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Distribution and Ontogeny of 3α-Hydroxysteroid Dehydrogenase in the Rat Brain

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3α-Hydroxysteroid dehydrogenase in the brain is responsible for production of neuroactive tetrahydrosteroids that interact with the major inhibitory gamma-aminobutyric acid receptor complexes. Distribution of 3α-hydroxysteroid dehydrogenase in different regions of the brain in rats was evaluated by activity assay and by Western immunoblotting using a monoclonal antibody against liver 3α-hydroxysteroid dehydrogenase as the probe. The olfactory bulb was found to contain the highest level of 3α-hydroxysteroid dehydrogenase activity, while moderate levels of the enzyme activity were found in other regions such as cerebellum, cerebral cortex, hypothalamus and pituitary. Some activities were found in the rest of the brain such as amygdala, brain stem, caudate putamen, cingulate cortex, hippocampus, midbrain, and thalamus. The protein levels of 3α-hydroxysteroid dehydrogenase in different regions of the brain as detected by Western immunoblotting are comparable to those of the enzyme activity. No sexual dimorphism was found in either the concentration levels or the activities of the brain 3α-hydroxysteroid dehydrogenase. At the time of birth, the rat brain already expresses a significant level of 3α-hydroxysteroid dehydrogenase; the levels of brain 3α-hydroxysteroid dehydrogenase activity in rats continue to rise during the first week after their birth, and reach a plateau thereafter.

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INTRODUCTION

Stress, as defined by Selye [1], develops in three stages: an initial alarm reaction, characterized by an immediate sympathoadrenomedullary discharge, a subsequent "stage of resistance", characterized by the activation of the hypothalamic–pituitary–adrenocortical axis (HPA), and, finally, by exhaustion. Two hypothalmic peptides which appear to play an important role in stress-related HPA activation are the corticotropin-release factor (CRF) and vasopressin (AVP) [2]. The secretion of both CRF and AVP to the portal system of the anterior pituitary during stress prompts an increase in the release of adrenocorticotropin (ACTH) into circulation [3, 4]. The elevated ACTH levels stimulate the release of glucocorticoids and progestins from the adrenal gland [5]. As these steroids reach the brain, they are quickly converted to tetrahydrosteroids by sequential reactions involving two brain enzymes: steroid 5α-reductase and 3α-hydroxysteroid dehydrogenase. The production of the tetrahydrosteroid metabolites is rapid and robust, but transient, and may only last an hour or so [6]. Recent studies have demonstrated that the steroid hormone metabolites, both tetrahydrodeoxycorticosterone (THDOC) and tetrahydroprogesterone (THP), serve as agonist-ligands for the major inhibitory $GABA_A$ receptor complex [7, 8]. Interaction between these tetrahydrosteroids and the $GABA_A$ receptor complex induces an anxiolytic response that may protect the neurons from overstimulation and therefore preserve the homeostasis of the central nervous system (CNS) [9].

Because the neuroactive tetrahydro-steroid metabolites induce anxiolytic, analgesic, anesthetic, and anticonflict behavior in animals and humans, it has been postulated that the lack of these steroid metabolites may cause adverse effects in brain functions [10]. As 3α-hydroxysteroid dehydrogenase is the key brain enzyme involved in the production of neuroactive tetrahydrosteroid hormones, its distribution in brain may have significant implications with regard to the physiology and pathology of brain functions. In order

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to characterize the enzyme we have previously made several monoclonal antibodies and used these monoclonal antibodies as probes to isolate a full-length cDNA encoding the rat liver 3α-hydroxysteroid dehydrogenase (3α-HSD) and several human cDNAs encoding enzymes structurally related to the rat 3α-HSD [11–14]. In this communication we studied the distribution of 3α-HSD by activity assays and by Western immunoblotting.

