Specific Binding of Dehydroepiandrosterone to the N Terminus of the Microtubule-associated Protein MAP2*

Emmanuelle Laurine†§, Daniel Lafitte†§, Catherine Grégoire‡§, Eric Sérécé‡, Erwann Loret‡, Soazig Douillard§, Bernard Michel**, Claudette Briand†, and Jean-Michel Verdier‡ ‡‡

From the †École Pratique des Hautes Études, Université Montpellier II, Place Eugène Bataillon, CC94, 34095 Montpellier cedex 05, France, ‡UMR CNRS 6632, Faculté de Pharmacie, 13385 Marseille cedex 05, France, and **Unité de Neurogériatrie, Hôpital Sainte-Marguerite, 13385 Marseille cedex 05, France

THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 278, No. 32, Issue of August 8, pp. 29979–29986, 2003

Printed in U.S.A.

© 2003 by The American Society for Biochemistry and Molecular Biology, Inc.

29979

This paper is available on line at http://www.jbc.org

Received for publication, March 28, 2003, and in revised form, May 12, 2003
Published, JBC Papers in Press, May 29, 2003, DOI 10.1074/jbc.M303242200

The effect of neurosteroids is mediated through their membrane or nuclear receptors. However, no dehydroepiandrosterone (DHEA)-specific receptors have been evidenced so far in the brain. In this paper, we showed by isothermal titration calorimetry that the DHEA specifically binds to the dendritic brain microtubule-associated protein MAP2C with an association constant of 2.7 × 10^7 M^-1 and at a molar ratio of 1:1. By partial tryptic digestions and mass spectrometry experiments, we found that the binding involved the N-terminal region of MAP2C. Interestingly, MAP2C displays homologies with 17β-hydroxysteroid dehydrogenase 1, an enzyme required for estrogen synthesis. Based on these sequence homologies and on the x-ray structure of the DHEA-binding pocket of 17β-hydroxysteroid dehydrogenase 1, we modeled the complex of DHEA with MAP2C. The binding of DHEA to MAP2C involved specific hydrogen bonds that orient the steroid into the pocket. This work suggests that DHEA can directly influence brain plasticity via MAP2C binding. It opens interesting ways for understanding the role of DHEA in the brain.

The microtubule-associated proteins (MAPs)1 are characterized by their ability to promote tubulin polymerization and to stabilize microtubules. MAP2 is one of the most abundant MAPs in the brain. A single gene containing 20 exons encodes multiple MAP2 isoforms, which are produced by alternative splicing of a pre-mRNA (1). MAP2 isoforms have been divided into two groups depending on their molecular weight. High molecular weight MAP2s include MAP2A and MAP2B, whereas low molecular weight MAP2s include MAP2C and MAP2D. Expression of MAP2 isoforms is regulated during development. MAP2A is mostly expressed in adult brain, whereas MAP2B is present all along the development of the nervous system (2). Conversely, MAP2C is expressed at early developmental stages but is also found in adult retina and olfactory bulb, tissues in which neurogenesis persists in the adult stage (3). MAP2 belongs to a family of cytoskeletal proteins and is predominantly expressed in dendrites of neurons (4). It regulates neurite extension (5) and is associated with the development of neuronal polarity (6). All in all, these observations suggest that MAP2 plays a significant role in neuronal plasticity.

Regulation of MAP2 involves steroids such as estradiol or progesterone (7). Interestingly, these steroids are also synthesized in the brain for its own use and are therefore called neurosteroids. In pioneering work, Baulieu and co-workers (8) showed in situ synthesis of DHEA and its sulfate form (DHEA-S) in the rat brain, independently of the peripheral endocrine glands. Moreover, they showed that major steroids, such as pregnenolone, pregnenolone sulfate, DHEA, and DHEA-S, are synthesized de novo from cholesterol in different brain cells such as glial cells or Purkinje cells (for reviews, see Refs. 9–11). As a consequence, these works opened an emerging field in the study of neurosteroid effects. Among these studies, many focused on the role of DHEA in the brain. Some concluded that DHEA displays a neuroprotective effect against anoxia (12), N-methyl-D-aspartate-induced injury (13), and amyloid-β toxicity (14) or even that DHEA stimulates neurogenesis in rat hippocampus (15).

Steroid hormones act mostly via binding to specific nuclear receptors, but they also modulate neurotransmitter receptor function at the membrane level. However, the mechanism of action of neurosteroids in the brain is not fully understood. Another recent breakthrough came from equilibrium binding studies, which evidenced that pregnenolone was able to bind MAP2 and to stimulate microtubule assembly (16, 17). These observations clearly suggested that neurosteroids could directly bind on neuronal cytoskeleton and influence its dynamics with potential implication for brain plasticity.

We report here that DHEA tightly binds to MAP2C. This binding involved polar and hydrophobic interactions and was essentially localized in the N-terminal extremity. Note that this N-terminal extremity is specific of MAP2 isoforms. It is absent in other MAPs, notably Tau, involved in neurodegenerative diseases. The direct interaction of DHEA with MAP2C raises the possibility that, in addition to the well known steroid hormone-receptor interactions, direct regulation by neurosteroids at the cytoskeleton level may participate in the plasticity of the brain.

