SUBSTRATE BINDING TO CYTOCHROME *P*-450 OF LIVER AND ADRENAL MICROSOMES

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1. Introduction

The binding of various substrates of the microsomal mono-oxygenase system to its terminal oxidase, cytchrome *P*-450, is associated with characteristic absorbance changes in the difference spectrum of the microsomes [1–3]. Thus, hexobarbital, aminopyrine, testosterone and laurate, as well as a number of other substances, elicit spectral changes characterized by a trough in the 420 nm region and a peak in the 390 nm region when added to a suspension of rat liver microsomes. This spectral change has been termed type I. The type II spectral change, on the other hand, has a peak at about 430 nm and trough at 390–400 nm and is likewise produced by a variety of substances, among them aniline.

In a recent paper we have suggested that the competitive inhibition that certain drugs, steroids and fatty acids exert on each other's oxidative metabolism in the liver microsomes may be related to the competition for binding to a common cytochrome *P*-450 species [4]. The substances used in that study, hexobarbital, aminopyrine, testosterone and laurate, were all potent inducers of type I spectra. However, it was not possible to decide whether all of these substrates may interact at a common or at different site(s) of the cytochrome *P*-450 molecule.

Furthermore, aminopyrine and hexobarbital, though slowly metabolized by guinea-pig adrenal microsomes, do not elicit detectable spectral changes in the difference spectrum of these particles [5]. That a binding of these drugs to adrenal microsomes might indeed take place was suggested by our recent finding that they interfere with the type I spectral change produced by cortisol [5], but it was not possible to decide whether or not the drugs and the steroids interacted at a common site on the cytochrome *P*-450 molecule.

The present investigation was designed to study in further detail the interaction between these substrates in their binding to cytochrome *P*-450 of rat liver and guinea-pig adrenal microsomes.

2. Materials and methods

Male Sprague-Dawley rats (ca. 200 g) and albino guinea-pigs (400–500 g) of local strain were used. The animals were starved overnight and killed by a blow on the head and decapitated. Rat liver and guinea-pig adrenal microsomes were isolated according to the procedure for liver microsomes described by Ernster et al. [6]. The microsomal pellets were rinsed with ice-cold 0.25 M sucrose solution and resuspended in this medium at a protein concentration of 10 mg/ml. Protein was measured by the method of Lowry et al. [7].

The sum of the absorbance changes at the wavelengths of the peak and trough of the type I spectral change caused by the addition of hexobarbital to liver microsomes was recorded at 390 nm minus 420 nm in a Phoenix Dual-Wavelength Scanning Spectrophotometer. The magnitude of the spectral change induced by the addition of cortisol-21-sodium succinate

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(cortisol succinate) to adrenal microsomes was measured at 385 nm minus 420 nm. The cuvette contained, in a final volume of 3 ml, liver or adrenal microsomes (3.5–5.6 mg protein), 50 mM tris Cl pH 5 and 15 mM KCl. 1–10 μl of aqueous solutions of hexobarbital 70 mM) or cortisol succinate (20 mM) were added stepwise (in 1 μl aliquots) and the change in absorbance recorded. The procedure was then repeated in the presence of various concentrations of other compounds previously shown to interfere with the type I spectral change induced by hexobarbital in liver microsomes (aminopyrine, testosterone and laurate [4]), or by cortisol succinate in adrenal microsomes (hexobarbital [5]).

The data obtained were treated as in the classical enzyme-substrate-inhibitor models, using the absorbance change in place of initial velocity, as recently suggested by Leibman et al. [8].

All chemicals employed were standard commercial products.

Table 1
Concentrations of substances required for half-maximal spectral changes (K_S) when added to suspensions of liver microsomes.

Substance	K_S* (μM)	Maximal absorbance ($\Delta OD_{390-420}$/3 ml/ 5.6 mg protein)
Hexobarbital	50	0.031
Aminopyrine	133	0.017
Testosterone	8	0.019
Laurate	4	0.023

* The K_S values were derived from double reciprocal plots of the sum of the absorbance changes at the wavelengths of the peak (390 nm) and trough (420 nm) associated with the addition of increasing concentrations of the respective substances to suspensions of rat liver microsomes.

