0013-7227/97/$03.00/0
Endocrinology

Vol. 138, No. 11
Printed in U.S.A.

Expression of Steroid Sulfatase during Embryogenesis*

NATHALIE A. COMPAGNONE†, EDUARDO SALIDO, LARRY J. SHAPIRO, AND SYNTHIA H. MELLON

Department of Obstetrics, Gynecology, and Reproductive Sciences (N.A.C., S.H.M.), the Metabolic Research Unit (S.H.M.), and the Department of Pediatrics (E.S., L.J.S.), University of California, San Francisco, California 94143; and Universidad de La Laguna (E.S.), Laguna, Spain

ABSTRACT

Neurosteroids are steroids that are synthesized *de novo* in the brain from cholesterol and, in general, mediate their effects through ion-gated channel receptors such as γ-aminobutyric acid$_A$ (GABA$_A$) and *N*-methyl-D-aspartate receptors rather than through classical nuclear steroid hormone receptors. Steroid hormones are known to exist not only as free compounds, but also as sulfated derivatives. Pharmacological studies indicate that unconjugated and sulfated steroids, such as pregnenolone and pregnenolone sulfate, may have opposite effects on GABA$_A$ receptors. Thus, pregnenolone acts as a potent positive allosteric modulator of γ-aminobutyric acid action at GABA$_A$ receptors, whereas pregnenolone sulfate acts as a potent negative modulator. Recent experiments also suggest that dehydroepiandrosterone and dehydroepiandrosterone sulfate may have distinct effects on growth of neurites from embryonic neocortical neurons *in vitro*. Thus, regulation of steroid sulfation may have profound behavioral and morphological effects on the nervous system. We, therefore, studied the developmental expression of the enzyme steroid sulfatase (STS), which converts sulfated steroids to free steroids. By *in situ* hybridization, STS messenger RNA was expressed in the embryonic mouse cortex, hindbrain, and thalamus during the last third of gestation. The sites of expression of STS were similar to those of P450c17, suggesting that these two enzymes may have concerted actions in similar functional processes. (*Endocrinology* **138:** 4768–4773, 1997)

STEROID sulfatase (STS) is an important enzyme in steroid metabolism because its activity increases the pool of precursors that can be metabolized by other steroidogenic enzymes to produce biologically active sex steroids (1, 2). The human STS gene has been cloned and mapped to Xp22.3 chromosome, proximal to the pseudoautosomal region (PAR), and the genetic aspect of this enzyme has been widely documented (3). A deficiency of STS activity results in severe ichthyosis caused by accumulation of steroid sulfates in the stratum corneum of the skin (4, 5). The main secretory product of the human fetal adrenal is dehydroepiandrosterone (DHEA) sulfate (DHEAS) (6). This steroid is not a substrate for the enzyme 3β-hydroxysteroid dehydrogenase in the adrenal and, therefore, is not metabolized further in the adrenal. DHEAS can be converted to 16α-hydroxy-DHEAS in the liver and other tissues, but it must undergo the sulfatase reaction in the placenta to be metabolized to estriol, the main estrogen of the placenta. Diminished or absent placental sulfatase activity reduces the pool of free DHEA available for placental conversion to estrogen, resulting in prolonged gestation and failure of induction of labor.

Previous studies have demonstrated STS activity in a variety of tissues other than the placenta, including the brain (7), and in virtually all tissues of the rhesus monkey (8), mouse macrophages (9), serum (10), human leukocytes (11), and testes (12). In general, high levels of STS activity have been found in tissues in which the biological activity of DHEA or its metabolites regulates cellular function.

We demonstrated that P450c17, the enzyme necessary for the synthesis of DHEA from pregnenolone, is transiently expressed in the neocortical subplate during embryogenesis (13). Furthermore, we recently demonstrated that DHEA and DHEAS affect the development of embryonic neocortical neurons *in vitro* (14). We further showed that these two steroids have different biological effects. Thus, regulation of the interconversion of these two steroids may play an important role in the development of the nervous system *in vivo*. We, therefore, studied the expression of STS in the developing rodent to characterize the distribution of this enzyme and thus determine the areas in the brain where sulfate hydrolysis may regulate neurosteroid activity. The rat and mouse STS have recently been cloned (15, 16), and we generated riboprobes from the mouse STS complementary DNA (cDNA) to study the expression of this enzyme by *in situ* hybridization. This report shows distribution of mouse STS during late embryogenesis. Our results show that the cortex, the hindbrain, and, interestingly, the thalamus, which is involved in supporting cortical differentiation during development (17), are sites in the central nervous system where STS is expressed during the last third of gestation. The sites of expression of STS are similar to those of P450c17, suggesting that these two enzymes may have concerted actions in similar functional processes.