EXPERIMENTAL PROCEDURES

DNA Construct—A full-length cDNA of rat MAP2C was constructed as previously described (18). In brief, the 5′ Ndel-PstI fragment of
protein expression was induced with 0.4 mM isopropyl-
dissolved in 50 mM phosphate buffer, pH 7.5, and dialyzed against the-
ranoside for 2 h. Bacteria were harvested by centrifugation at 6000
178
rpm for 45 min at 20,000 × g to remove bacterial debris. Overnight
anmation and subsequent fractionation (35% final) clarified the supernatant.
These pellets were worked up by centrifugation for 45 min at 20,000
rpm and protein concentration was determined by the Beckman model 6300 amino acid analyzer
(Beckman Coulter Inc.). Data were analyzed with the method of
Mavalan and Johnson (23). This method uses 32 reference proteins with
known secondary structures determined from high resolution x-ray
crystallography data to deduce the secondary structure.

Isothermal Titration Calorimetry (ITC)—For ITC experiments,
MAP2C was resuspended in 50 mM MES buffer, 1 mM dithiothreitol, pH 6.8, or in 50 mM phosphate buffer, pH 7.5, and dialyzed against
the same buffer for 2 h to remove any remaining trifluoroacetic acid
counters. Protein concentration was determined by amino acid analysis.
DHEA was dissolved in 100% isopropyl alcohol or in 100% methanol
and then diluted with the buffer to ensure a final concentration of 10%
isopropyl alcohol or 20% methanol was added to the protein solution.
Binding of MAP2C to DHEA was carried out at 25 °C using a
MicroCal MCS titration calorimeter (MicroCal LLC, Northampton, MA).
The enthalpy of binding (ΔH), the affinity constant (Kd), and the
molecular stoichiometry were determined with the ITC procedure. Ten-microliter aliquots of DHEA (2 × 10−5 M) were injected
with a 250-μl microsyringe into the 1.34-ml calorimeter cell containing
the protein solution (at about 2 × 10−5 M) to achieve a complete binding
isotherm. The heat of dilution was measured by injecting the ligand into
the buffer solution. The value obtained was subtracted from the heat
of reaction to obtain the effective heat of binding. Titration curves were
fitted using the MicroCal Origin software, assuming one set of sites. Changes in free energy ΔG and entropy ΔS were calculated from the
following relationship:

\[ ΔG = −RT ln K_d = ΔH − TΔS \]  

Tryptic Digests of MAP2C—MAP2C (50 μg) with and without DHEA
was mixed with 0.5 ng of trypsin and incubated at 37 °C. Digests were
stopped after 15 to 45 min by the addition of 5% formic acid.
Samples were then analyzed by matrix-assisted laser desorption
ionization spectrometry.

Secondary Structure Predictions—The profile network prediction
Heidelberg (PHD) method was used to predict MAP2C secondary
structure. This software developed by Rost and Sander (24, 25) predicts
the secondary structure of proteins from multiple sequence alignments
(availble on the World Wide Web at cubic.bioc.columbia.edu/predict-
proteins/). Secondary structure is predicted through neural networks,
based on 28 known three-dimensional protein structures, ratting to an
expected average accuracy >72% for the three states: helix, strand, and
loop. In our alignments, however, we used only predictions with an
average accuracy of >82% to be more confident with our results.

Sequence Alignment and Molecular Modeling—The DHEA-binding
pocket of the 17-β-hydroxysteroid dehydrogenase 1 (17β-HSD1) crystal
structure (Protein Data Bank code 3DHE) is made of six regions. These
six regions were well aligned with the MAP2C sequence by the ClustalX
program (26). Five of them aligned with the N-terminal sequence
of MAP2C (residues 1–14, 47–53, 58–61, 67–71, and 108–119), whereas
only one region aligned with the C-terminal sequence (residues 295–
310). We then used these six aligned regions to build the DHEA-binding
domain of MAP2C by structural homology with the 17β-HSD1
structure, according to the main principles outlined by Greer (27). For this,
we first built the MAP2C backbone of each of these six regions with the
software TITTO (Tool for Incremental Threading Optimization (28);
available on the World Wide Web at bioinfo.cbs.du.ac.uk). This software
allows one to generate the backbone of a protein of unknown
structure from a sequence homology with a protein of known structure.
However, at this step, only the backbone and not the lateral chains
was obtained. Therefore, in a second step, we used the MaxSprout software
(29) (available on the World Wide Web at www.ebi.ac.uk/maxsprout/)
for generating the amino acid side chains. Third, to construct the
final DHEA-binding site of MAP2C, we superimposed the six regions of
MAP2C with the homology models of the DHEA-binding pocket of
17β-HSD1. For that purpose, we used the Accelrys software InsightII
and Builder modules (San Diego, CA), run on a Silicon Graphics O2
workstation (SGI, Mountain View, CA). Then we positioned the DHEA
molecule in the pocket of MAP2C, as it appears in 17β-HSD1.
RESULTS

