DRUG-INDUCED CYTOCHROME P-450 OF RAT LIVER AND VITAMIN A INTAKE

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There is evidence in the literature that xenobiotics and, in particular, insecticides, polychlorinated biphenyls, phenobarbital, methycholanthrene, etc., can cause a marked decline in the vitamin A content of the liver [7-10, 15], and can thereby disturb the supply of this vitamin, which is essentially for a number of key metabolic processes [4]. Since a common property of the compounds listed above is their ability to induce a cytochrome P-450-dependent mono-oxygenase system [1, 3], and since vitamin A metabolism is evidently linked to some degree with the functioning of that system [13, 14], it has been suggested that the observed effects are based on acceleration of retinol metabolism in the liver through the participation of xenobiotic-induced cytochrome P-450 [9, 10].

It is generally accepted that the number of inducers of cytochrome P-450 includes many drugs widely used in daily clinical practice and, in particular, amidopyrine (aminopyrine) and rheopyrine (a mixture of phenylbutazone and aminopyrine) in equal proportions. However, data in the literature on the direct experimental assessment of the inducing effect of these compounds are extremely limited [5, 6, 12], and their effect on the vitamin A content of the liver has not been studied. It was therefore decised to study the ability of aminopyrine and rheopyrine to induce cytochrome P-450 and to compare it with the action of a "classical" inducer, namely phenobarbital (PB), and to undertake a parallel study of their possible effect on the vitamin A content in the liver.

EXPERIMENTAL METHOD

Experiments were carried out on adult male rats kept on a balanced, semisynthetic diet, including 20% (by weight) of casein, 60% of starch, 10% of sucrose, and 5% of sunflower oil, to which ergocalciferol (calciferol) in the ratio of 2000 IU/kg of diet, 4% of mixed salt, and 1% of a mixture of water-soluble vitamins were added. To standardize the experimental conditions vitamin A was not included in the composition of the diet, but was given to all animals via a gastric tube once a week in the form of an oily solution of retinal palmitate in strictly identical quantities (400 IU per rat). The animals were divided into four groups received solutions of PB (40 mg/kg), rheophrine (200 mg/kg) or amidopyrine (100 mg/kg) via a gastric tube. Animals of group 4 received water, also via the gastric tube (control). The animals were killed by decapitation 24 h after the last dose of the drugs, the liver was removed and weighed, and part of the liver (1 g) was used to prepare homogenates, in which the content of cytochrome P-450 was determined. The remainder of the liver was frozen and kept at –30°C until required for determination of the vitamin A content. Liver homogenates were prepared as described previously [2], using 0.25 M sucrose in Tris-HCl, pH 7.4, as the homogenization medium. The concentration of cytochrome P-450 in the homogenates was determined by the method in [11], using a molar extinction coefficient of 104 mmole·cm^{-1}. The vitamin A concentration in the liver was determined as described previously, using antimony trichloride [2].

EXPERIMENTAL RESULTS

Prolonged administration of high doses of the drugs to the animals did not affect their general state, behavior, or appetite, but reduced a little their gain in body weight during the experiment (Table 1). PB and, to a somewhat lesser degree, amidopyrine and rheopyrine

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TABLE 1. Body Weight and Weight of Liver of Rats Receiving Certain Drugs (M ± m; n = 5)

Experimental conditions	Body weight, g			Weight of liver, g	Relative weight of liver
	initial	before sacrifice	gain during expt.		
Control	275,0±11,0	352,0±8,4	77,0±4,9	9,96±0,42	2,84±0,14
PB	285,0±11,0	333,0±9,7	48,0±9,7*	11,52±0,66	3,44±0,12*
Rheopyrine	270,0±12,3	339,0±18,6	69,0±7,3	10,66±0,77	3,10±0,08
Amidopyrine	274,0±4,7	341,0±18,0	67,0±2,6	11,14±0,71	3,26±0,05*

Legend. Here and in Table 2, *p < 0.05.

increased the absolute weight of the liver, and increased its relative weight even more (Table 1).