Materials and Methods

Animals

Fresh frozen mouse embryos were obtained from BALB/c mice. The mating day (positive plug test) was referred to as embryonic day 0.5

Received May 14, 1997.

Address all correspondence and requests for reprints to: Synthia H. Mellon, Ph.D., Department of Obstetrics and Gynecology, University of California, Box 0556, San Francisco, California 94143-0556.

* This work was supported by NIH Grants HD-27970 and HD-11979, a grant from the Alzheimer's Association, and the Obstetrics and Gynecology Research and Education Foundation.

† Supported in part by funds from the Phillipe Foundation and the Singer Polignac Foundation.

(E0.5). Embryos were removed from the mother's uterus under anesthesia, dissected from the implantation site when possible (from E11.5), and quickly frozen in dry ice/2-methylbutane (isopentane). Frozen 8-μm sections were made on a cryostat.

Ribonuclease (RNase) protection assays

Mouse steroid sulfohydrolase (mSTS) cDNA fragments were subcloned into pSKII$^+$ (Stratagene, La Jolla, CA) for the synthesis of RNA probes. A 447-bp *Hin*dIII/*Bam*HI fragment of mouse STS cDNA (nucleotides 1289–1736) was used to prepare a 507-base mSTS probe. RNA was extracted from tissues using a Trizol messenger RNA (mRNA) extraction kit (Life Technologies, Grand Island, NY), and analyzed by RNase protection assays. Total RNA was analyzed from the following sources: E18.5 central nervous system tissues and E18.5 intestine (200 μg), E18.5 lung (150 μg), E18.5 skin (60 μg), and adult mouse cortex (160 μg). RNA was combined with 0.5×10^6 cpm ^{32}P-labeled riboprobe; precipitated with ethanol; resuspended in 80% formamide, 400 mM piperazine-*N*-*N*′-bis[ethanesulfonic acid] (pH 6.4), and 1 mM EDTA; boiled for 5 min; and incubated overnight at 42 C. Samples were diluted 10-fold with 10 mM Tris-HCl (pH 7.9), 300 mM NaCl, and 5 mM EDTA; treated with RNase A (30 μg/ml); incubated for 30 min at 37 C; treated with 0.25 μg/ml proteinase K and 0.5% SDS for an additional 15 min at 37 C; extracted once with phenol/chloroform; and precipitated with ethanol. Protected ^{32}P-labeled fragments were separated by electrophoresis on 6% polyacrylamide-7.5 M urea gels.

In situ hybridization

Mouse STS cDNA has 63% nucleotide identity with human STS and 75% nucleotide identity with rat STS (16). Furthermore, mouse STS does not share homology with other sulfatases, such as iduronate sulfate (mouse) and chondroitin sulfate (goat), because these sequences only have 36% nucleotide identity with the mSTS. A 447-bp *Hin*dIII-*Bam*HI (nucleotides 1289–1736) cDNA fragment of mSTS cDNA was subcloned into PSKII (Stratagene) for ^{35}S-labeled sense and antisense RNA probe synthesis. *In situ* hybridization was performed as previously described (18). Briefly, slides were fixed in 4% paraformaldehyde in PBS for 20 min at room temperature, and sections were treated with 1 μg/ml proteinase K in 100 mM Tris-HCl, pH 8, containing 50 mM EDTA and acetylated with acetic anhydride in 0.1 M triethanolamine buffer for 10 min at room temperature. Slides were hybridized overnight at 52 C with 10^6 cpm probe in buffer containing 50% formamide, 5 × SSC (1 × SSC = 0.15 M NaCl and 0.015 M sodium citrate), 10 mM β-mercaptoethanol, 10% dextran sulfate, 2 × Denhardt's solution (0.02% polyvinylpyrrolidone, 0.02% Ficoll, and 0.02% BSA), 250 μg/ml yeast transfer RNA, and 500 μg/ml salmon sperm DNA. Nonhybridized probe was washed from the slides with 2 × SSC containing 10 mM β-mercaptoethanol for 30 min at room temperature. Sections were then treated with 20 μg/ml RNase A at room temperature for 40 min, followed by two washes for 30 min each time at 55 C in 50% formamide-2 × SSC containing 1 mM EDTA and 10 mM β-mercaptoethanol and a wash for 2 min at room temperature in 0.2 × SCC. Slides were dipped in Ilford K5 nuclear emulsion (Warrington, PA), dried, and stored at 4 C until developed.