3. Results

Hexobarbital, aminopyrine, testosterone and laurate all induce type I spectral changes when added to a suspension of rat liver microsomes. The binding affinities for testosterone and laurate were considerably higher than those for hexobarbital and aminopyrine, as revealed by the K_S values presented in the table. It may be interesting to note the low K_S value obtained with laurate (4 μM) which is in good agreement with the previously reported K_m value of 6 μM for the ω-oxidation of laurate catalyzed by rat liver microsomes [9]. A similarity between K_S and K_m has also previously been observed for hexobarbital and aminopyrine, and it has been proposed that the two constants reflect the binding of the substrate to the enzyme [3].

In a previous investigation [4], we observed that aminopyrine, testosterone and laurate interfere with the type I spectral change induced in rat liver microsomes by hexobarbital. This finding was taken to indicate that all four substances may actually bind to the same cytochrome *P*-450 species. This phenomenon was now studied in further detail.

The spectral change elicited by the addition of increasing amounts of hexobarbital to rat liver microsomes was measured in the absence and presence of various concentrations of aminopyrine. Data in fig. 1 reveal that aminopyrine acts as a competitive inhibitor of the spectral change produced by the addition of hexobarbital, i.e. the apparent K_S for hexobarbital is increased in the presence of aminopyrine. Similar results were obtained when the titration of the spectral change with hexobarbital was performed in the presence of testosterone or laurate at various concentrations (figs. 2 and 3).

With adrenal microsomes the situation appears to be somewhat different. Titration of the type I spectral change by the addition of increasing concentrations of cortisol succinate rendered an apparent K_S value of 0.25 mM (fig. 4). In the presence of hexobarbital the spectral change induced by cortisol succinate was markedly inhibited. However, this inhibition was not competitive, but rather seemed to be of a mixed type.

4. Comments

The evidence available suggests that a number of substances undergoing hydroxylation in liver microsomes, such as certain drugs, steroids and fatty acids, interact with a common cytochrome *P*-450 species. The competitive inhibition that these substrates exert on each other's metabolism may thus be explained by a competition for their binding to the cytochrome. Although the mechanism of the binding underlying

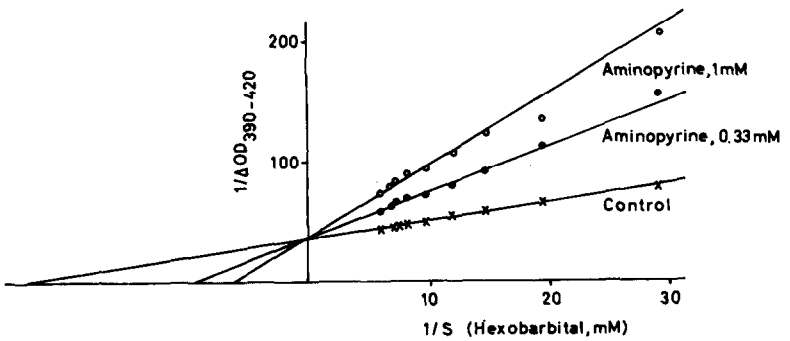

Fig. 1. Effect of aminopyrine on the type I spectral change induced by hexobarbital in rat liver microsomes.

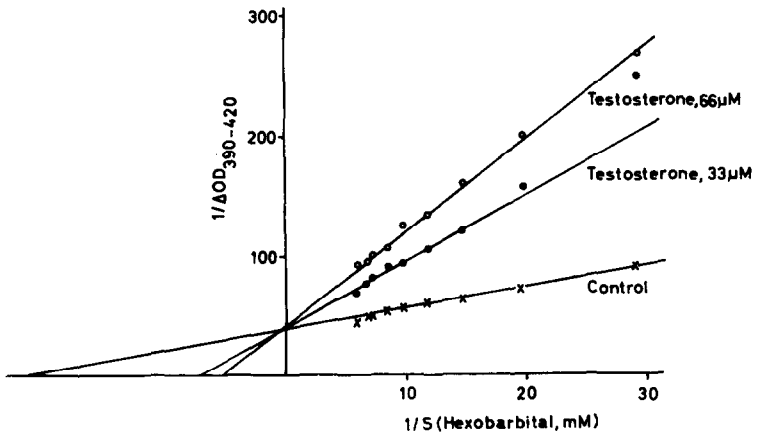

Fig. 2. Effect of testosterone on the type I spectral change induced by hexobarbital in rat liver microsomes.