It will be clear from Fig. 1 that administration of PB to the rats caused a marked increase in the cytochrome P-450 content in the liver and an even greater increase in its relative level, calculated per 100 g body weight. These data are in agreement with results obtained by Leo and co-workers [10], who studied the inducing effect of PB under similar experimental conditions. Long-term administration of rheopyrine and amidopyrine also was accompanied by an increase in the cytochrome P-450 content in the liver, but by a much lesser degree than when PB was given, and the difference was significant, moreover, only when the content of the hemoprotein was calculated per 100 g body weight (Fig. 1). Rheopyrine had a greater effect on the cytochrome P-450 content than amidopyrine, evidently because of the potentiating action of the phenylbutazone, a constitutent of rheopyrine, on amidopyrine. The results showing the effect of amidopyrine alone and of amidopyrine + phenylbutazone on the cytochrome P-450 content in the rat liver are in agreement with earlier results [5, 6], which are still the main source of information about the inducing potential of these compounds. The conclusions drawn by the authors cited, however, were based on data showing acceleration of the conversion of drugs in the liver when taken into the body. The results of the present investigations give more strictly accurate characteristics of the ability of these compounds to act as cytochrome P-450 inducers, based on the direct study of its level in the liver. These results, together with data in the literature [5, 6], show that the amidopyrine and phenylbutazone possess undoubted ability to induce cytochrome P-450, but they are essentially weaker inducers than PB. It will be clear from Table 2 that PB significantly lowered the vitamin A concentration in the liver (by 31%) and caused a marked tendency for its total content in the liver to decrease (by 19.3% compared with the control). The results are in agreement with data in the literature, although they indicate a smaller reduction in the retinol depot. This result may perhaps be due to the method of intragastric administration of vitamin A, which we used, by contrast with the natural method of giving the vitamin with the food, used by the other workers. Administration of rheopyrine to the rats also was accompanied by a signifi-

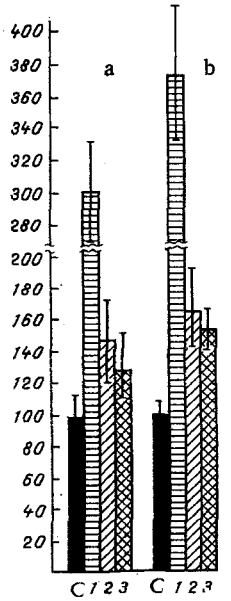

Fig. 1. Comparative study of inducing action of PB, rheopyrine, and amidopyrine on rat liver cytochrome P-450. Ordinate, cytochrome P-450 content in liver, % of control: a) nmoles/g liver; b) nmoles/100 body weight. C) Control. 1, 2, 3) PB, rheopyrine, and amidopyrine, respectively. Mean results of five experiments shown. Cytochrome P-450 content in liver of control rats was 44.2 ± 5.5 nmoles/g liver and 123.2 ± 10.9 nmoles/100 g body weight.

TABLE 2. Effect of Some Drugs on Vitamin A Content in Rat Liver (M ± m; n = 5)

Experimental conditions	Vitamin A concentration in liver		Total vitamin A content in liver	
	μg/g tissue	in % of control	μg	in % of control
Control	100,53±3,18[1]	100,0	1004,0±64,3	100,0
PB	69,37±3,51*	69,0	810,5±78,6	80,7
Rheopyrine	73,77±4,93*	73,4	815,7±32,9*	81,2
Amidopyrine	84,87±8,74	84,5	884,9±91,9	88,0

cant fall in the retinol concentration in the liver, the degree of which was similar to that induced by PB. It is important to emphasize that long-term administration of rheopyrine caused a significant reduction in the vitamin A reserves in the liver. Amidopyrine caused a smaller reduction of the retinol concentration and of its total content in the liver than PB or rheopyrine (Table 2), below the level of significance.

These experiments thus showed that long-term administration of PB and rheopyrine and, to a lesser degree, of amidopyrine to rats leads to induction of cytochrome P-450 in the liver and, at the same time, to a reduction in the concentration and total content of vitamin A in that organ. The results confirm the view that cytochrome P-450 plays an important role in the metabolism of retinol in the body. Meanwhile, under these experimental conditions, no clear dependence was found between the degree of induction of cytochrome P-450 and the fall in the retinol level in the liver. It can accordingly be postulated that although cytochrome P-450 plays a definite role in retinol metabolism in the liver, it also has other conversion pathways, which influence the retinol level in the liver.

It must be emphasized that, whatever the concrete biochemical mechanisms which lie at the basis of the fall of the vitamin A concentration in the liver during long-term administration of PB, rheopyrine, and amidopyrine, this fact is evidently of great importance in clinical practice, for it shows the potential risk of using these drugs, as factors capable of inducing a state of hypovitaminosis A. It must be pointed out that the present investigation was conducted on intact animals. Meanwhile these drugs are used for the treatment of patients with chronic diseases (collagenoses, nervous and mental diseases, and so on) which, in turn, can cause disturbances of the vitamin A supply to the body, thus aggrevating the similar effects of the drugs. This state of affairs evidently can explain recent observations of a sharp decline in the vitamin A concentration in the liver of patients treated for long periods with various psychotropic drugs, steroid hormones, etc. [10]. Accordingly, when patients receive long-term treatment with PB, butadione, and amidopyrine, a constant watch must be kept on the vitamin A balance of the body and, if necessary, additional doses of vitamin A preparations should be given.

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