Return of Drug-metabolizing Systems to Control Levels after Induction with 3-Methylcholanthrene

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The induction of drug-metabolizing systems by treatment of animals with xenobiotics such as phenobarbital and 3-methylcholanthrene has been extensively investigated for almost 20 years now.[1] The time course for the induction of cytochrome P-450, epoxide hydrolase, glutathione *S*-transferase(s) and other drug-metabolizing systems has been characterized. However, the time course with which these activities return to control levels after cessation of treatment with the inducer has received considerably less attention. The return of drug-metabolizing systems to control levels after induction must necessarily involve protein degradation and, in some cases, membrane degradation as well. Consequently, this return may provide a useful experimental system for answering questions about the mechanisms and control of protein and membrane degradation.

Treatment of rats with phenobarbital causes an approximately 4-fold increase in the specific activity of the cytochrome P-450 system in the hepatic endoplasmic reticulum, as well as an approximately 2–2.5-fold increase in the content of endoplasmic reticulum membranes per gram liver.[2] We have shown that after the final injection of phenobarbital the induced protein and phospholipid components of the endoplasmic reticulum return in parallel to control levels within five days.[3] In conjunction with results from other laboratories[4] this finding has led us to conclude that the endoplasmic reticulum induced by phenobarbital is degraded in large pieces by autophagic vacuoles after cessation of the treatment.

On the other hand, treatment of rats with 3-methylcholanthrene results in an approximately 4-fold increase in the content of cytochrome P-450 in the hepatic endoplasmic reticulum without causing proliferation of this organelle.[1,5] Thus, in this case it would seem to be highly wasteful to degrade whole segments of the endoplasmic reticulum during the process of return to control levels. In an initial attempt to characterize the return to control levels after induction with 3-methylcholanthrene, we have examined here the time course of this return for a number of different drug-metabolizing enzymes.

Male Sprague-Dawley rats weighing 180–200 g were injected intraperitoneally once daily for 1–5 days with 20 mg 3-methylcholanthrene/kg body weight in corn oil or with a corresponding volume of the vehicle alone. The animals were starved overnight before sacrifice by decapitation and the total microsomal and high-speed supernatant fractions were prepared in the usual manner.[6] Cytochrome P-450,[7] cytocgrome b_5,[7] glutathione *S*-transferase activity towards 1-chloro-2,4-dinitrobenzene,[8] and DT-diaphorase activity[9] were all assayed using published procedures.

In the experiments designed to characterize the excretion of 3-methylcholanthrene and its metabolites, 3 rats were injected intraperitoneally with 20 mg 3-methylcholanthrene/kg body weight in corn oil containing 185 MBq [^{14}C]-3-methylcholanthrene (The Radiochemical Centre, Amerham-Searle, England) once daily for 5 days. These animals were maintained in metabolism cages and their urine and feces collected in 24-h periods during the period of treatment and for 14 days after the final injection. The feces were dried and weighed, aliquots of both the urine and feces were bleached using H_2O_2, and the radioactivity present was determined by scintillation counting in Lumagel.

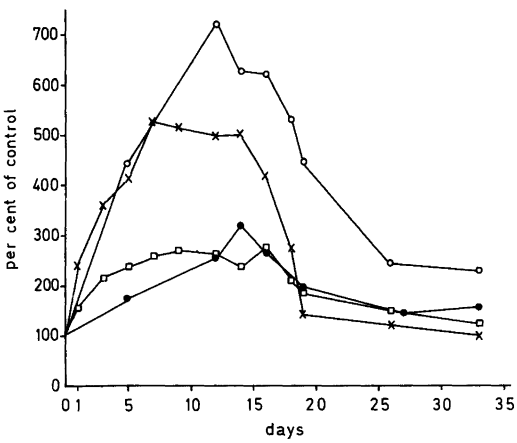

Fig. 1. Induction of drug-metabolizing systems by 3-methylcholanthrene and subsequent return to control levels. The rats were injected on days 0, 1, 2, 3 and 4. Each point represents an average value for 3–9 different animals. The symbols used are as follows: × = cytochrome P-450; □ = cytochrome b_5; ● = glutathione *S*-transferse(s); ○ = DT-diaphorase. For further details see the text.

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Fig. 1 shows the time course of induction of cytochromes P-450 and b_5, glutathione *S*-transferase(s) and DT-diaphorase by 3-methylcholanthrene and the return of these enzymes to control levels after cessation of the treatment. It can be seen that the amounts and/or activities of these enzymes present in the liver continue to rise for several days after the cessation of 3-methylcholanthrene treatment. Cytochrome P-450, cytochrome b_5, glutathione *S*-transferase(s) and DT-diaphorase do not return to control levels until 2–3 weeks after the final injection.

As mentioned above, the return of drug-metabolizing enzymes to control levels after induction with phenobarbital requires about 5 days.[3] This is also the case after induction with *trans*-stilbene oxide[10] or with 2-actylaminofluorene.[11] One possible explanation as to why the return to control levels requires considerably longer after induction with 3-methylcholanthrene may be that this xenobiotic is excreted only slowly and consequently remains in the rat's body for several weeks.

In order to investigate this possibility, the experiment illustrated in Fig. 2 was performed. It can be seen that about 75 % of the total amount of 3-methylcholanthrene injected into the animals has been excreted by the fifth day after the final injection. Thus, it does not seem likely that retention of 3-methylcholanthrene in the body can explain the relatively long time period required for return to control conditions. It can also be seen from Fig. 2

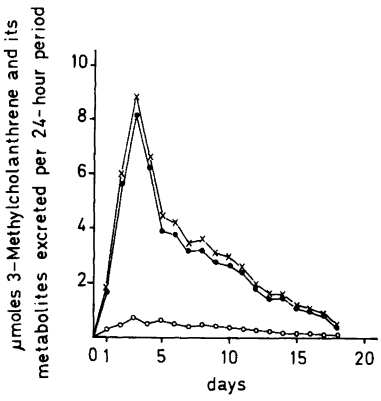

Fig. 2. Excretion of 3-methylcholanthrene and its metabolites by the rat. The animals were injected with [^{14}C]-3-methylcholanthrene on days 0, 1, 2, 3 and 4. Each point represents an average value for 3 different animals. The symbols used are as follows: ● = excretion in the feces; ○ = excretion in the urine; × = total excretion. For further details see the text.

that almost all the 3-methylcholanthrene is excreted in the feces. This may be due to the uptake of 3-methylcholanthrene from the peritoneal cavity directly into the intestine, followed by excretion, and/or to the excretion of conjugated metabolites of 3-methylcholanthrene into the bile. We are in the process of identifying the form in which the radioactivity is excreted.

Thus, the return of drug-metabolizing systems to control levels requires considerably more time after induction with 3-methylcholanthrene than after induction with other xenobiotics. This may indicate that a different mechanism of protein degradation is involved in the case of 3-methylcholanthrene. In addition, the mechanism for degrading membrane proteins such as cytochromes P-450 and b_5 may differ from the degradation of cytosolic enzymes such as glutathione *S*-transferase(s) and DT-diaphorase – *e.g.*, degradation of membrane proteins may require their selective removal from the membrane.

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