Results

RNase protection assay

STS mRNA was initially assayed using RNase protection assays. Due to the apparent low abundance of this mRNA in the developing embryo, it was necessary to use large quantities of RNA to detect STS mRNA in developing mouse brain regions (Fig. 1). On E18.5, we detected mSTS mRNA expression in the cortex and hindbrain, but we did not detect this mRNA in the basal ganglia or diencephalon of the mouse embryo. Using reverse transcription-PCR, we detected mSTS mRNA in the mouse diencephalon (not shown), suggesting that this mRNA was expressed at extremely low levels in this region.

We also studied the expression of mSTS mRNA in several

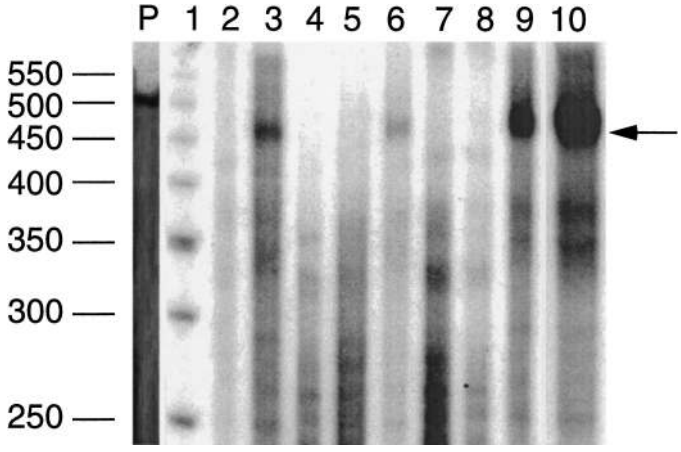

FIG. 1. RNase protection assay of STS mRNA in the mouse embryo. RNA from mouse E18.5 embryonic cortex (lane 3; 200 μg), basal ganglia (lane 4; 200 μg), diencephalon (lane 5; 200 μg), hindbrain (lane 6; 200 μg), intestine (lane 7; 200 μg), lung (lane 8; 150 μg), and skin (lane 9; 60 μg) as well as mouse adult cortex (lane 10; 150 μg) or transfer RNA alone (lane 2; 200 μg) was combined with 0.5×10^6 cpm ^{32}P-labeled mSTS complementary RNA probe (lane P), hybridized overnight, digested with RNase A, and separated on 6% acrylamide-7.5 M urea sequencing gels. Gels were exposed overnight. Lane 10 is from a shorter exposure (4 h) from an adjacent lane on the same gel. Mol wt markers (lane 1) are ^{32}P-labeled 50-bp DNA ladder fragments (Life Technologies), and their sizes are indicated on the *left* of the autoradiogram.

other tissues reported to have STS activity in the adult rhesus monkey (8). Expression of mSTS mRNA was detected in E18.5 intestine, but was not detected in E18.5 lung. STS mRNA was also not detected in skin.

In the adult rat, we detected STS mRNA in the cortex (using 160 μg total RNA). As the signal in the cortex was robust, we used less RNA from other regions of the adult brain (thalamus, hypothalamus, hindbrain, and cerebellum; 25–90 μg) and detected no STS mRNA in these regions.

In situ hybridization

We detected STS mRNA in cartilage primordia and in the liver on E14.5 (Fig. 2A). In the liver, STS mRNA was observed in restricted areas (Fig. 2E). In cartilage, expression of mSTS mRNA was first observed at the base of the skull and in the cartilage of the ribs and vertebrae (E14.5). Later in ontogeny (E16.5–E18.5), expression of mSTS mRNA was observed in the cartilage of the temporal and occipital regions of the head as well as in the facial region, the eye, and the ear (Fig. 2, B, C, F, and G). Figure 2D shows a higher magnification of the vertebral cartilage of the rib.

Later in ontogeny (E16.5), the strongest signal was observed in the spleen and the inner epithelial layer of the intestine (Fig. 2H). As expected, mSTS mRNA was strongly expressed in the skin of E16.5 embryos (Fig. 2I).

In the central nervous system, a hybridization signal was first observed on E16.5. At this time, STS mRNA was found in the thalamus (Fig. 3C). By E18.5, STS mRNA was found in the cortex, hippocampus, thalamus, cerebellum, and spinal cord (not shown), and expression in these regions persisted 9 days after birth (Fig. 3B). Expression of STS mRNA in the