Purification Procedures Respected the Functional Integrity of MAP2C—Procedures described in the literature generally use heat to purify microtubule-associated proteins. To avoid such treatment for preventing protein denaturation, we employed milder conditions in our purification scheme. As shown by SDS-PAGE, ion exchange chromatography produced an enriched fraction of MAP2C with an apparent molecular mass of 70 kDa (Fig. 1A, lane 1). This observation was confirmed by Western blot experiments with polyclonal antibodies to MAP2C (Fig. 1A, lane 2). This fraction was further purified by high pressure liquid chromatography to a high level of purity (Fig. 1A, lane 3). Electrospray mass spectrometry analysis (Fig. 1B) showed a MAP2C molecular mass of 49,172 ± 5 Da. This molecular mass corresponds to the full-length protein without the N-terminal methionine, which is often cleaved by bacteria. Nonetheless, this result indicated a discrepancy with the apparent molecular size observed by SDS-PAGE. This behavior suggested a nonglobular three-dimensional pattern of microtubule-associated proteins, as previously reported (19). The CD spectrum was characterized by a negative band at 200 nm, which is usually associated with random coil structures (Fig. 2). However, that the intensity of this band was low relative to CD spectra of peptides in full random coil (30) indicated that other secondary structures exist in this protein. Analysis of this spectrum according to the method of Manavalan and Johnson (23) indicated 31% β-turns, 21% β-sheet or extended structures, and 4% α-helices (Table I). The circular dichroism spectrum of an α-helix is characterized by three bands, a positive and a negative contribution at 190 and 207 nm, respectively, due to π-π* transitions and a negative contribution at 222 nm due to n-π* transitions (30). The MAP2C spectrum did not show a positive contribution at 190 nm, indicating a very low content in α-helices. However, the high content of β-turns and the negative contribution at 185 nm observed with type II β-turn might explain the absence of an α-helix positive contribution at 190 nm. Finally, to check the functionality of our purified preparation of MAP2C, we performed tubulin polymerization assays. Indeed, MAPs are characterized by their ability to promote tubulin polymerization and to stabilize microtubules. Therefore, we performed tests with and without MAP2C. As expected, with MAP2C, we observed a left shift of the spectrum, indicating that MAP2C increased microtubule nucleation and polymerization (Fig. 3). The increase in plateau and the slower decrease induced by lowering the temperature to 4 °C showed that MAP2C increased the stabilization of microtubules. These results indicated that purified MAP2C was functional. We therefore investigated the possible binding of DHEA to this functional MAP2C.

DHEA Binds to MAP2C—Binding of DHEA to MAP2C was studied by ITC. ITC is the method of choice for measuring interactions between proteins and small ligands. This method gives direct access to all thermodynamic parameters of the interaction: $K_a$ (affinity constant), $ΔG$ (free energy), $ΔH$ (enthalpy), $ΔS$ (entropy), and $n$ (number of sites). As a consequence, we can deduce the information about the type of interactions between the ligand and the protein. A negative $ΔH$ value reflects polar interactions and Van der Waals contacts, and a positive $ΔS$ reflects hydrophobic contacts. A typical set of data in 50 mM MES, pH 6.8, is shown in Fig. 4A. The upper plot represents the raw calorimetric data for the ligand-into-protein titration, and the lower plot represents the binding isotherm. DHEA bound to MAP2C with a strong affinity ($K_a = 2.7 \times 10^{7} M^{-1}$), which revealed the specificity of interaction, and with a stoichiometric of 1:1. In addition, DHEA binding resulted in a strong exothermic effect ($ΔH = -34 \pm 5$ kJ mol$^{-1}$). This high enthalpy was due to a combination of the binding itself, mainly via polar or Van der Waals interactions, and the buffer ionization. Therefore, to determine the contribution of buffer ionization to the thermodynamic values and to calculate the entropy ($ΔS$), we performed the same experiment in 50 mM phosphate buffer, pH 7.5, a buffer with no heat of ionization (versus 3.3 kJ mol$^{-1}$ for MES). With phosphate buffer, the difference between values measured in MES and phosphate buffers was attributable solely to proton exchange occurring during the
binding of DHEA. As illustrated in Fig. 4B, the enthalpy changed sharply for the binding of DHEA to MAP2C (ΔH = −10 ± 8 kJ mol⁻¹), whereas the affinity remained constant (Kᵣ = 9.7 × 10⁶ M⁻¹). We therefore concluded that several protons were exchanged during the binding of DHEA. However, their exact number was difficult to determine because the signal in phosphate buffer was extremely low and because it gave an inaccurate ΔH value. ΔH (ΔH = −10 ± 8 kJ mol⁻¹) and ΔS (ΔS = 0.101 ± 0.01 kJ mol⁻¹) values showed that the DHEA binding was both enthalpically and entropically driven, indicating that the number of polar and hydrophobic interactions increased due to DHEA binding. We therefore wanted to look at the region in which DHEA binds to MAP2C. We then looked for the DHEA binding domain of MAP2C by tryptic digestion experiments (see below).

