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Relationships between propranolol plasma protein binding, glycoprotein concentration, and enzyme induction following phenobarbital administration in the dog*

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We have reported previously on the induction of the plasma protein binding of propranolol by phenobarbital, phenytoin, and Arochlor 1254 [1]. The mechanism of this increased binding was increased synthesis of α_1-acid glycoprotein (AAG). The known properties of these three substances as inducers of cytochrome P-450 suggested that the stimulation of AAG might be directly associated with induced drug metabolism. On the other hand, the induction of AAG could be an incidental finding. In the present work, we have performed a parallel experiment which measures the extent of antipyrine metabolism after cessation of phenobarbital administration. Antipyrine metabolism is known to depend on the level of hepatic cytochrome P-450 [2]. In this way, the time course of drug metabolism could be compared to the time course of AAG production and propranolol binding.

Most of the experimental details were presented in our earlier work [1]. Briefly, four male littermate beagle dogs were the experimental subjects. Phenobarbital was administered for 15 days at a dose of 180 mg/day, p.o. Antipyrine (15 mg/kg, i.v.) was dissolved in 5 ml of distilled water and filtered through a Millex-GS filter. Blood samples for antipyrine assay were obtained by syringe and placed in glass tubes containing heparin. The plasma was separated and frozen until assayed.

Plasma antipyrine was assayed by the method of Brodie *et al.* [3]. Blood samples were obtained prior to the antipyrine dose and at 30, 60, 90, and 120 min after injection. One antipyrine experiment was done before the animals were begun on phenobarbital and is designated as control. The occasion of the last phenobarbital dose was called day 0. Other antipyrine kinetic experiments were then performed on days 0, 5, 9, and 12.

For a flow-independent system the $T_{\frac{1}{2}}$ for a drug reflects the enzymatic competence for the process acting on it; the linear relationship is between λ, the elimination rate constant, and Q, the quantity of enzyme [4]. These λ values were used as the data reflecting the induction of antipyrine metabolism by phenobarbital. The percent induction was calculated as:

$$\frac{\lambda(t)\text{-}\lambda\ (\text{control})}{\lambda(0)\text{-}\lambda\ (\text{control})} \times 100 \qquad (1)$$

where $\lambda(t)$ is the observed rate constant on day 0, 5, 9, or 12 post-phenobarbital. A similar manipulation of the data previously obtained for AAG concentration and propranolol binding was performed [1].

The plasma antipyrine concentrations during various experimental conditions are shown in Fig. 1. The half-lives calculated between 30 and 120 min for antipyrine after intravenous doses in the four dogs for the five experimental periods are presented in Table 1. They show the anticipated progression with the shortest occurring immediately after the phenobarbital treatment and the longest during the control period.

The time course of the recovery of antipyrine metabolism from its induced state towards a control value can be examined in two ways. The λ values can be adjusted to the amount of enzyme which has been induced by subtracting the λ for the control period from the λ values during the phenobarbital decay phase. These data are presented in a semilogarithmic plot in Fig. 2. This shows an apparent

* A portion of this work was presented at the Eighth International Congress of Pharmacology, Tokyo, Japan, July 1981.

Table 1. Antipyrine half-lives

Dog	Antipyrine half-life (min)				
		Days postphenobarbital			
	Control	0	5	9	12
1	136	39	30	70	91
2	99	24	23	50	86
3	112	20	35	51	75
4	152	27	25	74	91
Mean ± S.E.	125 ± 12*	27.5 ± 4.1*	28.2 ± 2.7*	61.2 ± 6.3*	86 ± 3.8*

* Every mean half-life is significantly different from each other at the $p < 0.05$ level or better except for days 0 and 5.