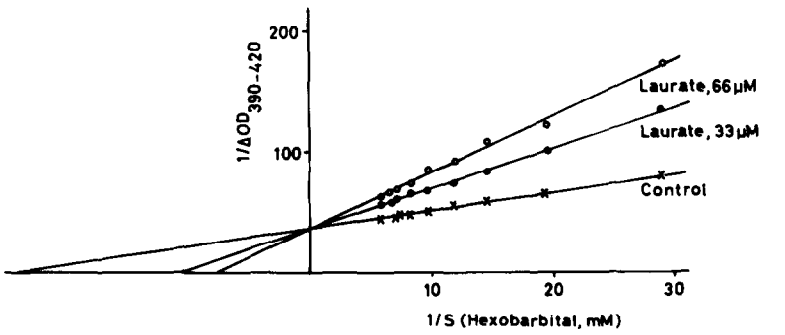

Fig. 3. Effect of laurate on the type I spectral change induced by hexobarbital in rat liver microsomes.

seems reasonable to assume that the substrates interact with the protein moiety which, in turn, gives rise to altered absorption characteristics of the heme [10]. It seems probable that the type I spectral change is a

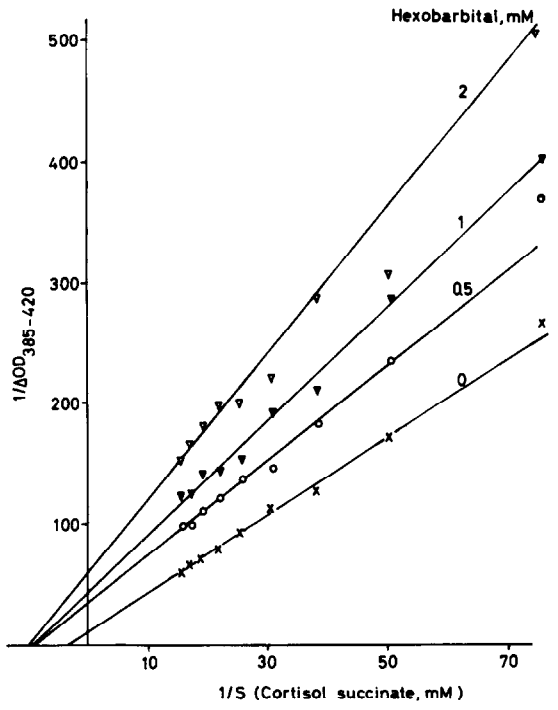

Fig. 4. Effect of hexobarbital on the type I spectral change induced by cortisol succinate in guinea-pig adrenal microsomes.

the type I spectral change is not yet elucidated, it reflection of the site on the protein with which the various substrates interact rather than an inherent attribute of the substrate molecule as such. Thus, testosterone gives rise to a type I spectral change when added to rat liver microsomes, whereas the same compound induces a type II spectral change with kidney cortex microsomes from the same species [11]. Furthermore, cortisol elicits a type I spectral change in guinea-pig liver microsomes but a type II spectrum in microsomes from rat liver [5]. Leibman et al. [8] have recently reported that barbiturates which elicit type I spectral changes in rat liver microsomes competitively inhibit each other's binding to cytochrome *P*-450. However, they postulated that this may be the case only when the substrates studied are structurally related, a conclusion which is not supported by the present data. Our observation that aminopyrine, testosterone and laurate act as competitive inhibitors of the type I spectral change induced by hexobarbital in rat liver microsomes adds further support for the existence of a common binding sites for these substrates on the liver-microsomal cytochrome *P*-450.

Although hexobarbital does not elicit detectable spectral changes with guinea-pig adrenal microsomes, it can bind to these particles, as revealed by its interference with the cortisol-induced type I spectral change [5]. In this case, however, the inhibition was not competitive. The present results are thus compatible with the hypothesis that structural differences exist between the cytochrome *P*-450 molecules of liver and adrenal microsomes, possibly in the form of different regulatory subunits, and that such differences may in fact be responsible for their different catalytic functions.

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