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Cytochrome P450 in the Brain: Neuroendocrine Functions

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The effectiveness of steroid hormone metabolites as sedatives and anesthetics has been known for many years. More recently, their interaction with neurotransmitter receptors has helped to elucidate their mechanism of action, but their physiological functions and their role in disturbances of behavior, anxiety, and sleep/wakefulness have yet to be elucidated. Until 1981 it was assumed that metabolites of steroid hormones arose from the adrenals and gonads and that their action on neurotransmitter receptors was a mechanism of communication between the brain and the periphery. The evidence that the brain could accumulate steroids independently of the adrenals and gonads in 1981 and later the evidence for the presence of the cholesterol side chain cleavage enzyme (P450scc) in the brain have challenged this concept and stimulated a great deal of interest in the possibility that the brain could be making its own steroids from cholesterol for some as yet undefined purpose. In this review we examine the data pertaining to the role of brain P450 in the synthesis and degradation of neurosteroids. We summarize the data on the presence of P450scc in the brain and try to answer the following questions: (1) Does P450scc in the brain contribute significantly to the synthesis of $GABA_A$ receptor active steroids? (2) Can the P450scc in the brain account for the accumulation of pregnenolone in the brain? (3) Is there evidence for special functions of the pregnenolone synthesized in the brain? (4) Is there a role for other forms of brain P450 in neurosteroid action? Key Words: steroid hormone metabolites; neurotransmitter; cytochrome P450.

INTRODUCTION

Since the discovery of aromatase (P45019) in the brain and its role in imprinting of sexual behavior (33), the possibility that various forms of P450 enzymes in the brain could be involved in novel functions has fascinated many neuroendocrinologists. In the past decade, multiple forms of P450 have been identified in the brain (for review see 49) and several novel functions have been suggested. These include: an important role in regulating the brain level of neurosteroids, steroids which are synthesized in the brain or in the periphery and which interact with $GABA_A$ receptors and influence mood, sleep/wakefulness, memory, and aggression (3, 7, 9, 37); synthesis and elimination of arachidonic acid metabolites which influence cerebral vascular tone (1, 4) and

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mediate the effects of dopamine on peptide release from neurons (18, 42); and metabolism, inactivation, and/or elimination of neurotoxins, substances which are of dietary or environmental origin or are toxic metabolites of endogenous compounds and which are thought to be causative agents in degenerative diseases of the CNS (46). In addition, it has become clear that the level and the pattern of P450 isozymes in the brain can be altered by physiological factors, environmental chemicals, and pharmaceutical agents (49). Since many of these induced forms of P450 metabolize steroid hormones, their induction in the brain is likely to influence the hormonal milieu of the brain and possibly feedback mechanisms at the level of the hypothalamus and pituitary.

In this review we examine the evidence for the existence of certain P450 enzymes in the brain, in particular those involved in neurosteroid metabolism. The major metabolites of the hormones progesterone and deoxycorticosterone (3α-hydroxy ring-A reduced pregnane steroids) are potent $GABA_A$ receptor agonists. They are present in the brain and their levels fluctuate in response to stress and during the menstrual cycle (13, 36). In this analysis we will not discuss the effectiveness of 3α,5α-C21 steroids as efficient, physiological ligands of the $GABA_A$ receptor but will focus on whether the ligands are synthesized from cholesterol in the brain and inactivated by brain P450s. If the brain makes signal molecules from steroids independently of the peripheral steroidogenic tissues, then it is unlikely that these molecules form part of the feedback communication between the brain and the adrenals and gonads. In fact, the brain would have to be able to distinguish between pregnenolone coming from the periphery and the pregnenolone it synthesizes for itself to accomplish "special tasks." In the only other case where the brain synthesizes a steroid (i.e., estrogen), the substrate is produced in the neonatal testis, and the brain and gonads communicate with each other to ensure that male sexual behavior is established in genetically determined males. The big question is, therefore, whether the brain does synthesize steroids from cholesterol and if so what is the function of these steroids.

We also examine the role of various forms of P450 in the brain in the synthesis and elimination of neurosteroids. We discuss the quantitation and localization of these enzymes, the importance of elucidating the mechanisms involved in their regulation, and some of the efforts to relate their presence in the brain to physiological functions.