MATERIALS AND METHODS

Preparation of cytosolic proteins: rats (200–250 g) were sacrificed by carbon dioxide euthanasia. Brains were removed and homogenized in a solution containing 0.25 M sucrose and 10 mM Tris–HCl, pH 7.2. The homogenate was centrifuged at 10,000*g* for 10 min. Supernatant containing cytosols and microsomes was recovered and subjected to a second centrifugation at 100,000*g* for 30 min. The supernatant containing cytosolic proteins was recovered for protein assay as described by Lowry [15].

3α-HSD assays

3α-HSD activities were measured by a radioactive procedure [16]. The reaction mixture contained 300 μg of brain cytosolic protein, 20 nmol of dihydrotestosterone, 20 nCi of [^{14}C 5α-dihydrotestosterone in 900 μl of 100 mM sodium phosphate buffer, pH 7.2. The reaction mixture was preincubated at 37°C for 3 min prior to the addition of 100 μl of 10 mM NADPH to start the reaction. The reaction was allowed to continue for 10 min and was stopped by the addition of 5 ml methylene chloride. Dihydrotestosterone and androsterone were extracted into methylene chloride using three successive 5 ml aliquots of the solvent. The solvent was dried under a stream of nitrogen. The dry residue was dissolved in 100 μl of methylene chloride and applied to a TLC plate. The plate was developed using a mixed solvent system of chloroform–ethyl acetate–ethanol 4:1:0.6, by vol). Radioactive spots were localized by autoradiography.

Immunoblotting

Proteins were subjected to electrophoresis in a 8% polyacrylamide–0.1% SDS gel and then electrotransferred to a nitrocellulose filter [11]. The filter was sequentially treated with 3% bovine serum albumin (BSA) in phosphate buffered saline (PBS), hybridoma supernatant and goat antimouse IgG–horseradish peroxidase conjugate. The proteins which interacted with monoclonal antibody were visualized by incubating the filter with a solution containing 4-chloronaphthol and hydrogen peroxide.

RESULTS

Immuno-cross reactivity between the rat liver and brain enzyme

Figure 1 shows the Western immunoblotting of the 3α-HSD in liver as well as in the brain using two monoclonal antibodies, MAbs 7D3 and 3G6, made against the liver enzyme. These two monoclonal antibodies have been shown to interact with the liver 3α-HSD at different epitopes [11]. Both MAbs 7D3 and 3G6 recognized a single protein in the liver, the olfactory bulb and the brain. The molecular size of the protein in these different tissues appear to be the same. The intensities of the protein bands detected by MAb 3G6 are much lighter than those detected by MAb 7D3, which is likely due to a lower binding affinity of MAb 3G6.

Distribution of 3α-HSD in different regions of the brain

The regional expression of the 3α-HSD in the brain was first analyzed by Western immunoblotting using MAb 7D3 as the probe. As shown in Fig. 2, expression of 3α-HSD protein was detectable in every part of the brain that was analyzed. Nonetheless, the levels of 3α-HSD protein as reflected by the intensities of the protein bands on the blots vary in different areas of the brain. We have performed at least five blots which showed reproducible band patterns. For example, the protein band in the olfactory bulb was the strongest. Other regions, such as cerebellum, cerebral cortex, hypothalamus, and pituitary, contained less but moderate amounts of the 3α-HSD protein. Much less 3α-HSD was found in amygdala, brain stem, caudate putamen, cingulate cortex, hippocampus, midbrain and thalamus.

A similar pattern of 3α-HSD activities was also seen in various regions of the brain. The brain tissues from four animals were used for the activity assays. As shown in Fig. 3, the olfactory bulb also contains the highest level of 3α-HSD activity. Moderate levels of 3α-HSD activity were also found in cerebellum, cerebral cortex, hypothalamus, and pituitary. This suggests that the differences in activities of 3α-HSD in various regions of the brain are due to their different levels of 3α-HSD enzyme.

Regulation of the brain 3α-HSD

In the following studies, the brain cytosols from at least four rats were prepared for the activity assays. The activities obtained from four different rats were averaged for comparison. The effect of androgen, estrogen and progestin on the expression of the brain 3α-HSD was evaluated by analyzing the 3α-HSD activities in the brains of the male, female and pregnant rats. No significant difference in the 3α-HSD activity was found in the brain of these three groups (data not shown), suggesting that steroid hormones are not involved in the regulation of the 3α-HSD in the brain.

