Cytochrome P-450 in the brain

MARIA STRÖMSTEDT, SHIN-ICHI HAYASHI,
MARGARET WARNER AND JAN-AKE GUSTAFSSON*
Department of Medical Nutrition, Karolinska Institute,
Huddinge University Hospital, Box 606, S-141 84 Huddinge,
Sweden

Introduction

Although it has been known for some time that there is cytochrome P-450 in the brain [1, 2], the major forms of the AMP-responsive elements in the bovine P450_{17a} and P450_{7a} genes are not related to the consensus CRE- or AP-2-binding sites associated with other genes whose transcription is regulated by cyclic AMP and we imagine that the steroid hydroxylases represent new examples of class 2 cyclic AMP-responsive genes.

Conclusions concerning cyclic AMP-mediated transcription of P450_{17a} and P450_{7a} genes

The P450_{17a} gene and the P450_{7a} gene are members of two distinct P450 gene families, CYP17 and CYP11A, respectively [1]. It is imagined that these two genes evolved from a common P450 progenitor gene several hundred million years ago. During evolution, these genes have diverged leading to gene products which each catalyse very specific and distinct chemical reactions. Furthermore, the P450_{17a} gene has evolved into one encoding a mitochondrial cytochrome P-450, while the P450_{7a} gene has evolved into one encoding a microsomal cytochrome P-450. Yet, over this same period of evolutionary time, both genes have retained, or developed, common cyclic AMP-dependent regulatory systems; common to the extent that ongoing protein synthesis is required for cyclic AMP responsiveness, and in neither case are the consensus CRE or AP-2 sequences involved in cyclic AMP-mediated transcription. Obviously, only upon purification and characterization of cyclic AMP-responsive trans-acting factors for these two genes will the degree of commonality become evident. Nevertheless, the preliminary analysis of the cyclic AMP-responsive cis-regulatory elements of these two different genes does permit some speculation concerning the common features of this transcription process.

In the case of the P450_{17a} gene, a 5'-AMP-responsive element has been located between -243 and -225 bp. When this 19 bp sequence is compared with that between -186 and -32 bp in the P450_{7a} gene, some similarity is found, but a perfect match of more than six base pairs [TTGATG] is not evident. Thus there is a possibility that a common cyclic AMP-responsive transcription factor is shared by these two genes, but proof of this possibility awaits the purification and characterization of the trans-acting factors. What is apparent, however, is that with respect to DNA sequence the cyclic AMP-responsive elements in the bovine P450_{17a} and P450_{7a} genes are not related to the consensus CRE- or AP-2-binding sites associated with other genes whose transcription is regulated by cyclic AMP and we imagine that the steroid hydroxylases represent new examples of class 2 cyclic AMP-responsive genes.

Received 11 August 1989


*To whom correspondence should be addressed.
brain cytochrome P-450 is probed with antibodies raised against the major hepatic forms of cytochrome P-450 (IA1, IA2, IB1, IIC11, IIC12, HE1, IIIA13) and (ii) the low level in the brain of catalytic activity typical of hepatic forms of cytochrome P-450, namely testosterone 6ß-, 16a- and 7a-hydroxylases, ethoxycoumarin- and ethoxyresorufin-O-de-ethylase and benzpyrene hydroxylase [4, 5].

Of the cytochromes P-450 known to be involved in steroid biosynthetic or degradative pathways, the only ones for which catalytic activity has been detectable in the brain are aromatase [6], oestradiol-2-hydroxylase [7] and 5a-androstene-3ß, 17ß-diol hydroxylase (3ß-diol hydroxylase) [8]. The first two enzymes represent a very small fraction of total brain cytochrome P-450, while 3ß-diol hydroxylase accounts for approximately 10% of total brain cytochrome P-450 and up to 50% of the cytochrome P-450 in the hypothalamus preoptic area.

Although cytochrome P450hol is reported to be present in the brain, the evidence is immunohistochemical and no catalytic activity has been detectable [9]. Arachidonic acid metabolites formed through cytochrome P-450-catalysed reactions have been detected in the hypothalamus and arc thought to mediate the effects of dopamine and growth hormone-releasing hormone on somatostatin release [10]. The forms of cytochrome P-450 involved in these reactions have not yet been characterized.