EVIDENCE FOR THE SYNTHESIS OF DHEA AND PREGNENOLONE IN THE BRAIN

In 1981 a landmark paper (6) was published by Baulieu and colleagues which led to the to term "neurosteroids." These steroids, mainly dehydroepiandrosterone (DHEA) and pregnenolone and their sulfate(s), appeared to be synthesized in the brain and this evidence stimulated a great interest in the brain as a steroidogenic organ. The pertinent data from this paper are summarized in Table 1. What the paper showed was that 15 days after the gonads and adrenals had been removed, the level of dehydroepiandrosterone sulfate (DHEAS) in the

DHEA Sulfate in Rats

	Intact	Sham 2 days	Sham 15 days	ORX + ADX 2 days	ORX + ADX 15 days
Plasma ng/ml	0.26	0.59	0.28	0.40	0.36
Brain ant. ng/g	1.56	5.77	1.19	4.35	1.63
Brain post. ng/g	4.89	13.19	2.98	8.99	1.92
Ratio brain/Plasma	6 (Ant.)	9.7	4.2	10	4.5
	18 (Post.)	22	10	22	5.3

Note. Data from (6).

posterior part of the brain decreased from the control value of 4.89 to 1.92 ng/g. In sham-operated animals the value was 2.98. Importantly, the plasma levels of DHEAS in controls, sham-operated rats, and animals which had been both adrenalectomized and castrated were 0.26, 0.28, and 0.36 ng/ml, respectively. Therefore, it can be safely concluded from these data that DHEAS accumulates in the brain. However, since the plasma level of this steroid did not decrease, it cannot be concluded that the brain levels are due to synthesis in the brain.

The source of the persistent plasma level of DHEAS in the absence of adrenals and gonads remains to be determined. It may result from slow release from fat stores. High concentrations of DHEA and other steroids have been found in human fat (8), which can act as a depot for steroids. Another contributing factor for the persistent level of this steroid could be a diminished capacity of the liver to eliminate steroids after gonadectomy in male rats (50).

In the case of pregnenolone, the level in the brain falls to 60% of the control level after adrenalectomy and gonadectomy, while the plasma level decreases from a control value of 2 to 0.2 ng/ml (39). However, high plasma levels of a pregnenolone–lipid conjugate persist up to 30 days after removal of the steroidogenic organs (39). The source of this pregnenolone–lipid conjugate has not been identified but its presence in plasma leaves open the possibility that maintenance of the level of pregnenolone in the brain is also due to transfer of the steroid to the brain from a pregnenolone depot. More recently, Koreyev *et al.* (19) have measured the level of DHEA and pregnenolone sulfate in the brains of adrenalectomized and castrated rats 2 days after surgery. They found the levels of DHEA in the brain to be unchanged and the concentration of pregnenolone sulfate to be 50% of that in sham-operated rats and claimed this as evidence for the production of these steroids in the brain "independent of a contribution from the peripheral endocrine glands." The Baulieu study (6) clearly showed that 2 days after surgery there is a substantial increase in the brain and plasma content of these steroids, probably due to the stress of the surgery. The high brain levels of these steroids in the Koreyev study (19) may be a reflection of this postsurgical stress and the claim that there is brain synthesis may not be warranted. Several laboratories have looked for more compelling evidence for synthesis of steroids from cholesterol in the brain. Much effort has been devoted to detection of the two forms of P450 involved in the synthesis of

pregnenolone and DHEA, i.e., the side chain cleavage enzyme, P450scc, and P45017α.

SEARCH FOR P450SCC AND P45017α IN THE BRAIN

To date there is no evidence for the presence of the 17α-hydroxylase or 17,20-lyase and, therefore, no evidence that DHEA can be made in the brain. Consequently, it must be assumed that high levels of this steroid in the brain are due to accumulation from the plasma. In the case of the synthesis of pregnenolone from cholesterol, there is immunohistochemical evidence for the presence of P450scc in the brain (25, 26), and its mRNA has been detected (32), but catalytic activity cannot be measured in brain slices, homogenates, or mitochondrial fractions. Indirect evidence for catalytic activity in the brain comes from the presence of P450scc in oligodendrocytes (14, 15, 17) and glioma cells in culture (10, 34) and from the accumulation of pregnenolone in the brain and in isolated brain mitochondria when cholesterol transport into mitochondria is stimulated (19, 40). What needs to be examined carefully is whether the activity which can be measured in oligodendrocytes is likely to account for the levels of pregnenolone in the brain; whether the accumulation of pregnenolone in mitochondria, when a stimulator of cholesterol transport is used, represents P450scc activity; and finally, whether the levels and distribution of P450scc in the brain are compatible with the postulated role of this enzyme in production of signal molecules involved in auto- or paracrine functions.