The N-terminal Extremity of MAP2 Binds DHEA—To look at the DHEA binding region of MAP2C, we performed limited proteolytic digestions with trypsin, with and without DHEA, and we analyzed the fragments by mass spectrometry (Table II). We found that, with DHEA, the entire N-terminus (residues 1–120) was more resistant to cleavage. With DHEA, Lys¹⁰ and Arg⁰¹ were transiently resistant to cleavage, whereas Lys¹¹² and Lys¹¹⁷ remained uncleaved even after 45 min of digestion. Because three of these amino acids (Arg⁰¹, Lys¹¹², and Lys¹¹⁷) were very close in the sequence, we looked more precisely around this region. Using PHD software that predicts protein secondary structure, we observed the presence of scarce α-helices and β-sheets localized at both extremities of MAP2C (Fig. 5). In addition, the region including the three amino acids Arg⁰¹, Lys¹¹², and Lys¹¹⁷ and encompassing the 84–120 region was predicted to be structured with α-helices. Interestingly, this 84–120 sequence is absent from Tau, which is unable to bind pregnenolone, a precursor of DHEA (16). We then synthesized the 84–120 N-terminal peptide to confirm these results by monitoring DHEA binding to MAP2C in this N-terminal region.

An Additional Region Is Required for the Binding of DHEA—Circular dichroism experiments showed that the 84–120 N-terminal peptide adopted a random coil structure in phosphate buffer. However, in the presence of moderate TFE concentrations (up to 23%), it adopted an α-helix structure (Fig. 6). This result indicated its propensity to fold in an α-helix as predicted by the PHD method. Isothermal titration calorimetry experiments with the 84–120 N-terminal peptide did not reveal any signal of DHEA binding (not shown). We therefore concluded that additional regions of MAP2C were necessary for DHEA binding. Most probably, the binding is ensured by the whole N-terminal and also other regions of MAP2C that confer a threedimensional structural motif. To identify these regions, we performed a sequence homology comparison between MAP2C and DHEA-binding proteins.

MAP2C Shared Sequence Homologies with Steroid-binding Proteins—To localize the DHEA binding site of MAP2C, we looked at the structure of steroid-binding proteins available in the Protein Data Bank. Interestingly, we found that 17β-HSD1 was co-crystallized with DHEA (31). 17β-HSD1 belongs to a family of enzymes required for the synthesis of active androgens and estrogens. Surprisingly, MAP2C showed sequence homologies with 17β-HSD1, especially with the DHEA binding site of 17β-HSD1 (Fig. 7). These homologies included five N-terminal regions of MAP2C (Met¹–Trp¹⁴, Gly¹⁷–Glu⁵³, Phe²⁵–His³⁰, Tyr³⁷–Lys³⁸, Gly¹⁰⁸–Asp¹¹⁹), which are localized in the 1–120 N-terminal sequence (Fig. 7A), and one additional C-terminal region of MAP2C (Ala²⁹⁵–Leu³¹⁰) (Fig. 7B). Indeed, Met¹–Trp¹⁴ and Gly¹⁰⁸–Asp¹¹⁹ regions contain amino acids previously identified by tryptic digestion (Lys¹⁰ and Arg¹¹², Arg¹¹⁷, respectively). These data suggested that these five N-terminal regions and one additional C-terminal region should together form the DHEA binding pocket of MAP2C. We therefore used the structure of 17β-HSD1 to build a model of the DHEA-binding site of MAP2C.

Reconstruction of the Hydrophobic MAP2C DHEA Binding Pocket by Homology Modeling—DHEA displays the common scaffold of steroids (i.e. the cholesterol nucleus) (Fig. 8). In addition, DHEA is able to form hydrogen bonds through its hydroxyl radical of the A ring and through the ketone radical of the D ring. In 17β-HSD1, DHEA binds to the hydrophobic pocket and is oriented in this pocket by hydrogen bonds involving A and D rings (31). The O-3 atom of the A ring of DHEA can form two possible hydrogen bonds, one with His²¹⁳ or one with Glu²⁹² of 17β-HSD1. The O-17 atom of the D ring can make one possible hydrogen bond with Tyr³⁵⁵ (Fig. 9A). By homology with 17β-HSD1, we built a model of the DHEA-binding pocket of MAP2C bound to DHEA (Fig. 9B). As for 17β-HSD1, the binding of DHEA in the pocket of MAP2C involved hydrophobic interactions and hydrogen bonds. Hydrogen bonds were established through O-3 and O-17 atoms from A and D rings, respectively. First, His¹¹⁶ or Asp¹¹⁸ could form hydrogen bonds with the O-3 atom of the A ring. Second, on the opposite side of DHEA, the O-17 atom could form one hydrogen bond with the His¹⁰³ of MAP2C. All of these hydrogen bonds were found in the N-terminal region of MAP2C. In addition, His¹¹⁵, His¹¹⁶, and Asp¹¹⁸ were localized in the regions Met¹–Trp¹⁴ and Gly¹⁰⁸–Asp¹¹⁹ involved in the binding, as suggested by tryptic digestion. This model is supported by the isothermal titration calorimetry experiments that showed proton exchanges during the binding of DHEA. At the experimental pH of isothermal titration calorimetry experiments, only histidine residues were able to exchange protons, suggesting the presence of histidine residues in the binding site. In conclusion, the binding of DHEA to MAP2C involved hydrophobic residues that formed a hydrophobic pocket with highly specific hydrogen bonds that oriented DHEA into the pocket.

### Table I

| Secondary structure analysis of MAP2C |  |
|-------------------------------------|--|
| α-Helix % | β-Sheet or extended structure % | Turn % | Other % |
| 4 | 21 | 31 | 44 |

**Fig. 3. Tubulin polymerization assay.** The tubulin assembly curve is given as a solid line, whereas tubulin polymerization with MAP2C is given as a dashed line. Polymerization is started by heating the samples to 37 °C. At the time indicated by the arrow, depolymerization was induced by cooling the samples to 4 °C.

