SPECTRAL STUDIES ON THE RAPID UPTAKE AND SUBSEQUENT BINDING OF DRUGS TO CYTOCHROME P-450 IN ISOLATED RAT LIVER CELLS

Christer von Bahr, Helena Vadi, Robert Grundin,

Peter Moldéus and Sten Orrenius

Department of Forensic Medicine and Department of Medicine at Huddinge Hospital, Karolinska Institutet, S-104 01 Stockholm 60, Sweden

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Summary

The rate of formation of the type I spectral change upon drug addition to a suspension of isolated rat liver cells was used to study factors that influence drug uptake by the hepatocytes. Although considerably slower than in liver homogenates and microsomes, drug combination with cellular cytochrome P-450 was still rapid and occurred within a few seconds. The effects of varying temperature and concentration and lipid solubility of the drugs studied as well as the lack of effect of preincubation of the cells with rotenone on the rate of formation of the type I spectral change, lead us to suggest that drug uptake into the hepatocytes occurs by a non-energy requiring diffusion process.

Introduction

In a previous communication (1), we have reported that a large fraction of cytochrome P-450 in isolated rat liver cells is present in the oxidized, non-substrate bound state and that the addition of various drugs to the medium produces the type I spectral change as the result of the formation of the cytochrome P-450-substrate complex (2). Since substrate binding to cytochrome P-450 in isolated liver microsomes is extremely rapid and takes place within milliseconds and since rat liver cells with - as far as we can judge - intact permeability properties can now be obtained, we have used this system to study factors that influence drug uptake into hepatocytes. It is con-

cluded that this occurs by a non-energy requiring diffusion process.

Methods

Unstarved male Sprague-Dawley rats weighing 200-220 g were used. The rats were killed by decapitation.

Liver homogenate and microsomes were prepared as described previously by Ernster et al. (3). Rat liver cells were isolated as reported earlier (4) with the following modifications: The liver was first perfused for 5 min with Locke's solution without glucose containing 0.5 mM EGTA, 2% bovine serum albumin and 2% (v/v) of washed bovine erythrocytes. Perfusion with the enzyme medium, containing 4 mM $CaCl_2$, was performed for only 10 min and the final incubation with enzymes for 5 min. The decreased preparation time resulted in a higher yield of viable liver cells, a liver of 10 g giving appr. 10 ml of a suspension of $3\text{-}4.5 \times 10^7$ cells/ml with a trypan blue exclusion frequency of 95-99%.

Cytochrome P-450 concentration and the type I spectral change were measured as described by Kupfer and Orrenius (5) using an Aminco DW-2 UV-VIS spectrophotometer. The cuvette was temperature-regulated and the cell suspensions were preincubated in a water bath to achieve the correct temperature. The drug was then added to the suspension of liver cells, homogenate or microsomes with a plunger (Aminco). Krebs-Henseleit buffer containing 2% bovine serum albumin was used for the suspension. In some experiments the cells were preincubated for 15 min at 37° with 10 µM rotenone in order to decrease the ATP level (4).

Results

As shown in Fig. 1, the rate of formation of the type I spectral change upon addition of hexobarbital to the medium

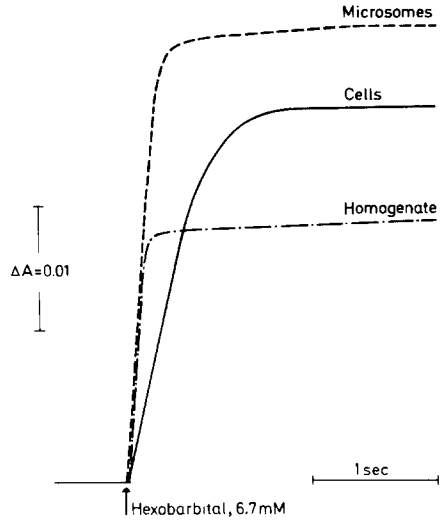

Fig. 1. Rate of formation of the type I spectral change upon addition of hexobarbital to liver homogenate, microsomes and isolated liver cells: Microsomes were suspended to contain 0.8 nmoles P-450 per ml, homogenate to 0.35 nmoles per ml and liver cells were suspended to contain $3x10^6$ cells per ml, equivalent to 0.5 nmoles P-450 per ml.

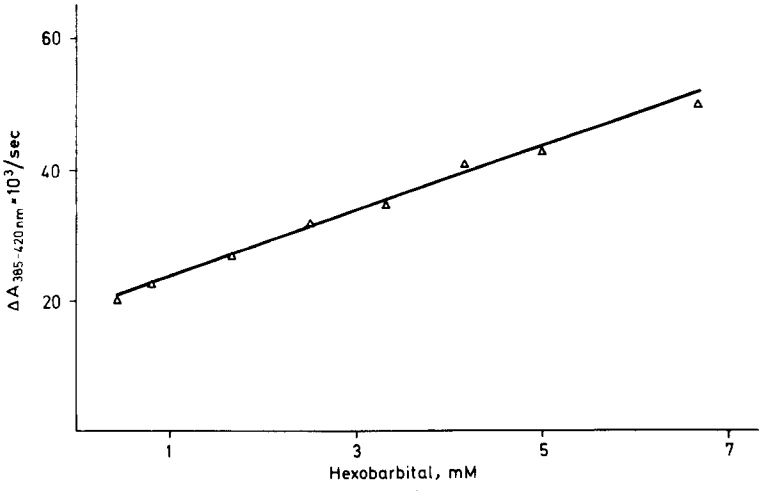

Fig. 2. Rate of formation of the type I spectral change upon addition of increasing concentrations of hexobarbital to isolated liver cells: Liver cells were suspended to a concentration of $3x10^6$ cells per ml. Temperature was maintained at + 37°C.