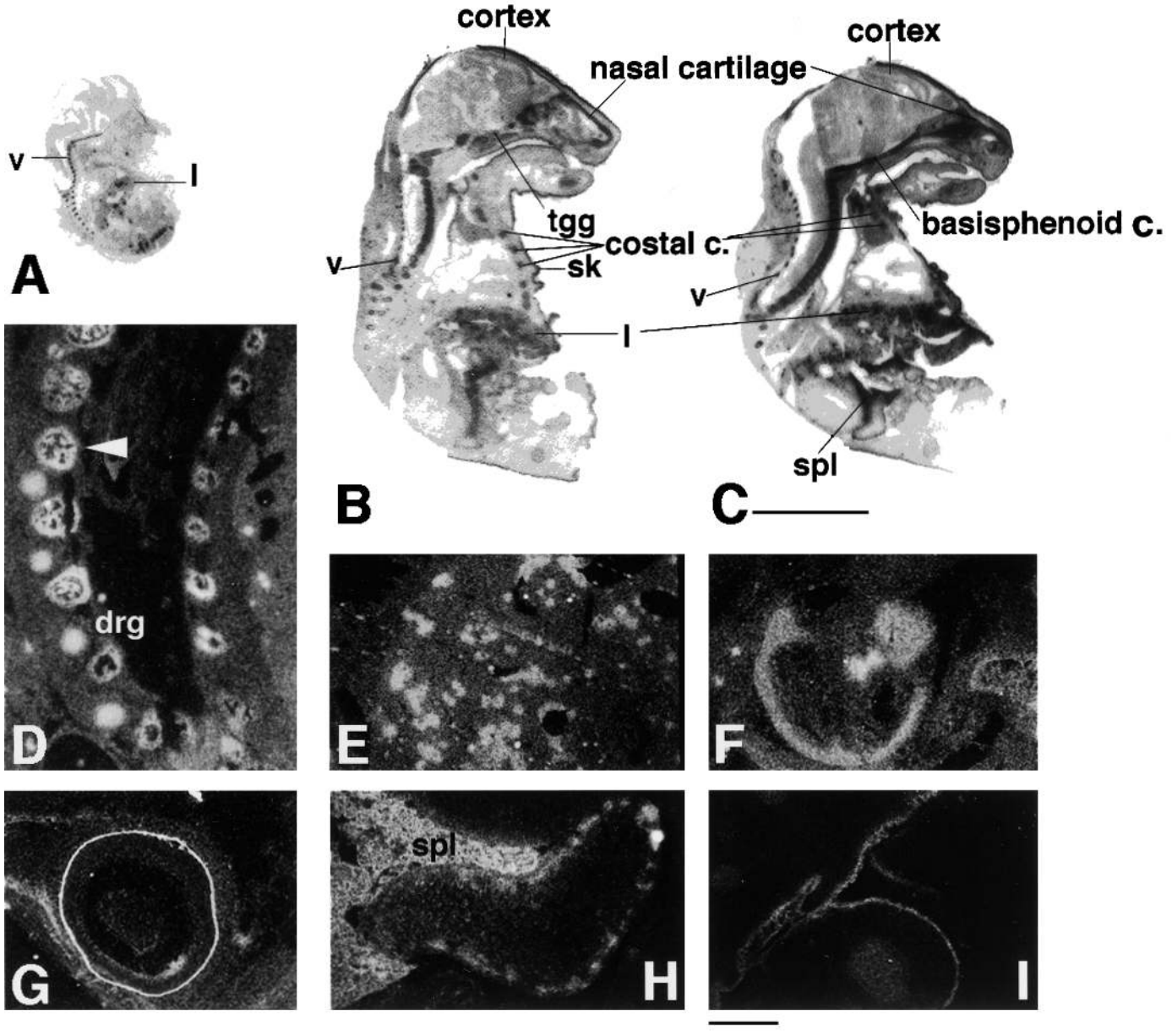

FIG. 2. *In situ* hybridization of STS mRNA in the mouse embryo. A–C, Autoradiogram of an *in situ* hybridization for mSTS mRNA in E14.5 mouse embryo (A) and E18.5 mouse embryo (B and C). A, Hybridization signal is observed in cartilage primordium in the skull and vertebrae (v). In the liver (l), a strong hybridization signal is localized in restricted areas. B and C, In E18.5 embryos, the hybridization signal was also observed in vertebrae (v) and liver (l) as well as in the trigeminal ganglia (tgg), skin (sk), spleen (spl), nasal cartilage, basisphenoid cartilage (basisphenoid c.), costal cartilage (costal c.), and cortex. D–I, Darkfield microscopy of E16 embryo sections after *in situ* hybridization for mSTS mRNA. D, Strong staining is observed in vertebral cartilage (*arrow*) as well as in dorsal root ganglia (drg). E, In the liver the hybridization signal is not evenly distributed. Note the presence of strong signals around blood vessels. F, Cochlea; staining of the ear cartilage and of the spiral ganglia. G, Signal in the eye cartilage and retina. H, Spleen (spl) and outer membrane of the intestine. I, Skin at the level of the paw. A–C are at the same magnification. The *bar* in C represents 1 cm. D–I are at the same magnification. The *bar* in I represents 1 mm.

cortex and cerebellum was diffuse, suggesting that mSTS mRNA may be expressed in cells not restricted to anatomical nuclei. By contrast, expression of mSTS mRNA in the thalamus was highly selective and was found in a small number of cells whose distribution suggested that its expression was restricted to a particular anatomical domain of the anterior thalamus (Fig. 3D). Hybridization signals for mSTS mRNA persisted in 9-day-old neonatal mouse brains (Fig. 3, B and D) in the (subicular area) CA1 region of the hippocampus and in the anterior thalamus.