If P450scc in the brain is involved in synthesis of signal molecules, the product of the enzymatic reaction would have to be formed in concentrations high enough to be of physiological significance. A low overall activity in the brain could be physiologically important if the enzyme were highly localized, probably in specific cells in the brain not accessible to pregnenolone from the periphery. The widespread distribution of P450scc in the white matter revealed in the immunohistochemical staining pattern suggests that this is not the case (25, 26). Immunohistochemical data are not sufficient proof for the presence of an enzyme in a tissue and are usually complemented with other supportive evidence for the presence of the protein. This is particularly true of P450scc in the brain, where the level is low and it must be rigorously demonstrated that the antibody is recognizing the antigen of interest. On Western blots with mitochondrial protein, Papadopoulos *et al.* (34) found that, with the same anti-P450scc antibody used in the immunohistochemical studies, signals obtained from C6-2B glioma cells were similar to those from Y-1 cells and MA-10 mouse Leydig cells. In view of the much lower catalytic activity in the glioma cells than that in Y-1 cells (see below), the strong signal on Western blots in the glioma cells is disturbing. It raises the possibility that the antibody is recognising another antigen in these cells or that there is a lot of catalytically inactive P450scc in these cells. With the same antibody, we could not detect signals of the correct size on Western blots when mitochondrial proteins from various

brain regions, up to 100 μg/lane, were loaded onto the gel. We were also unable to detect P450scc signals on Western blots with P450 prepared from brain regions of control rats (48). However, in pregnant rats signals of the correct size were apparent. Under the conditions of our Western blots, the antibody recognized several other bands on the gels. Despite the strong signal of the correct size, we could not detect P450scc activity in mitochondrial fractions from the brain regions of pregnant rats. Our inability to detect a P450scc signal on Western blots with brain mitochondria is consistent with the results in other laboratories, which show that the catalytic activity cannot be measured in brain slices or brain mitochondria (26). Therefore, from the information available in the literature and from our own studies, it is not possible to be certain that the staining pattern revealed in the immunohistochemical studies represents that of P450scc.

Evidence for P450scc catalytic activity in the brain has been obtained by two methods. In one case, radiolabeled mevalonate (a precursor of cholesterol), or cholesterol itself, was added to oligodendrocytes in culture and the production of radiolabeled pregnenolone and its metabolites assessed by their R_f on TLC systems (14, 15, 17). The concentration of mevalonate used in these studies was 33 p*M* and the products were measured 12 h or more after addition of the substrate. The data are expressed as fmol pregnenolone and 20-hydroxypregnenolone produced/mg DNA and this can be converted to a value of 7.5 fmol/mg cellular protein/12 h, a very low activity. When oligodendrocyte mitochondria from 21-day-old Sprague–Dawley male rats were incubated with 100 n*M* [^{3}H]cholesterol, [^{3}H]pregnenolone and [^{3}H]pregnene-3β,20α-diol were both formed at a rate of 2.5 pmol/mg protein/h. These are not initial rates and the substrate concentrations are not optimal. The experiments were done to demonstrate the presence of P450scc in oligodendrocytes and not to study the kinetics or quantitate the amount of enzyme. Clearly there is P450scc activity in oligodendrocytes, but it is doubtful whether this low activity can account for the levels of pregnenolone in the rat brain after adrenalectomy and gonadectomy.

The alternative method of measuring pregnenolone synthesis is to incubate mitochondria under appropriate conditions and measure the change in the mitochondrial content of pregnenolone after some defined time. The basal rate of pregnenolone synthesis may not reflect the P450scc content of the mitochondria since the rate-limiting step in the overall reaction is the transport of cholesterol into the mitochondria (21, 35). In mitochondria isolated from the brains of adrenalectomized and castrated rats, the rate of synthesis expressed as pmol pregnenolone formed/mg mitochondrial protein/min is 2 (19). The corresponding rates of synthesis in mitochondria from glioma cells (34) and in Y-1 adrenal cells (21) are 1 and 3, respectively. A more meaningful comparison is obtained after maximal stimulation of cholesterol uptake into mitochondria. This is usually done by addition of ligands for the peripheral benzodiazepine receptor, a receptor system which is involved in regulating cholesterol uptake and transport from the outer to the inner mitochondrial membrane (22, 35).