**Reconstruction of the Hydrophobic MAP2C DHEA Binding Pocket by Homology Modeling—**DHEA displays the common scaffold of steroids (i.e. the cholesterol nucleus) (Fig. 8). In addition, DHEA is able to form hydrogen bonds through its hydroxyl radical of the A ring and through the ketone radical of the D ring. In 17β-HSD1, DHEA binds to the hydrophobic pocket and is oriented in this pocket by hydrogen bonds involving A and D rings (31). The O-3 atom of the A ring of DHEA can form two possible hydrogen bonds, one with His²¹³ or one with Glu²⁹² of 17β-HSD1. The O-17 atom of the D ring can make one possible hydrogen bond with Tyr³⁵⁵ (Fig. 9A). By homology with 17β-HSD1, we built a model of the DHEA-binding pocket of MAP2C bound to DHEA (Fig. 9B). As for 17β-HSD1, the binding of DHEA in the pocket of MAP2C involved hydrophobic interactions and hydrogen bonds. Hydrogen bonds were established through O-3 and O-17 atoms from A and D rings, respectively. First, His¹¹⁶ or Asp¹¹⁸ could form hydrogen bonds with the O-3 atom of the A ring. Second, on the opposite side of DHEA, the O-17 atom could form one hydrogen bond with the His¹⁰³ of MAP2C. All of these hydrogen bonds were found in the N-terminal region of MAP2C. In addition, His¹¹⁵, His¹¹⁶, and Asp¹¹⁸ were localized in the regions Met¹–Trp¹⁴ and Gly¹⁰⁸–Asp¹¹⁹ involved in the binding, as suggested by tryptic digestion. This model is supported by the isothermal titration calorimetry experiments that showed proton exchanges during the binding of DHEA. At the experimental pH of isothermal titration calorimetry experiments, only histidine residues were able to exchange protons, suggesting the presence of histidine residues in the binding site. In conclusion, the binding of DHEA to MAP2C involved hydrophobic residues that formed a hydrophobic pocket with highly specific hydrogen bonds that oriented DHEA into the pocket.
DISCUSSION

Our work provides evidence that MAP2C is able to bind DHEA. One molecule of DHEA bound to one molecule of MAP2C within a hydrophobic pocket resembling those of steroid-binding dehydrogenases. This binding involved both the N terminus and the C terminus, which closes the pocket, through hydrophobic interactions and hydrogen bonds that orient the steroid into the pocket. These hydrogen bonds are necessary for the binding to be specific and for the hormones to be discriminated (31).

Historically, MAP2 has been described as a flexible protein with few secondary structures that displays an extended structure with a majority of random coils (32, 33). However, random coil does not mean unwinding proteins. Indeed, true random coil structures do not exist, even under strongly denaturing conditions (34). These observations may explain why MAP2 obtained by boiling purification procedures still retains its tubulin polymerization and steroid binding activity (16). In addition, very recently, Malmendal et al. (35) showed by partial tryptic digestion and NMR studies that there is a nascent structure in the N-terminal region of MAP2C. They suggested that this nascent structure could be stabilized by a cofactor.

Fig. 4. DHEA specifically binds MAP2C. The upper plot shows the raw calorimetric data for DHEA binding: The lower plot represents the isotherm of binding. Experiments were done in 50 mM MES, pH 6.8 (A), or in 50 mM phosphate buffer, pH 7.5 (B). Molar ratio is DHEA/MAP2C.

Fig. 5. Secondary structure prediction of MAP2C. Predictions were done with the PHD method (see “Experimental Procedures”). Only predictions with a probability of 82% or more were retained. Helix predictions are represented by dark gray boxes, and β-sheets are shown by light gray boxes.

Fig. 6. The 84–120 N-terminal peptide of MAP2C can adopt an α-helical structure. Circular dichroism of the 84–120 N-terminal peptide of MAP2C (0.30 mg/ml) in 20 mM phosphate buffer, pH 7.0, without TFE (thin black line), in 9% TFE (gray line), or in 23% TFE (thick black line).

Table II

| Amino acid | MAP2C 15 min | MAP2C 45 min | MAP2C + DHEA 15 min | MAP2C + DHEA 45 min |
|------------|--------------|--------------|---------------------|---------------------|
| Lys10      | +            | -            | +                   | -                   |
| Arg93      | +            | +            | -                   | +                   |
| Lys112     | +            | +            | -                   | +                   |
| Lys117     | -            | +            | -                   | +                   |

Table II

Variations of MAP2C tryptic digestion without and with DHEA
A plus sign indicates cleavage, and a minus sign indicates absence of cleavage.
further support of these observations, preincubation of MAP2 with tubulin increases more than 8 times the number of steroid binding sites present in a calf purified MAP2 preparation (16). These experiments suggest that the fixation of MAP2 to tubulin increases its own structuring. Like Baulieu and co-workers (16), we believe that neurosteroids operate through a novel mode of action. Neurosteroids are thought to exert their effects through binding to nuclear receptors in neurons, which triggers the regulation of gene transcription, and through binding at the membrane level to neurotransmitter receptors, like N-methyl-D-aspartate or \( \gamma \)-aminobutyric acid receptors. Despite continuous research, no specific DHEA receptors have been identified in the central nervous system (for review, see Ref. 36). The hypothesis that DHEA interacts directly with cytoskeleton components is supported by the observation that, in ovariectomized rat, MAP2 protein content is induced by exogenous estradiol or progesterone at physiological doses (7). All in all, these results suggest that steroids could specifically stabilize MAP2 via direct binding and thereby modulate MAP2 content in neurons.