From E16.5–E18.5, mSTS mRNA was also expressed in the peripheral nervous system. A signal less intense than that in the thalamus was observed in cranial ganglia (trigeminal ganglia and spiral ganglia; Fig. 2F) and in dorsal root ganglia (Fig. 2D) as well as in the inner neural layer of the retina (Fig. 2G).

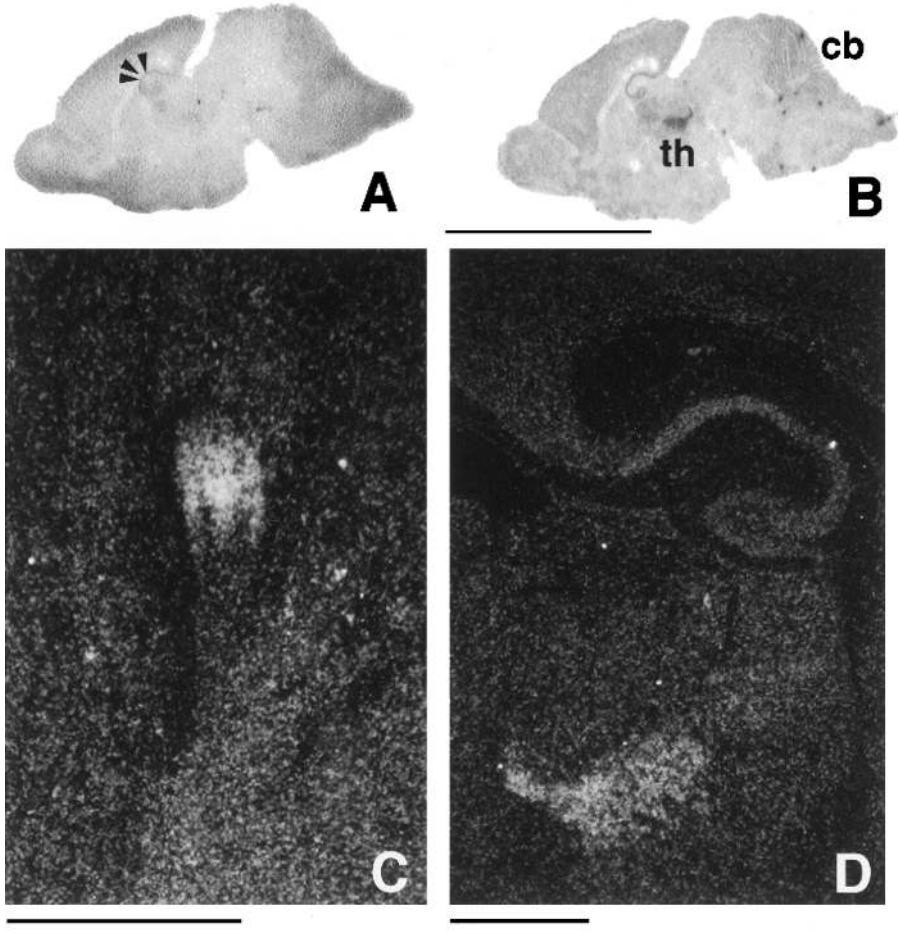

FIG. 3. *In situ* hybridization of STS mRNA in the thalamus. Autoradiogram (A and B) and darkfield microscopy (C and D) of mSTS *in situ* hybridization in 9-day-old (P9; A, B, and D) and E16.5 (C) thalamus. A, Nonspecific hybridization signal observed after annealing reactions with the sense mSTS probe. Some nonspecific binding is observed in hippocampus (*arrowheads*). B, Sister section after hybridization with the antisense mSTS probe; a strong signal is observed in the thalamus (th) and the subicular region (CA1) of the hippocampus, and a less intense signal is seen in the cerebellum (cb). In the subicular area of the hippocampus, the density of silver grains with the antisense probe (B) is greater than that with the sense probe (A). C, Hybridization signal in the thalamus is first observed on E16.5. D, Darkfield microscopy and higher magnification of the thalamic staining in the neonate (P9). The signal is mainly located in the anterior thalamus. A and B are at the same magnification; the *bar* in B represents 7 mm. The *bars* in C and D represent 1 mm.

