ACTION OF AMOBARBITAL ON MICROSOMAL AND MITOCHONDRIAL RESPIRATORY STATE IN PERFUSED RAT LIVER WITH AND WITHOUT PHENOBARBITAL INDUCTION

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1. Introduction

It is well known that amobarbital acts as a potent inhibitor of mitochondrial respiration at the NADH site. This has been recently confirmed for whole hemoglobin-free perfused rat liver in titrations of the respiratory rate, and surface fluorescence [1,2], and also by spectral observation of respiratory pigments [1]. On the other hand, amobarbital serves in the liver as an external substrate of microsomal hydroxylation. An insight into the interrelation between these two oxygen-consuming systems can be derived (a) by comparison of the effects of amobarbital to those of aminopyrine, the latter acting solely at the microsomal site, and (b) by induction of the microsomal hydroxylation capacity which results from repetitive phenobarbital injections [3].

2. Experimental

The perfusion procedure and the dual wavelength technique, as well as the determination of the ratio (cytochrome P-450/cytochrome $(a+a_3)$) have been described [1,4,5]. Oxygen uptake was followed polarographically with teflon-shielded Ag-Pt(20μm)-microelectrodes, inserted in the perfusion circuit directly before and after the liver. For induction, 0.05 g/kg phenobarbital was administered i.p. once daily: the number of injections is indicated in the figures.

3. Results and discussion

3.1. *Non-induced liver*

The respiratory rate rises by about 6% when 0.2 mM aminopyrine is added (fig. 1, on the ordinate). Also, a small O.D. increment at (450–463 nm) is observed. This corresponds to about 2–5% of the increment caused by anoxia. These spectral changes are considered to reflect a true reduction because the

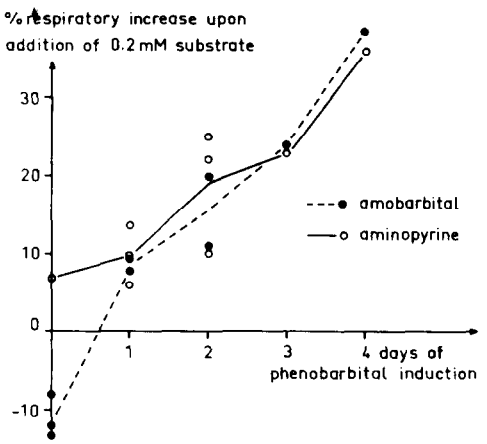

Fig. 1. Effect of aminopyrine (0.2 mM) and amobarbital (0.2 mM) on respiratory rate of perfused rat liver, dependent upon phenobarbital treatment (0.05 g/kg daily). Steady normoxic oxygen uptake was 1.62 ± 0.4 matoms O per hour per organ.

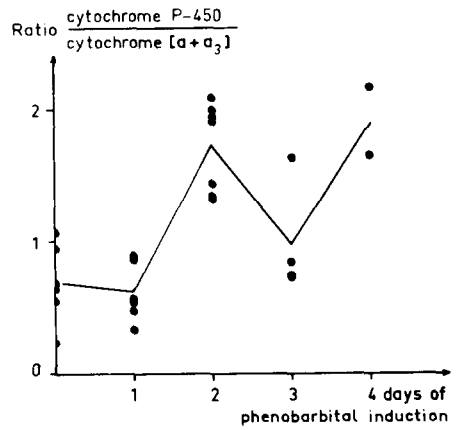

Fig. 2. Increase of ratio (cytochrome P-450/cytochrome ($a+a_3$)) after induction with phenobarbital. The ratio was calculated for each experiment from spectrophotometric readings according to ref. [5].

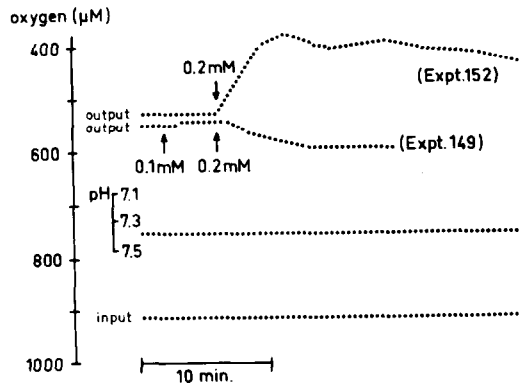

Fig. 3. Superposed records of two perfusion experiments. One rat was treated with phenobarbital for four days (expt. 152), the other was not treated (expt. 149). Upon addition of 0.2 mM amobarbital, the respiratory rate shows inverse movements: in the treated liver, respiration rises by 38%, while it decreases by 12% in the non-treated liver.

substrate binding exhibits no significant (450–463 nm)-signal [6]; this is also valid for amobarbital. Recently, we reported that the redox state of P-450 in normoxic livers ranges at 6% reduction or lower [7]. This was concluded from CO titration studies when no external substrate was added. With external substrate present, the degree of reduction therefore amounts to maximally 10%. The small increase in reduction of P-450 after substrate addition is interpreted to reflect an increased flux in terms of an increased deviation from equilibrium. When mitochondrial pigments are followed with the dual wavelength method, no redox change is observed after aminopyrine addition. In contrast, amobarbital (at the same concentration of 0.2 mM) produces such changes, and a decrease of the respiratory rate by 12% is observed (fig. 1, on the ordinate), in agreement with the earlier findings mentioned in the introduction.