From the data of Kreuger and Papadopoulos (21), if cholesterol transport is stimulated with ligands for the peripheral benzodiazepine receptor, the value for pregnenolone synthesis in adrenal Y-1 cells increases from 3 to approximately 16. If in addition, the cells are pretreated with ACTH, the value is close to 40. The same laboratory has shown that in C6-B2 glioma cells the rate of pregnenolone accumulation upon addition of a peripheral benzodiazepine receptor ligand is increased to 3 pmol/mg mitochondrial protein/min from a control value of 1 pmol/mg mitochondrial protein/min (34). The surprising data are from Romeo *et al.* (40), who found a basal level for pregnenolone synthesis in brain mitochondria of 2 pmol/mg mitochondrial protein/min and a stimulated value of 19. This high rate of synthesis of pregnenolone in brain mitochondria is very puzzling. It is similar to that in adrenal Y-1 cells. Since pregnenolone was measured by RIA, the issue of the specificity of the antibody must be raised. This issue has been discussed by Morfin *et al.* (32) because the antibody recognized something in the sciatic nerve which could not be identified as pregnenolone by GC-MS. It remains possible that something other than pregnenolone is also recognized in the brain.

In another study, Korneyev *et al.* (19) found an increase in the brain level of pregnenolone upon administration of 4′-chlorodiazepam (a ligand for the peripheral benzodiazepine receptor) to rats *in vivo*. In these rats, the level of pregnenolone in the brain increased from a control value of 10 to a value of 25 ng/g in 5 min. The accumulation was blocked by α-amino glutethimide, a P450scc inhibitor, indicating that it was synthesized from cholesterol. The accumulation of pregnenolone was transient and, after reaching a maximum increase at 10 min, decreased to control values by 30 min. These data indicate a dynamic pool of pregnenolone in the brain with a high rate of synthesis and utilization. The change in the brain content of pregnenolone was 15 ng/g tissue in 5 min. If the yield of mitochondria is 20 mg/g, then the rate of synthesis of pregnenolone would have to be 150 pmol/mg mitochondrial protein/min. This is an extremely high rate, and this raises the question of whether the increase in the brain level is due to mechanisms other than P450scc activity. One possibility is that the ligands for the benzodiazepine receptor affect the uptake of pregnenolone into the cell and mitochondria. The experiment which argues against this explanation of the data is the inhibition of the accumulation of pregnenolone by aminoglutethamide, an inhibitor of P450scc. However, aminoglutethamide inhibits several other enzymes which metabolize steroids so it cannot be excluded that the drug could bind to and inhibit the activity of a pregnenolone transport protein. The rapid disappearance of the extra pregnenolone from the brain deserves a comment. The pregnenolone disappeared, although its conversion to progesterone was blocked by the presence, during the experiments, of trilostane, an inhibitor of 3β-hydroxysteroid dehydrogenase isomerase. Interestingly, no pregnenolone accumulated in the absence of trilostane, indicating that conversion to progesterone is an important pathway for the disposition of pregnenolone.

BRAIN NEUROSTEROID SYNTHESIS AND CONTROL OF BEHAVIOR

Administration of neurosteroids to rats or human beings results in changes in behavior (9, 12, 30). What is more difficult to demonstrate is that neurosteroids synthesized in the brain from cholesterol have effects on behavior. Several attempts have been made to demonstrate such effects. If male rats are habituated to the company of other males and then exposed to the scent of females for 7 days, there is a decrease in the amount of pregnenolone in the olfactory bulbs. This phenomenon has been observed in two independent laboratories (5, 23). It has been suggested that there may be involvement of pregnenolone in an olfactory pathway related to sex recognition (26). In one case where only pregnenolone was measured, the decrease was by 45%, and in the other case, where both pregnenolone and pregnenolone sulfate were measured, the decrease was by 74%. There did not appear to be any significant changes in brain regions other than the olfactory lobes. Before the significance of these changes in the pregnenolone content of the olfactory lobes can be evaluated, it is necessary to know if the release of pregnenolone from the adrenals is diminished upon exposure of male rats to the scent of females. Exposure to the scent of females is probably a normal condition for a male rat while exclusive exposure to males may be considered a more stressful condition and could lead to increase in the adrenal secretion of pregnenolone and corticosterone. It is well documented that, in rats, there is a circadian variation in the plasma levels of pregnenolone with a peak which precedes that of corticosterone (39). The question of whether there are changes in the levels or the secretion pattern of steroids from the adrenal when males are exposed to females would have to be verified before the data can be evaluated. Since the olfactory lobes have a higher content of pregnenolone than other brain areas, they may be more sensitive to changes in plasma levels of this steroid.