We have been investigating the physiological functions of brain cytochrome P-450 and trying to characterize the forms of the enzyme present in this tissue. The approaches involve: (i) studying the endocrine regulation of the cytochrome P-450 content of the brain; (ii) characterizing the substrate specificity of 3ß-diol hydroxylase and (iii) finding novel forms of cytochrome P-450 by screening cDNA libraries from the brain and other extrahepatic tissues, using oligonucleotides from the haem-binding region of microsomal cytochromes P-450 as probes under low-stringency conditions.

**Results**

**Endocrine regulation of brain cytochrome P-450.** The yield of cytochrome P-450 from brain microsomes was 0.04 ± 0.009 mmol/g of tissue and did not vary over different regions of the cerebrum. In the hypothalamus preoptic area during lactation and in the olfactory lobes during pregnancy, the yield of cytochrome P-450 increased 8-10-fold to values of 0.35 and 0.50 mmol/g of tissue, respectively. Implantation of rats with silastic tubes (4 cm long, 2 mm internal diameter) containing crystalline dihydrotestosterone caused a 5-10-fold induction of cytochrome P-450 in both the olfactory lobes and the hypothalamus preoptic area after 3 weeks of treatment. The cytochrome P-450 content of the rest of the cerebrum did not change under these conditions. No catalytic activity of the cytochromes P-450 known to be present in the brain increased in parallel with the increase in the cytochrome P-450 content.

**Distribution, regulation and substrate specificity of 3ß-diol hydroxylase.** 3ß-Diol hydroxylase was distributed throughout the brain of male and female rats in the cortex, cerebellum, brain stem, hypothalamus preoptic area and olfactory lobes at a level of between 70 and 153 nmol of triol formed/g of tissue per h. Catalytic activity was not influenced by adrenal-cotistone, castration or dihydrotestosterone treatment of animals, nor was there any change during pregnancy or lactation.

In addition to 3ß-diol, brain microsomes also catalysed the hydroxylation of 5a-androstene-3ß, 17ß-diol, dehydroepiandrosterone and dihydrotestosterone. The respective catalytic rates were 73, 15, 13 and 3 nmol of product formed/g of tissue per h. The catalytic activities were competitively inhibited by 5a-androstene-3ß, 17ß-diol. When

### Table 1. Oligonucleotide (51 mer) mix used for screening rat cDNA library

<table>
<thead>
<tr>
<th>Cytochrome P-450 family</th>
<th>Trivial name</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA2</td>
<td>d</td>
</tr>
<tr>
<td>IIA1</td>
<td>a</td>
</tr>
<tr>
<td>IB1</td>
<td>b</td>
</tr>
<tr>
<td>IIC1</td>
<td>15ß</td>
</tr>
<tr>
<td>HE1</td>
<td>j</td>
</tr>
<tr>
<td>IIIA1</td>
<td>pen1</td>
</tr>
<tr>
<td>IVA1</td>
<td>LA90</td>
</tr>
<tr>
<td>XVIIA1</td>
<td>17a</td>
</tr>
<tr>
<td>XXIIA1</td>
<td>C21</td>
</tr>
</tbody>
</table>

3ß-diol hydroxylase was purified from the prostate, a single form of cytochrome P-450, in a reconstituted system, catalysed the hydroxylation of all four substrates.

**Cross-hybridization experiments.** Rat brain and prostate cDNA libraries were screened with the mixture of oligonucleotides shown in Table 1. Under these conditions, a cDNA with 70% sequence similarity can be picked up. Ten positive clones were obtained from the brain library after screening 150,000 plaques and three positives from the prostate library after screening of 250,000 plaques.

So far, one of the prostate clones has been characterized. It is a member of the cytochrome P-450 family IV and the coding region so far sequenced shows 70-80% sequence similarity with hepatic lauric acid ω-hydroxylase (LaO). There is no sequence similarity between the prostate clone and lauric and ω-hydroxylase in the 3' untranslated region.

Northern blots revealed strong signals in the kidney and retina and weak signals in the prostate and liver.

### Conclusions

Cytochromes P-450 in the hypothalamus preoptic area and olfactory lobes are regulated by the endocrine status of rats, since their levels increase several fold during pregnancy, lactation and dihydrotestosterone treatment of rats. The substrates of these induced cytochromes P-450 are not known, but do not appear to be steroids.

The major steroid hydroxylase in the brain, namely 3ß-diol hydroxylase, is not regulated by the endocrine status of rats. The function of this enzyme remains unclear, but the possibility exists that its physiological substrate is not the 3ß-diol, but may instead be a 'neurosteroid', such as dehydroepiandrosterone. Such neurosteroids do not act through intracellular steroid hormone receptors, but exert their action on neurons through interaction with γ-amino butyric acid receptors [11].