Neither did short term administration (4 days) of progesterone, corticosterone or deoxycorticosterone affect the levels of 3α-HSD in adult male rats (data not shown). Adrenalectomy which intended to eliminate the production of steroid hormones also did not affect the 3α-HSD activities in the rat brain.

Figure 4 shows the brain 3α-HSD activities at different developmental stages. At the time of birth the newborn rats already expressed a modest level of 3α-HSD. During the first week after the birth the 3α-HSD activity rises by approx. 40%. The activities remained the same thereafter.

DISCUSSION

Over the last 50 years since Selye [10] demonstrated that 3α-tetrahydrosteroids induced anxiolytic, analgesic and anticonvulsive effects in animals, the mechanism by which these neuroactive steroids interact with neurons at molecular levels has been well delineated. As demonstrated by competitive ligand binding, these 3α-tetrahydrosteroids interact with the major inhibitory $GABA_A$ receptor complex near the binding site for barbiturates [15]. Upon binding to the $GABA_A$ receptor, tetrahydrosteroids induce a prolonged opening of the chloride channel leading to the transport of chloride ions and hyperpolarization of the neurons. As a result of the modulating function of 3α-tetrahydrosteroids on $GABA_A$ receptor complex, it has been speculated that some psychiatric disorders, such as depression, premenstrual syndrome, and anxiety, may be due to abnormality in the metabolism of neuroactive steroid metabolites [17, 18]. Since 3α-HSD is one of the key enzymes responsible for the production of the neuroactive tetrahydrosteroids, its expression and regulation may have direct implication to various functions of the