Interestingly, MAP2 also interacts with other members of signal transduction pathways such as Src and Grb2 (37) or protein kinase A (PKA) (38). Interaction with PKA, which modulates the MAP2 phosphorylation state, results in profound effects on microtubule dynamics (39) and cellular morphology. Furthermore, such binding allows PKA to be anchored in dendrites and allows the PKA signal transduction pathway to be activated (40). Because the MAP2 PKA-binding domain (residues 82–113) and the MAP2 DHEA-binding pocket (residues 108–119) partially overlap, DHEA binding may interfere with PKA binding and may block its activity. Note, however, that none of the phosphorylated Ser/Thr in MAP2 isoforms belongs to the DHEA binding site. Most of these sites are localized in the central or in the C-terminal domain of MAP2. Only one phosphorylation site was found in the N-terminal domain (Ser136). These observations suggest that these known phosphorylation sites on Ser/Thr should not affect DHEA binding. By contrast, the DHEA-binding pocket of MAP2C contains a tyrosine (Tyr67) that is a potential phosphorylation site as predicted by the NetPhos software (41) (available on the World Wide Web at www.cbs.dtu.dk/services/NetPhos/). Hence, if Tyr67 is phosphorylated in vivo, it should avoid the binding of DHEA by introducing a negative charge in the hydrophobic pocket.

We believe that high molecular weight MAP2 isoforms also bind DHEA for the two following reasons. First, all of the six regions forming the DHEA binding pocket of 17β-HSD1 (Protein Data Bank code 3DHE) is aligned with the N-terminal (residues 1–122) (A) and with the C-terminal (residues 295–383) (B) sequences of MAP2C showing homologies with 17β-HSD1. Conserved residues (*) and semiconserved residues are represented in dark gray. Six regions in the DHEA binding pocket of 17β-HSD1 aligned well with MAP2C. Five of them corresponded to N-terminal sequences, residues 1–14, 47–53, 58–61, 67–71, and 108–119, and only one with the C-terminal sequence (residues 295–310) (in light gray). These regions were used to model the DHEA binding site of MAP2C. Lysine and arginine residues identified by tryptic digestion and MALDI experiments. The N-terminal peptide (residues 84–120) of MAP2C is underlined. Numbering corresponds to the MAP2C sequence.

Fig. 7. MAP2C displays block homologies with the DHEA binding site of 17β-HSD1. Human 17β-HSD1 (Protein Data Bank code 3DHE) is aligned with the N-terminal (residues 1–122) (A) and with the C-terminal (residues 295–383) (B) sequences of MAP2C showing homologies with 17β-HSD1. Conserved residues (*) and semiconserved residues are represented in dark gray. Six regions in the DHEA binding pocket of 17β-HSD1 aligned well with MAP2C. Five of them corresponded to N-terminal sequences, residues 1–14, 47–53, 58–61, 67–71, and 108–119, and only one with the C-terminal sequence (residues 295–310) (in light gray). These regions were used to model the DHEA binding site of MAP2C. Lysine and arginine residues identified by tryptic digestion and MALDI experiments. The N-terminal peptide (residues 84–120) of MAP2C is underlined. Numbering corresponds to the MAP2C sequence.

Fig. 8. Chemical structure of DHEA. DHEA is formed by the hydrophobic cyclopentenophenanthrene scaffold common to steroid hormones. Four rings, A, B, C, and D, compose this scaffold. The two oxygen atoms O-3 (OH in position 3) and O-17 (C=O in position 17) can establish hydrogen bonds.

Stabilization of MAP2 via direct binding and thereby modulate MAP2 content in neurons.

Interestingly, MAP2 also interacts with other members of signal transduction pathways such as Src and Grb2 (37) or protein kinase A (PKA) (38). Interaction with PKA, which modulates the MAP2 phosphorylation state, results in profound effects on microtubule dynamics (39) and cellular morphology. Furthermore, such binding allows PKA to be anchored in dendrites and allows the PKA signal transduction pathway to be activated (40). Because the MAP2 PKA-binding domain (residues 82–113) and the MAP2 DHEA-binding pocket (residues 108–119) partially overlap, DHEA binding may interfere with PKA binding and may block its activity. Note, however, that none of the phosphorylated Ser/Thr in MAP2 isoforms belongs to the DHEA binding site. Most of these sites are localized in the central or in the C-terminal domain of MAP2. Only one phosphorylation site was found in the N-terminal domain (Ser136). These observations suggest that these known phosphorylation sites on Ser/Thr should not affect DHEA binding. By contrast, the DHEA-binding pocket of MAP2C contains a tyrosine (Tyr67) that is a potential phosphorylation site as predicted by the NetPhos software (41) (available on the World Wide Web at www.cbs.dtu.dk/services/NetPhos/). Hence, if Tyr67 is phosphorylated in vivo, it should avoid the binding of DHEA by introducing a negative charge in the hydrophobic pocket.