To ensure the specificity of the hybridization signal, we performed *in situ* hybridizations on sister sections of postnatal day 9 mice brains using a sense RNA probe (Fig. 3A). The sense probe did not show any hybridization signal in the thalamus. There was a weak signal in the dentate gyrus and in Ammon's horn, which probably represents nonspecific RNA binding, but no signal was observed in the subicular area, suggesting that the hybridization signals observed using the STS antisense RNA probe were specific.

Discussion

Sulfated steroids are ubiquitously distributed compounds with many putative biological functions (reviewed in Ref. 3). Sulfated forms of 3β-hydroxysteroids (such as dehydroepiandrosterone) have been thought to be inert forms of more active hormones that are released by the action of STS. Our results show a restricted expression of STS mRNA during mouse embryonic development. The sites of STS expression provide more precise indications of the target tissues in which active, nonsulfated steroid hormones resulting from STS activity may act during embryogenesis. Our results are also consistent the demonstration of STS activity in various tissues of the adult rhesus monkey that have been suggested to be able to form biologically active androgens and estrogens (8).

In addition to the known roles of STS in the placenta and skin, our results emphasize other possible roles for STS in the function of other organs during embryogenesis. In the liver the signal is clustered in restricted areas resembling blood islands, suggesting that STS may be active in the hematopoiesis signaling processes. Spleen expression of mSTS is very high, and this strongly suggests a role for mSTS in lymphocyte production. Sulfatase activity, recently found in endothelial cells and monocytes, has been shown to be an important regulator of the inflammatory response, as the efficiency of this response relies on the ability of leukocytes, platelets, and/or endothelial cells to degrade the subendothelial basement membrane, which is considered to be an important barrier to the entry of leukocytes into inflammatory sites (19). Sulfatase activity in endothelial cells, but not in monocytes, could be induced by tumor necrosis factor, interleukin-1, and interleukin-8 (19).

Expression of STS mRNA in the developing cartilage early during embryogenesis coincides with the appearance of ossification centers (E14–E14.5), suggesting that this enzyme may participate in the differentiation of the skeletal system. Consistent with our observation of mSTS mRNA expression in developing ossification centers, STS may continue to play an important role in the maintenance of bones throughout life. The conversion of estrone sulfate to estradiol plays an important role in modulating bone turnover. Estrone sulfatase activity was detected in the MG63 human osteoblast cell

line (20), suggesting that development of osteoporosis in postmenopausal women may be due to a reduction in the activity of the enzymes responsible for local estrogen production.

Expression of mSTS mRNA in the skin only appeared on E16.5, concomitant with the formation of the multilayered structure of the epidermis and with a peak in expression of cholesterol sulfatase, another sulfatase involved in epidermal differentiation (21).

In the central and peripheral nervous systems, the expression of mSTS mRNA may be related to the expression of steroidogenic enzymes involved in neurosteroid synthesis. We have previously shown the expression of P450c17 in restricted areas of the developing nervous system (13), particularly in the neocortex, and demonstrated a role for both DHEA and DHEAS in neocortical neuron differentiation. Although DHEA promotes axonal growth, DHEAS promotes dendrite growth and cell clustering (14). The expression of STS in the neocortex, hippocampus, and thalamus may regulate the ratio of DHEA/DHEAS. Furthermore, as the thalamus has been shown to influence the survival of the cortical subplate (17), where we detected P450c17-expressing neurons, the ratio of these two steroids may play crucial roles in controlling axonal *vs.* dendritic growth of cortico-thalamic fibers during neocortex organization. The afferent and efferent connections of the cerebral neocortex develop simultaneously toward the end of embryogenesis. At this stage, the neocortex comprises two main cell-dense layers: the thicker and more superficial cortical plate (future layers 2–6) and the thinner underlying subplate. Many early thalamocortical projections temporarily innervate the subplate before leaving it to locate their ultimate targets in the overlying cortical plate. The subplate then disappears. In addition, subplate neurons have been shown to prime thalamic connections in the cortex (22, 23). Hence, we propose that thalamic conversion of DHEA to DHEAS in the thalamus may be a regulatory mechanism controlling axonal growth of thalamic fibers during neocortex cytoarchitectural organization. Furthermore, as DHEA has been shown to modulate $GABA_A$ and *N*-methyl-D-aspartate receptor activities (24–29), STS activity in the thalamus might be considered a regulatory process of the thalamic neurosecretion.