In adult male rats after acute swim stress, allopregnanolone and allotetrahydrodeoxycorticosterone (THDOC) levels were increased in the cerebral cortex (38). For allopregnanolone the increase was from 5 to 12 ng/g. In stressed adrenalectomized rats the brain level was 3 ng/g. THDOC was not detectable in the brains of adrenalectomized stressed rats, but in stressed intact rats the brain level was 0.7 ng/g. As discussed above, there is a measurable level of pregnenolone in the brain 14 days after adrenalectomy. Since the level of alloprogesterone in these animals was below that in the nonstressed rats, it can be concluded that the pregnenolone in the brain is not used for the synthesis of allopregnanolone.

In contrast to this study, Romeo *et al.* (41) have presented evidence that the brain of adrenalectomized and castrated rats does synthesize allopregnanolone and that there is a role of this brain-synthesized neurosteroid in post-training, long-term memory consolidation. These experiments involved use of adrenalectomized and castrated rats in three behavioral studies: (1) passive avoidance experiments, (2) training of rats to exit a water maze, and (3) training of rats to obtain food in a radial maze. Once the rats had been trained, the administration of an NMDA receptor antagonist impaired the learned behavior. The effect

of the NMDA antagonist could be prevented by the administration of drugs which increased cholesterol uptake into mitochondria or by administration of pregnenolone sulfate, allopregnanolone, or THDOC. The beneficial effect of pregnenolone sulfate was found to be dependent on the activity of steroid 5α-reductase, indicating that the active molecule was allopregnanolone. Although these complicated pharmacological manipulations provide evidence for an effect of neurosteroids on memory, there is no conclusive evidence that these steroids are synthesized from cholesterol in the brain. The possibility exists that the precursor of these steroids is pregnenolone taken up from the plasma. It is not known whether there is increased uptake of pregnenolone from the plasma in response to the pharmacological agents which were used to increase cholesterol transport into mitochondria. The notion that there is regulation of pregnenolone transport into the brain is not totally speculative, since a pregnenolone binding protein has been described in the brain (24) and a role for such a protein in storage and transport of pregnenolone has been suggested (44).

The beneficial effect of neurosteroids on post-training, long-term memory consolidation is unexpected since GABA agonists produce cognition deficits. The authors (41) propose an explanation for the unexpected role of neurosteroid GABA receptor function in memory. The neurosteroids are proposed to act on astroglial $GABA_A$ receptors because of their high content of γ-1 subunits. This interaction with astroglial receptors would lead to secretion into the extracellular space of substances which can modulate GABAergic or glutaminergic synaptic functions. These experiments and their interpretation reflect the complexity of the model chosen and the difficulty of demonstrating physiological functions of neurosteroids.

If GABA receptor ligands are produced in the brain as a result of exposure to stress, this metabolism might well represent an important pathway for modulation of the stress response, and the factors which regulate the level of these steroids need to be understood. Pregnenolone is not only a precursor of GABA receptor agonists, but also the precursor of a potent GABA receptor antagonist, pregnenolone sulfate (27, 28). The mechanisms involved in regulating the balance between the levels of GABA receptor agonists and antagonists remain to be elucidated. Perhaps in order to obtain a clearer picture of the changes in GABA receptor activity in response to stress, pregnenolone sulfate levels as well as those of pregnenolone, allopregnanolone, and THDOC should be measured.

A BRAIN P450 INVOLVED IN THE HYDROXYLATION OF 3β,5α STEROIDS: ROLE IN REGULATING NEUROSTEROID LEVELS

The levels of 3α,5α steroids in the brain are determined both by their rate of synthesis and by their inactivation and/or elimination. We propose that inactivation occurs by *in situ* metabolism through the activity of a specific form of brain cytochrome P450.