We believe that high molecular weight MAP2 isoforms also bind DHEA for the two following reasons. First, all of the six regions forming the DHEA binding pocket are conserved in high molecular weight isoforms of MAP2. Only one phosphorylation site was found in the N-terminal domain (Ser136). These observations suggest that these known phosphorylation sites on Ser/Thr should not affect DHEA binding. By contrast, the DHEA-binding pocket of MAP2C contains a tyrosine (Tyr67) that is a potential phosphorylation site as predicted by the NetPhos software (41) (available on the World Wide Web at www.cbs.dtu.dk/services/NetPhos/). Hence, if Tyr67 is phosphorylated in vivo, it should avoid the binding of DHEA by introducing a negative charge in the hydrophobic pocket.

We believe that high molecular weight MAP2 isoforms also bind DHEA for the two following reasons. First, all of the six regions forming the DHEA binding pocket are conserved in high molecular weight isoforms (Fig. 10). Second, Murakami et al. (16) showed that high molecular weight MAP2 isoforms purified from calf were able to bind pregnenolone, the direct precursor of DHEA. This result demonstrates that the steroid binding site is conserved in high molecular weight isoforms of
In conclusion, DHEA binding (and, more generally, steroid binding to MAP2) is of importance for both fetal (MAP2C) and adult (MAP2A and MAP2B) brain isoforms.

In addition, Reyra-Neyra et al. (7) showed that the expression of MAP2 was modified by estradiol or progesterone, whereas Tau content was not. Tau is another related MAP preferentially expressed in axons, which does not bind steroids (16). Interestingly, Tau lacks the N-terminal region involved in the binding of DHEA and is involved in several pathologies of the central nervous system known as tauopathies, like Alzheimer’s disease. In these diseases, Tau forms fibrillar deposits in the brain of patients. Conversely, so far, MAP2 has never been shown to form fibrillar deposits in these pathologies (42). The difference between the N-terminal sequence of MAP2 and Tau could account for the difference in their aggregative properties. This hypothesis is supported by recent observations that showed the importance of the N terminus of Tau in the aggregation process (43). In this way, steroid binding to the N-terminal of MAP2 could be a protective event against fibrillar aggregation and may influence neuronal plasticity.

Acknowledgments—We thank V. Peyrot for help with microtubule polymerization experiments and B. Charvet for pMAP2c cloning. We also thank D. Lesuisse (Aventis) for the gift of DHEA. Plasmids pCG2b33 and pJBMap2c were both gifts by Dr. C. Garner.

**Fig. 9.** The DHEA binding site of MAP2C shows hydrophobic interactions and specific hydrogen bonds. DHEA is represented in red. The backbone is in white. Aromatic (Tyr, Phe, Trp, and His) and basic (Arg and Lys) residues are in blue. Residues that form hydrogen bonds with DHEA are in yellow (His, Tyr, Asp, and Glu). The DHEA binding site of 17β-HSD1 (A) and MAP2C (B) is composed of hydrophobic and aromatic residues that bind the steroid ring of DHEA. In the 17β-HSD1 binding site, His221, Glu282, and Tyr155 form possible hydrogen bonds with the O-3 and the O-17 atoms of DHEA that orient the steroid into the pocket. In the MAP2C binding site, these hydrogen bonds with DHEA are preserved, but they are those of His116, Asp118, and His13, Lys10, Lys112, and Lys117 identified by tryptic digestion and MALDI experiments are localized around the pocket.

**Fig. 10.** Structure of MAP2 isoforms and Tau. A. MAP2 isoforms are divided in two groups: high molecular weight isoforms (HMWMAP2), including MAP2A and MAP2B, and low molecular weight isoforms (LMWMAP2), including MAP2C. LMWMAP2 isoforms lack the projection domain (PD). They are made of the N- and the C-terminal domains of high molecular weight isoforms linked together. The tubulin-binding domain (TBD) is formed by three or four repeated sequences (black boxes). The five N-terminal sequences and the C-terminal sequence that we identified as involved in the DHEA binding are in hatched boxes. B. Tau C-terminal domain is highly homologous to MAP2 isoforms, whereas its N-terminal domain is entirely different. Therefore, the N-terminal sequences constituting the DHEA-binding pocket of MAP2C are not present in Tau.
REFERENCES