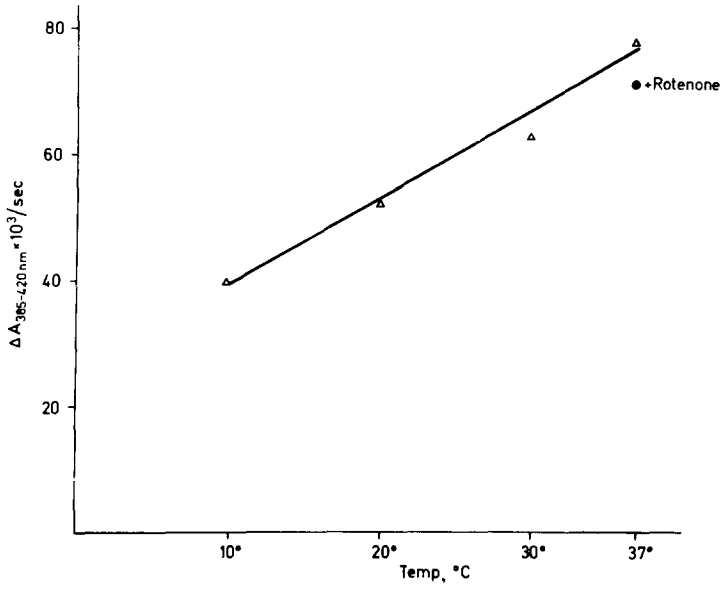

Fig. 3. Rate of formation of the type I spectral change upon addition of hexobarbital to isolated liver cells at different temperatures: liver cells were suspended to a concentration of $3x10^6$ cells per ml. Hexobarbital was added in a concentration of 6.7 mM. In some experiments preincubation with rotenone was performed according to methods.

was considerably slower in isolated liver cells than with liver homogenate and microsomes. The initial rate of appearance of the spectral change was dependent on the concentration of hexobarbital and showed no evidence of saturation at the concentrations studied (Fig. 2).

Further, the rate of formation of the spectral change increased by a factor of about 1.3 at a temperature increase of 10° between 10° and 37° (Fig. 3). Preincubation of the liver cells with rotenone to lower the ATP level caused no marked decrease in the rate of formation of the spectral change (Fig. 3).

Finally, using four different barbiturates the differences in rate of formation of the type I spectral change in isolated liver cells were found to be related to differences in

TABLE 1

Effect of lipid solubility on the rate of uptake of various barbiturates into isolated rat liver cells

Compound	k (o/w)[x]	K_s, μM[x]	$\Delta A_{385-420\ nm} \times 10^3$/sec
Hexobarbital	7.62	80	43
Amobarbital	4.85	65	36
Heptabarbital	3.44	40	32
Butobarbital	1.84	100	26

Cells were suspended in Krebs-Henseleit buffer, pH 7.5, to a final concentration of 3×10^6 cells/ml. Final concentration of the barbiturates was 5.3 mM. Rate of drug uptake was measured at 37°. The maximal magnitude of the type I spectral change was about the same for the different compounds.

x Data from Jansson et al. (6).

lipid solubility (Table I). No such relationship was found between the rate of formation of the type I spectral change in liver cells and the affinity of the barbiturates for binding to microsomal cytochrome P-450, as judged by their apparent spectral dissociation constants (K_s) (Table I and ref. 6). However, it can not be excluded that such a relationship can be found at lower substrate concentrations.

Comments

This study was primarily designed to resolve whether hepatic drug uptake occurs by passive diffusion or involves an energy consuming process. The results support the former alternative. Thus, preincubation of the isolated liver cells with rotenone, which decreases the cellular ATP concentration to 5-10% (4), had litt-

le effect on the rate of formation of the hexobarbital-produced type I spectral change. Further, the moderate temperature effect (Q_{10}=1.3) on this rate and its apparent relationship with the lipid solubility of the drug studied favor the conclusion that drug uptake into hepatocytes is not energy dependent.

Whether a non-energy requiring carrier is involved in the uptake of hexobarbital cannot be completely ruled out on the present data. However, the finding that there was no saturation of the rate of formation of the type I spectral change upon increasing hexobarbital concentration argues against the involvement of a carrier-mediated transport and supports the hypothesis that drug uptake into hepatocytes, in the concentration range studied, occurs by passive diffusion.

Acknowledgements

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