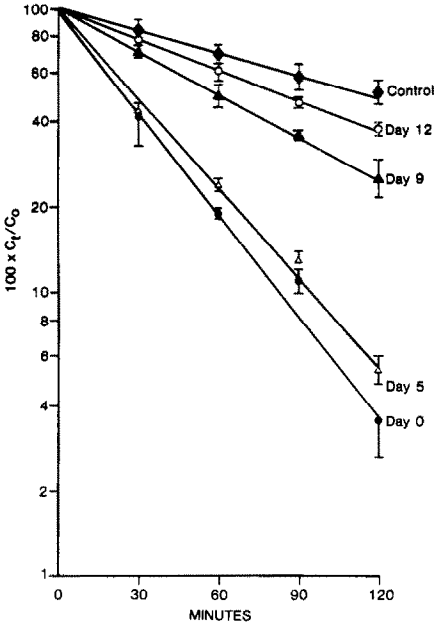

Fig. 1. Plasma antipyrine concentrations: the effect of discontinuing phenobarbital treatment. The data shown are the means and standard errors for four dogs on each of five occasions and are expressed as a percentage of the extrapolated initial concentration. The days are counted from the last phenobarbital dose.

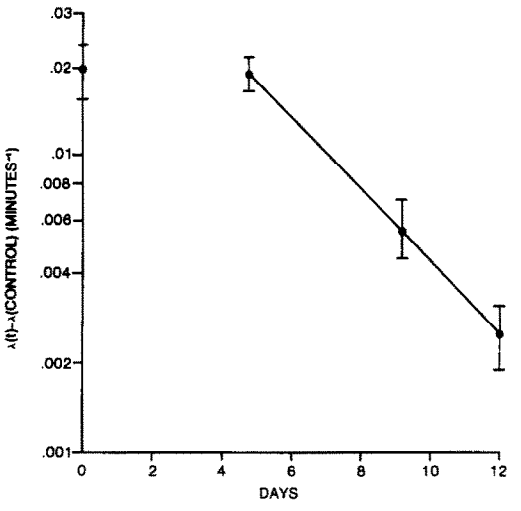

Fig. 2. Kinetics of the recovery from induction of antipyrine metabolism following the cessation of phenobarbital dosing. The data shown are the means and standard errors for four dogs. The line drawn has a slope of 0.29 day^{-1}.

turnover rate constant of 0.29 day^{-1} or a $T_{\frac{1}{2}}$ of 2.4 days. The decay did not begin on day 0, but appears to have lagged by about 5 days.

Alternatively, the fractional antipyrine induction after ceasing phenobarbital therapy can be linearly plotted as a function of time. Figure 3 shows this set of data along with analogous data for the concentration AAG, and the bound/free concentration ratios for propranolol in these same dogs after cessation of phenobarbital treatment [1].

For our analysis of antipyrine metabolism to be valid, flow-independent kinetics must apply. The antipyrine clearance ranges between 20 and 100 ml/min, based on the equation Cl = Dose/auc and the data in Fig. 1. In these dogs, the hepatic blood flow was 540 ml/min in control experiments and was unchanged after phenobarbital administration [5]; thus, our assumption appears to be valid.

There are two alternative hypotheses to explain our previous observations that inducers of drug metabolism also induce AAG production. There could be one "receptor" site for an inducer which initiated an elevated production rate for both cytochrome P-450 and AAG. Alternatively, there could be two separate sites, one for induction of metabolism, the other for the glycoprotein. The present data show similarity in the behaviour of P-450 and AAG after phenobarbital treatment is withdrawn. As will be discussed later, this recovery is governed by the sensitivity of each process towards induction.

Figure 2 is very similar to Fig. 4 in Ref. 1 where the decline of induced AAG was examined. There the apparent lag was about 3 days. In each case, the explanation lies in a K_m sufficiently below the initial phenobarbital concentration that the production rate of the P-450 or AAG continues at a nearly maximal rate despite the decay of the inducer. In these dogs, the maximum concentration of the inducer, phenobarbital, was 25 μg/ml and its half-life was 33 hr. A simple monoexponential decay was used to estimate its plasma concentration following its last dose on day 0. If the K_m for phenobarbital-inducing antipyrine metabolism was 3 μg/ml, it would take 4.2 days to reach this level after its dosing was stopped, a time frame consistent with the lag period before the induction appears to decay.

