oligonucleotide. Changes in the metabolism of DHEA to androgen and of androgen to estrogen were evaluated by measuring A_4 and E_2 levels in the culture medium by RIA. Trilostane at a concentration of 50 ng/ml inhibited the conversion of DHEA (10^{-6} M) to A_4 by 83% compared with that in controls, where astrocytes cultured alone (Fig. 7A). The effect of trilostane on 3βHSD activity in astrocytes was further confirmed by the ability of trilostane to inhibit the conversion of P_5 to P_4 (Fig. 7B).

Treatment of astrocytes with the antisense oligonucleotide to P450arom cDNA resulted in inhibition of aromatization of T to E_2 in a dose-dependent manner (Fig. 8). At a dose of 10 μM, the antisense oligonucleotide inhibited the conversion of T (10^{-7} M) to E_2 by 50-fold compared with that in controls where astrocytes cultured alone or with 10 μM sense oligonucleotide (Fig. 8). To confirm the specificity of the antisense oligonucleotide action, semiquantitative RT-PCR analysis using β-actin as an internal standard housekeeping gene was employed to assess the effects of the oligonucleotide on the mRNA level of P450arom in astrocytes. Treatment of cells with the oligonucleotide resulted in a dose-dependent inhibition of P450arom mRNA, whereas the sense oligonucleotide was without effect (data not shown).

Discussion

The present study demonstrated for the first time that astrocytes isolated from cerebral cortex and hypothalamus of neonatal rat brains are able to convert P_5 into DHEA. Cerebral cortical neurons also had the ability to produce small, but measurable, amounts of DHEA. The production of DHEA by astrocytes and neurons was confirmed by the expression of P450c17 mRNA, as demonstrated by RT-PCR/Southern blot analysis. The P450c17 enzyme in astrocytes and neurons is similar to that found in adult rat ovary and testis, as restriction digestion analysis of PCR products showed identical DNA fragments. The expression of P450c17 and the production of DHEA by astrocytes and neurons were further supported by the ability of these cells to convert P_4

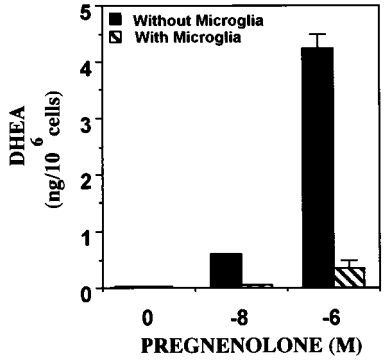

FIG. 5. Effect of residual microglia cells on DHEA production by cortical astrocytes, as determined by RIA. **, $P < 0.001$ (significant difference). Significant differences of at least $P < 0.001$ in DHEA production were found among the different groups of cortical astrocytes (in the presence and absence of residual microglia cells) treated with various doses of P_5.

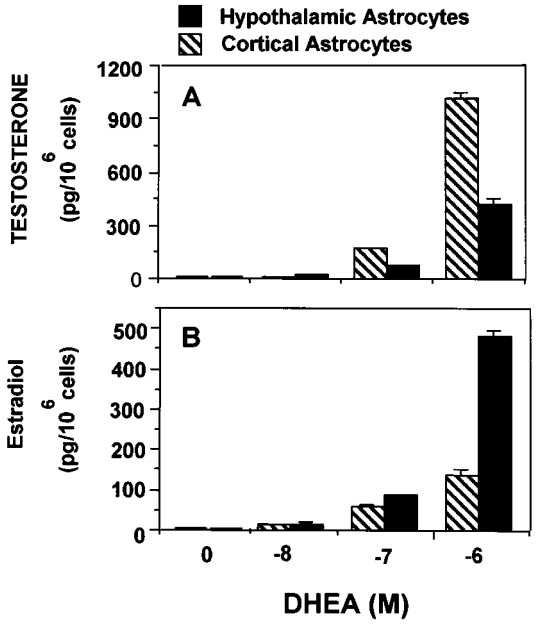

FIG. 6. Metabolism of DHEA to T (A) and E_2 (B) by hypothalamic and cortical astrocytes of neonatal rat brain, as determined by RIA. ns, No significant difference. *, $P < 0.05$; **, $P < 0.001$ (significant differences). Significant differences of at least $P < 0.05$ in T and E_2 production were found among different groups of cortical or hypothalamic astrocytes treated with various doses of DHEA.

to A_4, confirming the data reported by Ficher and Baker (38), who demonstrated the ability of cat brain tissues to metabolize P_4 to A_4. The expression of P450c17 and conversion of P_5 to DHEA by astrocytes and neurons were not artifact, as under the same culture conditions oligodendrocytes negatively expressed P450c17 mRNA and were unable to convert P_5 into DHEA. Moreover, addition of ketoconazole to astrocytes in cultures inhibited DHEA production in a dose-dependent manner. Ketoconazole is known to be a potent inhibitor of cytochrome P450 steroidogenic enzymes, including P450c17 (39–42).