3.2. *Induced liver*

The respiratory increase upon aminopyrine addition correlates with the number of phenobarbital injections (fig. 1), as was found in isolated microsomes [8]. The respiratory increment comes up to approximately one additional third of the normal respiration on the fourth day. The rise in the ratio (cytochrome P-450/cytochrome ($a+a_3$)) is shown in fig. 2. The induction kinetics is not linear; a drop is seen on the third day. Figs. 1 and 2 show that there exists no precise correlation of respiratory increment and the (P-450/($a+a_3$)) ratio, suggesting an influence of additional factors on microsomal activity. Furthermore, there is a large scatter of data in fig. 2 for non-induced as well as induced animals, in contrast to the more stable ratios measured for the various mitochondrial cytochromes (paper in preparation).

With amobarbital (0.2 mM) as substrate, an increase in overall respiration by about 8% one day after a single phenobarbital injection can be demonstrated (fig. 1). This remarkable fact suggests that after a single dose of inducing agent the mitochondrial site of action is no longer accessible to 0.2 mM amobarbital; it appears to act now solely as a microsomal substrate. Two experimental records with addition of 0.2 mM amobarbital to non-induced and induced liver are shown in fig. 3. Only when the concentration is increased (to 0.4 mM or higher), the respiratory rate diminishes again. However, the maximal amobarbital inhibition is less than in non-induced liver.

3.3. *Oxygen stoichiometry*

Addition of 40 μMoles amobarbital to the perfusion in a closed circuit of an induced liver caused an additional consumption of 120 μMoles O_2 (1.7

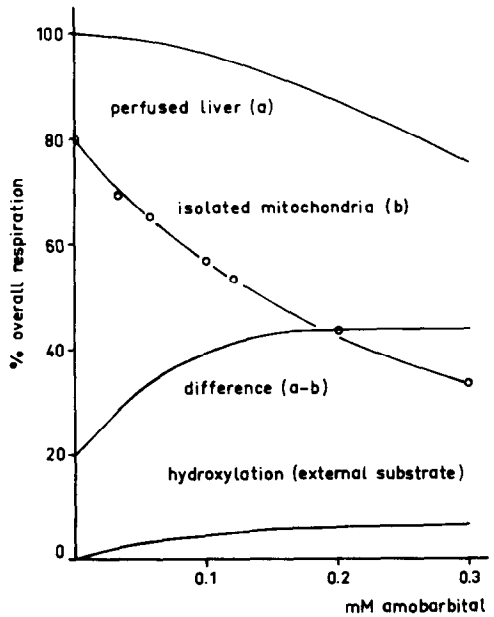

Fig. 4. Amobarbital titration curves. (a): overall respiration of perfused liver (from ref. [2], figs. 3 and 4); (b) curve (from unpublished data of H.Versmold) representing isolated state 3 mitochondria, respiring glutamate-malate-malonate, assumed to comprise 80% of overall liver respiration [1]; (c): (a) – (b); (d): microsomal titration curve, roughly estimated from fig. 1 and half-maximal concentration.

μMoles O_2/min per liver in the beginning) until recovery of the original respiratory rate (21.4 μMoles O_2/min per liver, after 82 min) in the mean of three experiments. That is, about 3 moles of oxygen were consumed per mole of amobarbital catabolized.

3.4. *Amobarbital effects and overall respiratory rate*

For the non-induced hemoglobin-free perfused liver, the plot of the respiratory rate against the concentration of amobarbital in the perfusion fluid has been found to be sigmoid [2,9], while the corresponding curve observed for isolated liver mitochondria is hyperbolic (fig. 4). The concentrations for half-maximal inhibition are 0.45 mM [2] and 0.2 mM, respectively. According to the findings reported here, some of the difference between the effect on the whole organ and that on the mitochondria is explained by the microsomal hydroxylation of amobarbital. Here, the half-maximal rate is observed already with 0.05 mM amobarbital. About one fourth of that difference can be accounted for by microsomal hydroxylation of the inhibitor. Screening of the mitochondria against the inhibitor by surrounding microsomes may be considered with respect to the residual difference. In fact, electron micrographs of perfused liver show that even after three hours of perfusion the mitochondria are tightly surrounded by endoplasmic reticulum. This consideration is supported by the observations of non-inhibition after induction shown in figs. 1 and 3.

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