In our earlier publication [1], we developed the following

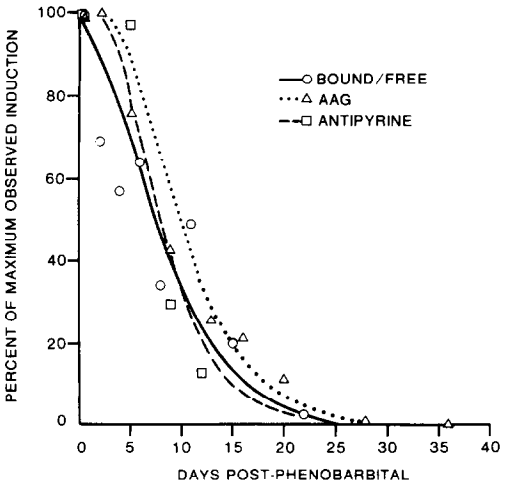

Fig. 3. Fractional recovery from induction following the cessation of phenobarbital dosing. The data shown are for antipyrine metabolism calculated from the adjusted reciprocal half-lives (equation 1). The data for the concentration of AAG and the propranolol bound/free concentration ratios were obtained from Ref. 1. Each datum is the mean from four dogs.

equation to describe the effect of the pharmacokinetics of an inducing agent on the subsequent behaviour of the induced substance. For the recovery towards control values of a process "X" following withdrawal of a chronically administered inducer "I",

$$dX/dt = V_{max} \cdot I(t)/(Km + I(t)) + R_0 - k_e \cdot (X) \quad (2)$$

where V_{max} is the maximum rate of stimulated production of substance X, K_m is the Michaelis constant for its stimulation by I, $I(t)$ is the concentration of I at time t, R_0 is the unstimulated rate of production of X, and k_e is the turnover rate constant for X. This equation defines a sigmoidal curve if $I(0) \gg K_m$ and $I(t)$ decreases exponentially with time.

By plotting $\lambda(t) - \lambda$ (control) in Fig. 2, the term R_0 is removed. The turnover rate constant obtained, 0.29 day^{-1}, was similar to the turnover rate constant of AAG, 0.20 day^{-1} [1]. This apparent k_e is likely an underestimate of the true turnover rate constant because of the simultaneous decay of phenobarbital. In the case of the glycoprotein, this caused the true elimination rate constant of 0.2 day^{-1} to appear as 0.15 day^{-1} for over 2 weeks after discontinuing phenobarbital dosing [1].

The k_e for enzymes metabolizing antipyrine is equal to a half-life of 2.4 days. This is similar to the 2.0 day $T_{\frac{1}{2}}$ for turnover of radiolabeled microsomal heme from rat liver [6], or the 1.9 day $T_{\frac{1}{2}}$ for removal of covalently bound microsomal radioactivity from rats treated with propranolol [7]. We assume that all these measurements reflect the same catabolic process.

If the turnover rate constants for antipyrine metabolism and AAG production are similar, the major influence on the rate of recovery of AAG, propranolol binding or antipyrine metabolism should be the K_m for the stimulation of each particular process by phenobarbital. The lines in Fig. 3 were obtained by integrating equation 2 with the value of K_m for phenobarbital induction which best described each data set. Those K_m values were 3 μg/ml for antipyrine metabolism, 2 μg/ml for AAG, and 5 μg/ml for B/F. Previously, we obtained a K_m of 1.2 μg/ml for AAG from these same data [1].

In an earlier abstract*, we concluded that there was a notable difference in the K_m which fit the metabolism data and the percentage of propranolol binding as a function of time. While superficially true, we erred in attempting to fit equation 2 to the free fraction of propranolol. If propranolol plasma protein binding were unsaturated, then B/F would be linearly related to protein concentration by the equation $B/F = nKa \cdot P$ where B and F refer to the bound and free propranolol concentrations, and K_a is the association constant for propranolol binding to the protein with n sites which is present at concentration P. Under our experimental conditions, this relationship was shown to be valid [1]. The free fraction is $F/(B + F)$, or $(B/F + 1)^{-1}$. Thus, the observed free fraction of propranolol is not a linear function of AAG concentration, and it is invalid to fit equation 2 to these data. It is true that the point in time where propranolol binding has returned halfway to normal, 17 days, is substantially delayed from the time where antipyrine metabolism is halfway recovered. What Fig. 3 shows is that the time course of recovery of the primary process which was stimulated, AAG concentration, was similar to the time course of antipyrine metabolism.

These data are insufficiently precise to definitively answer the question initially posed: is the induction of glycoprotein production different from the induction of drug metabolism? We do show that there is not a large difference in the main parameter governing the sensitivity of each towards phenobarbital.

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