STS expression most likely persists beyond embryonic development into adulthood. In earlier studies assaying STS activity, investigators detected abundant quantities of STS activity in the central nuclei of the thalamus of two adult males, aged 44 and 63 yr, who died of lymphatic leukemia and pulmonary embolus, respectively (30). The thalamus had the greatest amount of STS activity, which was about twice as much activity as any other region per g tissue. The hippocampus and hypothalamus had lesser, but still substantial, amounts of activity. Many other regions of the brain contained less activity. Thus, the thalamus may continue to be a central region for coordinating the relative abundance of sulfated and free steroids in the brain throughout life.

In addition to possible roles in axonal and dendritic growth during embryogenesis, others have proposed a role for DHEAS in memory in adult humans and rodents (31–35). Chemical inhibition of STS activity *in vivo* was associated with changes in memory in adult rats (31, 36). Thus, regulation of STS activity in the thalamus and hippocampus may have implications for learning and/or memory enhancement throughout life.

The distribution of STS mRNA in other regions of the central and peripheral nervous systems is compatible with a concerted action of both STS and P450c17 in similar processes during embryogenesis. The concept of functional regulation of some neurosteroids by their sulfation or sulfate hydrolysis, suggested by our previous data (14) and others (reviewed in Ref. 37) is thus supported by the localization and ontogeny of mSTS mRNA.

References

1. **Shapiro L,** The genetics of the steroid sulfatases. International Symposium on DHEA Transformation into Androgens and Estrogens in Target Tissues: Intracrinology, Quebec, Canada, 1995
2. **De Meio R** 1975 Sulfate activation and transfer. In: Greenberg DM (ed) Metabolism of Sulfur Compounds. Academic Press, New York, vol 7:287–359
3. **Ballabio A, Shapiro LJ** 1995 Steroid sulfatase deficiency and X-linked ichthyosis. In: Schriver CR, Beaudet AL, Sly WS, Valle D (eds) The Metabolic and Molecular Bases of Inherited Disease. McGraw-Hill, New York, pp 2999–3022
4. **Mohandas TK, Stern HJ, Meeker CA, Passage MB, Muller U, Page DC, Yen PH, Shapiro LJ** 1990 Steroid sulfatase gene in XX males. Am J Hum Genet 46:369–376
5. **Shapiro LJ** 1982 Steroid sulfatase deficiency and x-linked ichthyosis. In: Stanbury JB, Wyngaarten JB, Fredrickson DS, Goldstein JL, Brown MS (eds) The Metabolic Basis of Inherited Diseases, ed 5. McGraw-Hill, New York, pp 1027–1034
6. **Miller, W, Tyrell J** 1994 The adrenal cortex. In: Felig P, Baxter J, Frohman L (eds) Endocrinology and Metabolism. McGraw-Hill, New York, pp 555–711
7. **Iwamori M, Moser HW, Kishimoto Y** 1976 Steroid sulfatase in brain: comparison of sulfohydrolase activities for various steroid sulfates in normal and pathological brains, including the various forms of metachromatic leukodystrophy. J Neurochem 27:1389–1395
8. **Martel C, Melner MH, Gagne D, Simard J, Labrie F** 1994 Widespread tissue distribution of steroid sulfatase, 3 beta-hydroxysteroid dehydrogenase/delta 5-delta 4 isomerase (3 beta-HSD), 17 beta-HSD 5 alpha-reductase and aromatase activities in the rhesus monkey. Mol Cell Endocrinol 104:103–111
9. **Hennebold JD, Daynes RA** 1994 Regulation of macrophage dehydroepiandrosterone sulfate metabolism by inflammatory cytokines. Endocrinology 135:67–75
10. **Sugawara T, Honke K, Gasa S, Tanaka T, Fujimoto S, Makita A** 1994 Serum levels of steroid sulfatase protein in gynecologic carcinomas. Clin Chim Acta 226:13–20
11. **Miyakawa I, Kawano Y, Taniyama K, Mori N** 1994 Steroid sulfatase activity in human leukocytes. Gynecol Obstet Invest 38:191–193
12. **Valencia-Sanchez A, Ortega-Corona BG, Dominguez-Vargas O** 1993 Effectiveness of calcium and magnesium on testicular sulfatase activity. Arch Androl 30:129–136
13. **Compagnone NA, Bulfone A, Rubenstein JLR, Mellon SH** 1995 Steroidogenic enzyme P450c17 is expressed in the embryonic central nervous system. Endocrinology 136:5212–5223
14. **Compagnone NA, Mellon SH,** Dehydroepiandrosterone: a potential signalling molecule for neocortical organization during development. Proc Natl Acad Sci USA, in press
15. **Li XM, Salido EC, Gong Y, Kitada K, Serikawa T, Yen PH, Shapiro LJ** 1996 Cloning of the rat steroid sulfatase gene (Sts), a non-pseudoautosomal X-linked gene that undergoes X inactivation. Mamm Genome 7:420–424
16. **Salido EC, Li XM, Yen PH, Martin N, Mohandas TK, Shapiro LJ** 1996 Cloning and expression of the mouse pseudoautosomal steroid sulphatase gene (Sts). Nat Genet 13:83–86
17. **Price DJ, Lotto RB** 1996 Influences of the thalamus on the survival of subplate and cortical plate cells in cultured embryonic mouse brain. J Neurosci 16:3247–3255
18. **Compagnone NA, Bulfone A, Rubenstein JLR, Mellon SH** 1995 Expression of the steroidogenic enzyme P450scc in the central and peripheral nervous systems during rodent embryogenesis. Endocrinology 136:2689–2696
19. **Bartlett MR, Underwood PA, Parish CR** 1995 Comparative analysis of the ability of leucocytes, endothelial cells and platelets to degrade the subendothelial basement membrane: evidence for cytokine dependence and detection of a novel sulfatase. Immunol Cell Biol 73:113–24
20. **Purohit A, Flanagan AM, Reed MJ** 1992 Estrogen synthesis by osteoblast cell lines. Endocrinology 131:2027–2029
21. **Kagehara M, Tachi M, Harii K, Iwamori M** 1994 Programmed expression of cholesterol sulfotransferase and transglutaminase during epidermal differentiation of murine skin development. Biochim Biophys Acta 1215:183–189