Identification of P450c17 mRNA in astrocytes and neurons coupled with the ability of these cells to convert P_5 to DHEA reveals *de novo* biosynthesis of DHEA in brain via a P450c17-dependent mechanism and strongly supports the idea that DHEA is a neurosteroid. However, a hypothetical biochemical pathway for DHEA formation in brain was also suggested depending on the findings that treatment of brain tissues (43) and brain glial tumor cell lines (C6 rat glioma cells) (44) with various chemicals, especially $FeSO_4$, liberated P_5 and DHEA. The P450c17 inhibitor, SU-10603, did not reverse $FeSO_4$-induced DHEA formation in C6 cells, suggesting that $FeSO_4$ effect is mediated via a P450c17-independent mechanism (44). However, our data did not exclude the possibility that biosynthesis of DHEA in brain is also induced by another unknown enzyme(s).

The discrepancy between our data and those reporting the inability of mixed glial cells and astrocytes in cultures to metabolize P_5 to DHEA (12) and express P450c17 (11) may be attributed to the purity of cells, particularly to the presence of contaminant microglia cells in the cultures. In this study we attempted to eradicate microglia cells from glial cell cultures by treating cells with L-leucine methyl-ester, followed by successive plating of cells with systematic changing of culture flasks. By combining these successive purification methods, no cell expressing microglia/macrophage-specific protein marker could be detected. However, no effort was made by other studies (11, 12) to remove microglia from cell cultures. Residual microglia cells was reported in the primary astrocyte cultures (12). Our data presented evidence that the presence of microglia cells in astrocyte cell cultures resulted in a dramatic inhibition of the conversion of P_5 to DHEA. However, the mechanism of microglia action is not known. In gonads, NO, TNFα, and IL-1β are mediated gonadal macrophages-induced inhibition of androgen production by Leydig and thecal-interstitial cells (14–19) via inhibition of P450c17 gene expression (14–18). As microglia cells secrete high concentrations of NO, TNFα, and IL-1β in brain, it is possible that these factors may also mediate the microglia effect in inhibition of DHEA biosynthesis in astrocytes. However, further studies are required to determine the role of these macrophage/microglia cell-secretory factors in the regulation of steroidogenesis in astrocytes.

Brain tissues are able to metabolize DHEA to its hydroxylated metabolites, 7α-hydroxy-DHEA and 7β-hydroxy-DHEA, leading to the formation of androstenediol and androstenetriol. Astrocytes were reported to be responsible for this metabolic activity in the brain (45). In the present study we have demonstrated that astrocytes from hypothalamus and cerebral cortex are also capable of metabolizing DHEA to T and subsequently to E_2. This process requires the enzymatic activities of 3βHSD, 17βHSD, and P450arom enzymes that are responsible for the conversion of DHEA to A_4, A_4 to T, and T to E_2, respectively. The gene expressions and activities of 3βHSD (46), 17βHSD (Zwain, I., and S. S. C. Yen, unpublished data), and P450arom (27) have been demonstrated in astrocytes of rat brains, supporting the ability of these cells to metabolize DHEA to sex steroid hormones. The specificity of the metabolism of DHEA by astrocytes was confirmed by the ability of trilostane and antisense oligonucleotide to P450arom cDNA to inhibit the metabolism of DHEA to androgen and androgen to estrogen, respectively.

Hypothalamic astrocytes appear to be more active than

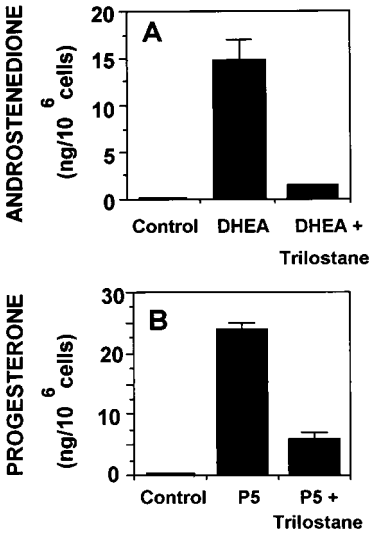

FIG. 7. Effect of trilostane on the conversion of DHEA to A_4 (A), and of P_5 to P_4 (B) by cortical astrocytes of neonatal rat brains, as determined by RIA. **, $P < 0.001$ (significant difference). Significant differences of $P < 0.001$ in A_4 and P_4 production were found among the different groups of astrocytes cultured alone, with steroid substrate, or with steroid substrate plus trilostane.