The technique of cross-hybridization of cDNAs is likely to prove a powerful tool in the identification of novel forms of brain cytochrome P-450.

This study was supported by a grant from the Swedish Medical Research Council (No. 13X-06807-07A).

Molecular mechanisms of cytochrome P-450 gene regulation

PETER GOLDFARB
Molecular Toxicology Research Group, Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH, U.K.

The role of the mixed function oxidases in cellular metabolism has been studied for more than 30 years. Recently, the use of cDNA cloning and the techniques of molecular biology have resulted in major advances in our understanding of this multi-gene superfamily of isoenzymes. The molecular biology of the cytochromes P-450 has been extensively reviewed [1-4] and aspects of their regulation discussed. As might be expected in a gene superfamily of this size, a variety of regulatory strategies has been observed. This is not surprising when one considers both the tissue specificity of expression shown by most of the cytochrome P450 genes, and the fact that, in many instances, expression is inducible by endogenous and/or exogenous molecules. However, for the non-expert, this variety of levels of regulation can be confusing, especially if the topic is reviewed from the substrate, inducer or gene classification point of view.

This short review, therefore, describes the various levels at which regulation of gene expression can take place in eukaryotes and indicates how the control of cytochrome P450 genes fits in with this general scheme.

The basics of gene expression

DNA in the nucleus of a eukaryotic cell is found in the form of chromatin, in which both histone and non-histone proteins are bound to the genetic material. For a gene to be available for expression, it must be in the activated state so that transcription can take place. The transcribing molecule, RNA polymerase, is then able to synthesize a pre-mRNA copy of the gene, including both the coding (exon) and non-coding (intron) regions. (The exon—intron structure of the gene is the result of gene building during evolution, the exons often coding for different functional domains in the apoprotein.) The pre-mRNA is then processed by a nuclear splicing mechanism so that the introns are removed and the exons joined together to produce the mature mRNA molecule. This is then transported to the cytoplasm where it is translated by ribosomes into the polypeptide apoprotein. This is usually modified to produce active enzyme which is then targeted to its functional site within the cell. Over the last 10 years, the study of gene regulation in many systems has demonstrated that virtually any of the stages of gene expression can be a site of regulatory control.

Abbreviations used: TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; Ah, aromatic hydrocarbon.

Gene structure and polymorphism

Quite clearly, an inherited deletion or mutation in a gene can lead to the production of an aberrant mRNA or a defective protein. Such inherited differences between individuals are termed polymorphisms and are of great interest as they may reflect the basis of inter-individual differences in drug or xenobiotic metabolism. Polymorphism may also occur in the control DNA region flanking the gene and result in alteration in the basal or induced rate of mRNA synthesis. A number of rodent polymorphisms have been described [4] and human polymorphisms have been reported in the P450IC, IID, IIIA and XXIA subfamilies [5-9]. Polymorphism has also been demonstrated at the aromatic hydrocarbon (Ah) receptor locus in rodents and correlated with differences in I/A1 inducibility [2].

Gene activation

The processes of determination and differentiation during the development of an organism involve the activation of genes by the unwinding of condensed, inactive chromatin. This is brought about by a combination of gene-specific demethylation events and the selective binding of activating proteins to the DNA. Thus, while all the cells in an organism will contain the information for the synthesis of the cytochromes P-450, only in some cells will the various genes be in an activated form available to make mRNA when required. To date, developmental stage-specific gene activation has only been clearly demonstrated for rra IIE1 [10], demethylation occurring shortly after birth in the neonate. The possibility that sex, tissue or developmental stage-specific expression of other constitutive or inducible forms of cytochrome P-450 is also controlled at this level, has yet to be demonstrated.

Transcriptional control

Regulation at the level of mRNA synthesis is dependent on two factors: the presence of cis-acting regulatory DNA sequences adjacent to or within the gene, and the synthesis or activation of gene-specific DNA-binding proteins. The DNA-binding proteins dock specifically with their target regulatory DNA sequences and facilitate the entry of RNA polymerase molecules into the mRNA promoter site of the gene. These DNA-binding proteins, or trans-acting factors as they are sometimes known, are the ultimate effectors in a wide variety of regulatory cascades controlling cellular mRNA synthesis. They are present in both the nucleus and cytoplasm and can be activated by ligand binding (drugs or hormones), by phosphorylation (second messenger signalling systems) or, in some instances, by metabolite-induced dimerization/dis-