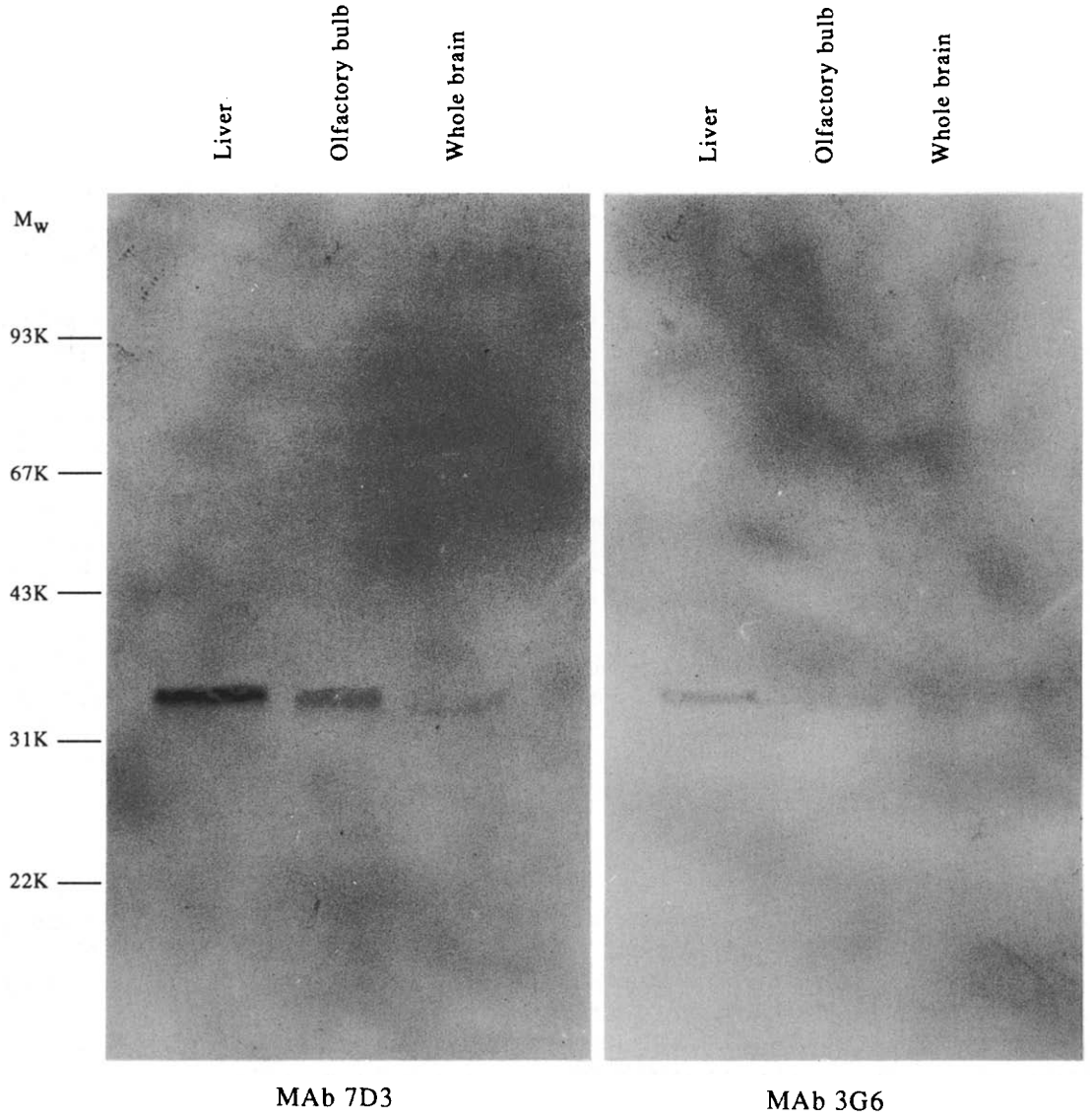

Fig. 1. Immunological cross-reactivity between the liver and the brain 3α-HSD. 2 μg of the liver cytosols were used for the immunoblot, whereas 300 μg of the olfactory bulb and the whole brain cytosols were used. The positions of the protein size markers are shown on the left side of the blots.

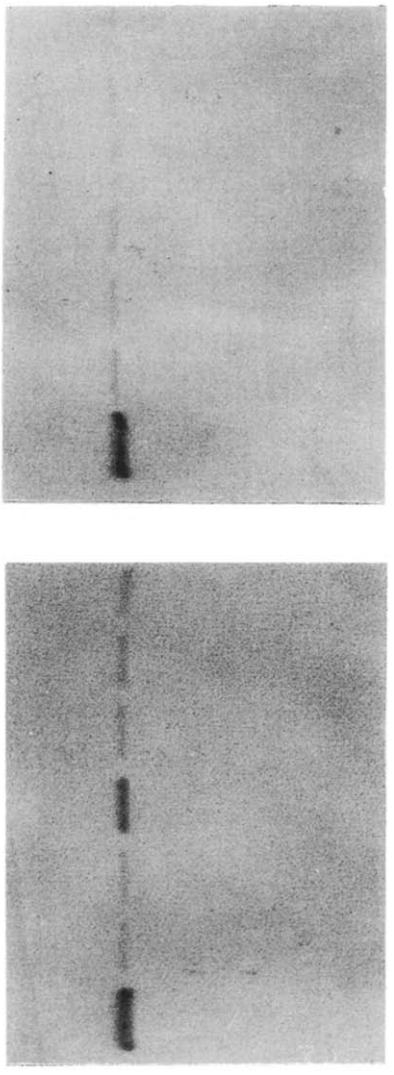

Fig. 2. Western immunoblotting of 3α-HSD in different regions of the brain using monoclonal antibody MAb 7D3. Each lane contains 300 μg of the cytosolic proteins. The positions of the protein size markers are shown on the left side of the blot.