22. **McConnell SK, Ghosh A, Shatz CJ** 1989 Subplate neurons pioneer the first axon pathway from the cerebral cortex. Science 245:978–982
23. **McConnell SK, Ghosh A, Shatz CJ** 1994 Subplate pioneers and the formation of descending connections from cerebral cortex. J Neurosci 14:1892–1907
24. **Harrison NL, Simmonds MA** 1984 Modulation of GABA receptor complex by a steroid anesthetic. Brain Res 323:284–293
25. **Majewska MD, Harrison NL, Schwartz RD** 1986 Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. Science 232:1004–1007
26. **Majewska MD, Schwartz RD** 1987 Pregnenolone-sulfate: an endogenous antagonist of the gamma-aminobutyric acid receptor complex in brain? Brain Res 404:355–360
27. **Majewska MD** 1990 Steroid regulation of the GABAA receptor: ligand binding, chloride transport and behaviour. Ciba Found Symp 153:83–97
28. **Puia G, Santi MR, Vicini S, Pritchett DB, Purdy RH, Paul SM, Seeburg PH, Costa E** 1990 Neurosteroids act on recombinant human GABAA receptors. Neuron 4:759–765
29. **Fahey JM, Lindquist DG, Pritchard GA, Miller LG** 1995 Pregnenolone sulfate potentiation of NMDA-mediated increases in intracellular calcium in cultured chick cortical neurons. Brain Res 669:183–188
30. **Perumal AS, Robins E** 1973 Regional and subcellular distribution of aryl-and steroid sulfatases in brain. Brain Res 59:349–358
31. **Li PK, Rhodes ME, Burke AM, and Johnson DA** 1997 Memory enhancement mediated by the steroid sulfatase inhibitor (*p*-*O*-sulfamoyl)-*N*-tetradecanoyl tyramine. Life Sci 60:L45–L51
32. **Diamond DM, Branch BJ, Fleshner M** 1996 The neurosteroid dehydroepiandrosterone sulfate (DHEAS) enhances hippocampal primed burst, but not long-term, potentiation. Neurosci Lett 202:204–208
33. **Flood JF, Smith GE, Roberts E** 1988 Dehydroepiandrosterone and its sulfate enhance memory retention in mice. Brain Res 447:269–278
34. **Flood JF, Roberts E** 1988 Dehydroepiandrosterone sulfate improves memory in aging mice. Brain Res 448:178–181
35. **Yoo A, Harris J, Dubrovsky B** 1996 Dose-response study of dehydroepiandrosterone sulfate on dentate gyrus long-term potentiation. Exp Neurol 137:151–156
36. **Li PK, Rhodes ME, Jagannathan S, Johnson DA** 1995 Reversal of scopolamine induced amnesia in rats by the steroid sulfatase inhibitor estrone-3-*O*-sulfamate. Brain Res Cogn Brain Res 2:251–254
37. **Majewska MD** 1991 Neurosteroids: GABA-agonistic and GABA-antagonistic modulators of the $GABA_A$ receptor. In: Costa E, Paul SM (eds) Neurosteroids and Brain Function. Thieme, New York, vol 8:109–117