1. Kalcheva, N., Albala, J., O’Guin, K., Rubino, H., Garner, C., and Shaft-Zagardo. B. Proc. Natl. Acad. Sci. U. S. A. 92, 10894–10899, 1995
2. Riederer, B., and Matus, A. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 6006–6009
3. Charriere-Bertrand, C., Garner, C., Tardy, M., and Nunez, J. (1991) J. Neurochem. 56, 385–391
4. Matus, A. (1994) Trends Neurosci. 17, 19–22
5. Boucher, M., Belanger, D., Baulieu, E., and Leclerc, N. (1999) Cell Motil. Cytoskeleton 42, 257–273
6. Gonzalez-Billault, C., Engelke, M., Jimenez-Mateos, E. M., Wandosell, F., Caceres, A., and Avila, J. (2002) J. Neurosci. Res. 68, 713–719
7. Reyna-Neyra, A., Camacho-Arroyo, I., Ferrera, P., and Azcunagui, B. (1991) J. Neurosci. Res. Bull. 30, 273–283
8. Riederer, B., and Matus, A. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 104–19679
9. Robel, P., and Baulieu, E. E. (1995) Crit. Rev. Neurobiol. 9, 383–384
10. Baulieu, E. E. (1997) Recent Prog. Horm. Res. 52, 1–32
11. Tsutsui, K., Ukeda, K., Usai, M., Sakamoto, H., and Takase, M. (2000) Neurosci. Res. 36, 261–273
12. Marx, C. E., Jarskog, L. F., Lauder, J. M., Gilmore, J. H., Lieberman, J. A., and Morrow, A. L. (2000) Brain Res. 871, 104–112
13. Kimonides, V. G., Khatibi, N. H., Svendsen, C. N., Sofroniew, M. V., and Herbert, J. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 1852–1857
14. Cardouzel, A., Riegelson, W., and Kalimi, M. (1999) Proc. Soc. Exp. Biol. Med. 222, 145–149
15. Kairisma, K. K., and Herbert, J. (2002) Eur. J. Neurosci. 16, 445–453
16. Murakami, K., Fellous, A., Baulieu, E. E., and Robel, P. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 3578–3584
17. Plassart-Schiess, E., and Baulieu, E. E. (2001) Brain Res. Brain Res. Rev. 37, 133–140
18. Gamblin, T. C., Nachmanoff, K., Halpain, S., and Williams, R. C., Jr. (1996) Biochemistry 35, 12576–12586
19. Kindler, S., Schulz, B., Goedert, M., and Garner, C. C. (1990) J. Biol. Chem. 265, 19679–19684
20. Berling, B., Wille, H., Roll, B., Mandelkow, E. M., Garner, C., and Mandelkow, E. (1994) Eur. J. Cell Biol. 64, 120–130
21. Barany, G., and Merrifield, R. B. (1980) The Peptide: Analysis, Synthesis, Biology (Gross, E., and Meinhofer, J., eds) Vol. 2, pp. 1–284, Academic Press, Inc., New York
22. Barbier, P., Peyrot, V., Leynadier, D., and Andreu, J. M. (1998) Biochemistry 37, 758–768
23. Manavalan, P., and Johnson, W. C., Jr. (1987) Anal. Biochem. 167, 76–85
24. Rost, B., and Sander, C. (1993) J. Mol. Biol. 232, 584–599
25. Hobohm, U., and Sander, C. (1994) Protein Sci. 3, 522–524
26. Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G. (1997) Nucleic Acids Res. 25, 4876–4882
27. Greer, J. (1991) Methods Enzymol. 202, 239–252
28. Labesse, G., and Moren, M. (1998) Bioinformatics 14, 206–211
29. Holm, L., and Sander, C. (1991) J. Mol. Biol. 218, 183–194
30. Johnson, W. C., Jr. (1995) Methods Biochem. Anal. 31, 61–163
31. Han, Q., Campbell, R. L., Gangloff, A., Huang, Y. W., and Lin, S. X. (2000) J. Biol. Chem. 275, 1105–1111
32. Voter, W. A., and Erickson, H. P. (1982) J. Ultrastruct. Res. 80, 374–392
33. Hernandez, M. A., Avila, J., and Andreu, J. M. (1986) Eur. J. Biochem. 154, 41–48
34. Shortle, D. (1996) FASEB J. 10, 27–34
35. Hohfeld, T., and Eisele, F. (1998) Biochem. Biophys. Res. Commun. 301, 136–142
36. Wold, O. T., and Kirschbaum, C. (1999) Brain Res. Brain Res. Rev. 30, 264–288
37. Lin, E. W., and Halpain, S. (2000) J. Biol. Chem. 275, 20578–20587
38. Davare, M. A., Dong, F., Rubin, C. S., and Hell, J. W. (1999) J. Biol. Chem. 274, 30280–30287
39. Alexa, A., Schmidt, G., Tompa, P., Ogueta, S., Vazquez, J., Kulcsar, P., Kovacs, J., Dombradi, V., and Friedrich, P. (2002) Biochemistry 41, 12427–12435
40. Harada, A., Teng, J., Takai, Y., Oguchi, K., and Hirokawa, M. (2000) J. Mol. Biol. 296, 541–549
41. Blom, N., Gammeltoft, S., and Brunak, S. (1999) J. Mol. Biol. 294, 1351–1362
42. Eisele, F., and Eisele, F. (1998) FEBS Lett. 252, 891–946
43. Gamblin, T. C., Berry, R. W., and Binder, L. I. (2003) Biochemistry 42, 2252–2257
Specific Binding of Dehydroepiandrosterone to the N Terminus of the Microtubule-associated Protein MAP2
Emmanuelle Laurine, Daniel Lafitte, Catherine Grégoire, Eric Séréé, Erwann Loret, Soazig Douillard, Bernard Michel, Claudette Briand and Jean-Michel Verdier

J. Biol. Chem. 2003, 278:29979-29986.
doi: 10.1074/jbc.M303242200 originally published online May 29, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M303242200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 42 references, 10 of which can be accessed free at http://www.jbc.org/content/278/32/29979.full.html#ref-list-1