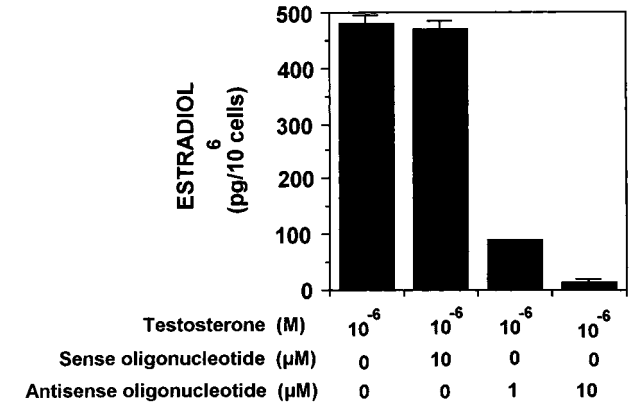

FIG. 8. Effect of an antisense oligonucleotide specific to P450arom cDNA on the aromatization of T to E_2 by hypothalamic astrocytes of neonatal rat brains as determined by RIA. ns, No significant difference. **, $P < 0.001$ (significant difference). Significant differences of $P < 0.001$ in E_2 production were found among the different groups of astrocytes given the various treatments.

cortical astrocytes in the metabolism of DHEA to estrogen, as reflected by the accumulation of E_2 in the cultured medium. These data are consistent with studies reporting high P450arom activity in hypothalamic tissues compared with other regions of rat brain (47). The high rates of production and metabolism of DHEA by hypothalamic astrocytes suggest that this hormone may be involved in the regulation of hypothalamic neuronal function, particularly GnRH neurons, whether directly or indirectly through its metabolite, E_2. However, further studies are required to address this issue.

Although DHEA sulfate (DHEAS) was abundantly present in brain tissues, DHEA sulfotransferase (D-Stase), the enzyme responsible for sulfonation of DHEA, was reported to be absent in whole human and rat brain tissues (48). The absence of D-STase protein (49) and its mRNA (50) was also reported in human brain tissues using immunohistochemical and Northern blot analyses, respectively. However, a low activity of D-STase was recently detected in several regions of brain, with the highest level in the hypothalamus and pons (51). The question remains to be answered whether this low activity of D-STase is responsible for the formation of DHEAS at high concentrations in the brain (51). Thus, biosynthesis of DHEAS in the brain remains an possibility. However, DHEAS was reported to be produced by human adrenal tumor tissues from P_5 sulfate (P_5-S) via a P450c17-dependent mechanism (52). As P_5-S is produced in high concentrations in brain, it is therefore possible that DHEAS is synthesized in the brain via the same mechanism. Further investigations are required to validate this suggestion using purified microglia-free astrocytes and neurons.

The exact role and significance of the biosynthesis of DHEA in brain are not yet known. However, several functional activities have been reported for DHEA in the central nervous system, being an inhibitor of the γ-aminobutyric acid$_A$ receptor (53) and an activator of *N*-methyl-D-aspartate (54) and σ-receptor (54). DHEA was also shown to increase memory and learning of adult rats (55). This effect may be explained by the ability of DHEA to increase neuronal communication in brain by increasing the extension of neuronal processes, enhancing the connection between isolated neurons, and increasing neuronal and glial survival and differentiation (55, 56). An antiaggression effect for DHEA was also reported; administration of DHEA to castrated male mice resulted in a decrease in the aggressive behavior of these animals toward lactating intruders (57). In a recent study, DHEA and DHEAS have also been shown to protect embryonic rat hippocampal neurons against excitatory amino acid-induced neurotoxicity, suggesting that these neurosteroids may be used as neuroprotective agents to reduce risk of age-related neurodegenerative disorders (58). A beneficial effect was reported for DHEA in reducing the risk of Alzheimer's disease (59). It has not yet been determined whether this effect is mediated by DHEA itself or by its metabolite, E_2, which has been demonstrated to decrease the risk of senile dementia associated with Alzheimer's disease in premenopausal women and to improve their cognitive performance (60). A remarkable correlation between blood DHEA/DHEAS and a feeling of well-being has also been reported by clinical studies of people 65 yr of age and older (61). The *de novo* biosynthesis of DHEA in brain, as demonstrated by the present study, may reveal a crucial role for this steroid hormone in the regulation of neuronal function in the central nervous system.

In conclusion, these results demonstrated for the first time that hypothalamic and cortical astrocytes *in vitro* express P450c17 steroidogenic enzyme and are able to synthesize and secrete DHEA and metabolize this hormone to testosterone and estradiol. Cortical neurons *in vitro* are also able to express a low level of P450c17 mRNA and produce a small amount of DHEA. However, it is not known whether astrocytes and neurons *in vivo* have the same capacity to produce DHEA as they do *in vitro* or whether these cells possess adequate enzymatic activity to metabolize DHEA to testosterone and estradiol *in vivo*. Further studies are required to address these

issues. The data of the present study suggest that, as in gonads and adrenal, the P450c17 enzyme is responsible for the biosynthesis of DHEA in the brain.

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