Cytochrome P450 is present in the brain at a concentration of approximately 1% of the liver level (49). In the liver steroid 16β- and 16α-hydroxylases are

abundant. They have a broad substrate specificity in that they accept estrogens and 5α-reduced or Δ5 steroids and represent elimination pathways for steroid hormones. These hydroxylations are undetectable in the brain. In contrast, one of the major forms of P450 in the brain is a very specific steroid hydroxylase with a requirement for substrates with the 3β,5α configuration (47). This enzyme is not detectable in the liver and kidney, but is abundant in the prostate, pituitary, and brain (11, 16, 45). It does not accept 3α-hydroxy steroids, testosterone, or androstenedione, and dihydrotestosterone (DHT) is a poor substrate. When the steroids in the brains of adult rats, rabbits, and dogs were extracted and identified, 3α- and 3β-dihydroprogesterone (3α-OH-DHP and 3β-OH-DHP) were present in approximately equal amounts (29); yet major metabolites found in rat brain after injection of [^{3}H]progesterone are DHP or after *in vitro* incubation of brain tissue with [^{3}H]progesterone are 3α-OH-DHP and 20α-OH-DHP (2, 20). One explanation for the reported absence of 3β-OH-DHP formation in adult brain could be that, in analogy with androgen metabolism in prostate (16), hydroxylation of 3β-OH-DHP represents an important elimination pathway and it is rapidly converted to polar metabolites.

We have investigated the role of brain steroid 3β,5α-hydroxylase in regulating the levels of the anesthetic steroid, 3α-OH-DHP (43). In analogy with the elimination of androgen from its target tissues, it is the 3β- and not the 3α-OH-DHP which represents the major pathway for the formation of more polar metabolites and thus the elimination of the 5α-reduced metabolites of progesterone from target tissues. No polar metabolites were formed when 3α-OH-DHP was incubated with microsomal fractions prepared from rat brain, but 3β-OH-DHP was hydroxylated at the 6α- and 7α positions. The rate of this P450-catalyzed reaction is 200 pmol/mg microsomal protein/min. This should be compared to the activity of aromatase in the brain which is 0.6 pmol/mg microsomal protein/min and P450scc which, as was discussed above, is at most 20 pmol/mg mitochondrial protein/min.

To test the hypothesis that the hydroxylation of 3β-OH-DHP represents a pathway for regulation of the level of 3α-OH-DHP in the brain, the effect of inhibition of the hydroxylation of 3β-OH-DHP on the duration of 3α-OH-DHP-induced anesthesia was examined. The nonanesthetic steroid, 5α-androstane-3β,17β-diol (3β-Adiol) was used as a competitive inhibitor of the metabolism of 3β-OH-DHP. The duration of anesthesia upon iv administration of 3α-OH-DHP was increased by 33% when 3β-Adiol was coadministered. It seems likely that, in the central nervous system, P450-catalyzed hydroxylation of 3β-OH-DHP is a degradative pathway which could play an important role in regulation of the levels of the neuroactive steroid 3α-OH-DHP.

CONCLUSIONS

We set out to examine the available data and determine whether brain P450 plays a role in regulating the level of neurosteroids in the brain. We must conclude that although neurosteroids have powerful actions on the brain, the

evidence for their synthesis from cholesterol in the brain is not compelling. Instead, it seems that the precursors of neurosteroids are pregnenolone, progesterone, and glucocorticoids synthesized in the periphery. Part of the physiological response to stress is release of glucocorticoids from the adrenal cortex. Subsequently these steroids are converted in the brain to the $GABA_A$ receptor agonists which probably modulate the stress response by reducing anxiety. Allopregnanolone could be synthesized from pregnenolone or progesterone and pregnenolone sulfate can be formed from pregnenolone sequestered from plasma. One important question is therefore, what regulates the level and duration of action of these powerful neuronal regulators. If metabolites of pregnenolone can be both $GABA_A$ receptor agonists and antagonists, what determines the balance between the two? The answer to this question is not yet available but part of the answer may be found in the specific brain P450, steroid 3β,5α-hydroxylase. This enzyme, one of the most abundant forms of P450 in the brain, can influence the concentration of 3α,5α steroids in the brain by elimination of the 3β,5α steroids. The cellular localization, regulation, and interindividual variation of this P450 may be factors in the regulation of neurosteroid levels in the brain.

Finally, from the foregoing analysis of the data in the literature, is there experimental evidence for a physiological function of P450scc in the brain? Since the mRNA of P450scc is detectable, the likelihood is that some protein can be expressed in the brain but P450scc mRNA abundance in brain or primary glial cultures was approximately 0.01% of that found in the adrenal. Whether under normal conditions this results in enough enzyme to produce physiologically relevant concentrations of pregnenolone still remains an open question.

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