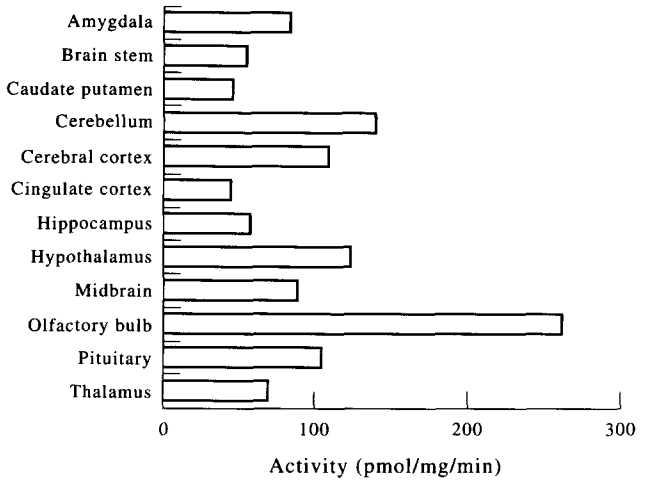

Fig. 3. 3α-HSD activities in various regions of the rat brain. Assay of 3α-HSD activity is described in Materials and Methods.

tetrahydrosteroids in the brain. It is rather interesting that the olfactory bulb contains a much higher concentration of the 3α-HSD than any other area of the rat brain. Since $GABA_A$ receptors in the olfactory bulb are involved in odor processing, it is possible that the neuroactive steroid metabolites may play a role in the odor coding pathway [19].

Deprivation of steroid hormones by adrenalectomy did not affect the levels of 3α-HSD in the brain; neither did administration of various steroid hormones affect the enzyme activity in the brain. No sex difference was found in the brain 3α-HSD activity. In contrast, the liver 3α-HSD has been shown to exhibit sexual dimorphism. These results suggest that the regulation of brain enzyme, which is steroid hormone-independent, is different from that of the liver enzyme.

Several groups of researchers have suggested the existence of multiple 3α-HSD in the liver [20, 21]. Penning *et al.* [22] have suggested that the brain enzyme is much less active than the liver enzyme. However, in this study we found that the liver and the brain enzyme are immunologically identical. Using monoclonal antibodies made against the liver enzyme to screen a λgt11 cDNA library derived from the rat brain we have isolated a cDNA clone that showed identical sequence to that of the liver enzyme (data not shown). Our studies, therefore, suggest the enzymes expressed in the liver and the brain are identical.

It was recently found that the syndrome of Apparent Mineralocorticoid Excess may be due to deficiency in 3α-HSD activity [23]. In order to understand the molecular genetics of 3α-HSD deficiency we have isolated and sequenced several cDNAs and their corresponding genes encoding human enzymes that are structurally related to rat 3α-HSD [13]. Since these human cDNAs are also expressed in the brain, it is tempting to speculate that a defect in the brain 3α-

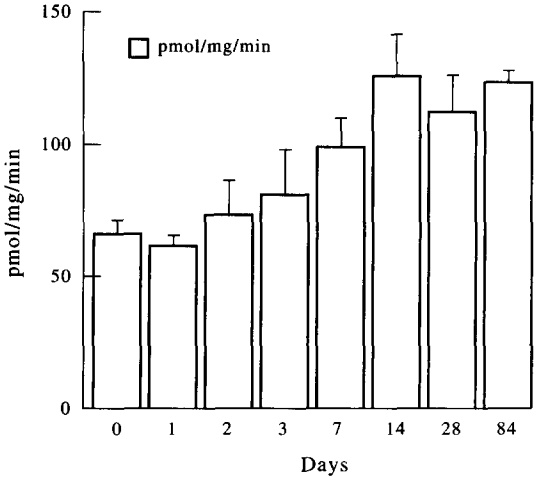

Fig. 4. Ontogeny of the 3α-HSD in the brain. Each solid bar represents the mean ± standard deviation of at least four individual assays. Assay of the enzyme activity is described in Materials and Methods.

HSD may cause adverse effects in the development of the CNS and/or psychiatric dysfunction. Future research on the polymorphism of human 3α-HSD and its relevance to brain function may shed light on the physiological role of 3α-HSD in the brain.

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