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Developmental differences in basal and induced aryl hydrocarbon (benzo[*a*]pyrene) hydroxylase activity in chick embryo liver and lung *in ovo*

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The chick embryo has been shown to have hepatic aryl hydrocarbon (benzo[*a*]pyrene) hydroxylase (AHH)* activity equal to or greater than that of the adult from 3 days of incubation (DI) through hatching [1]. AHH in chick embryo liver is inducible by 3,4,3′,4′-tetrachlorobiphenyl (TCB) [2] and other polycyclic aromatic hydrocarbons [1–6] to levels of activity equal to or greater than maximum induction in the adult from 7 DI through hatching [1]. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) was first shown to be a potent inducer of AHH activity in 17 DI chick embryo liver [3].

Preliminary studies indicated that AHH was only inducible in the liver of early chick embryos [1]. This was surprising since, in adult mammals, the lung generally has the highest AHH activity of the non-hepatic tissues, and this activity is induced over a similar dose range, and to a similar percent increase over control, as in liver [7–12]. We therefore compared the developmental patterns of basal and inducible AHH activities in the chick embryo liver and lung during late embryonic and neonatal development, particularly with regard to two considerations. First, since AHH inducibility in the liver develops concomitantly with morphological differentiation of the hepatocyte [1, 13, 14], AHH inducibility in the lung might also be expected to develop concomitantly with its functional differentiation, i.e. during late embryonic development or at hatching. Second, marked increases in both basal and induced levels of AHH activity in the liver at 14 DI and at 1 day post-hatching (PH) suggest developmental changes in the chick embryonic mixed-function oxidase (MFO) system at these times [1, 14, 15]. Such developmental factors might also cause parallel changes in basal and/or inducible AHH activity in the lung. To investigate these points, basal and TCB-induced AHH activity was measured in the liver and lung of chick embryos and chicks on each day of development from 14 DI through 4 days PH, at 10 days PH, and at maturity.

Eggs from the Cornell K-strain [16] were incubated at 37.5° and 85% humidity and rotated once an hour. Fertile eggs were selected by candling. Chick embryos and newly hatched chicks were injected with 5×10^{-6} moles/kg body weight TCB (Ultra Scientific Co., Hope, RI.) in 10 μl acetone as previously described [1] and killed 24 hr later by cervical dislocation; their livers and lungs were quickly removed and rinsed in ice-cold Tris–sucrose buffer (0.05 M Tris–HCl, 0.25 M sucrose, 3 mM $MgCl_2$, 1 mM EDTA, pH 7.4). Liver microsomes were prepared as previously described [1, 17]. Lungs were homogenized in Tris–sucrose buffer, (1:3, w/v), first in a Waring blender and subsequently in a glass-teflon homogenizer. Lung microsomes were prepared from the homogenate in the same manner as liver microsomes. Pellets were stored at −85° for up to 7 days before being assayed. Microsomes were resuspended in AHH buffer (0.1 M potassium phosphate, 5 mM $MgCl_2$, 1 mM EDTA, pH 7.4) and adjusted to 2 mg/ml protein according to the method of Lowry *et al.* [18], using bovine serum albumin as the standard. AHH activity was determined using the direct fluorimetric technique of Yang and Kicha [19] and is expressed as nanomoles benzo[*a*]pyrene metabolized per min per mg microsomal protein. This assay measures total benzo[*a*]pyrene (BaP) metabolism and has been shown to have several distinct advantages over other assays of AHH metabolism [1, 17, 19]. For the earlier embryonic time points, livers or lungs from several individuals were pooled in the homogenizer and treated statistically as a single individual. This ranged from ten individuals/sample at 14 DI to one individual/sample at 18 DI and older. Each sample was assayed a minimum of two times, and replicate samples were run for each time point.

The dose of 5×10^{-6} moles/kg body weight TCB was determined previously to be the lowest dose which causes maximum hepatic AHH induction in the chick throughout the embryonic and neonatal period [1]. Studies on induction in mice and rats have shown liver and lung AHH to have similar dose-responses to inducers, within a strain [7–12]. Hepatic AHH activity was equal to or greater than adult activity from 14 DI through 10 days PH and inducible by TCB to levels equal to or greater than maximum induction in the adult (Table 1, Fig. 1a). AHH activity increased through the hatching period to a maximum, at 1 day PH, of 2–3 times adult activity, and it remained high for several days after hatching. At 14–15 DI and 20 DI–2 days PH, the maximum induced AHH activity was 50% higher than in the adult. TCB-induced liver preparations had 10–30 times the activity of corresponding controls throughout the study.

We had reported previously that the chick embryo exhibits AHH activity equal to adult levels as early as 3 DI, that the liver AHH is inducible by TCB as early as 6 DI, and that the onset of inducibility is concomitant with hepatocyte differentiation [1]. Chick embryo liver MFO activities have been shown to be inducible by TCDD, TCB, 3-methylcholanthrene, phenobarbital, β-naphthoflavone and other compounds known to cause induction in mammals [1–6]. As shown here, the precocious development of basal and inducible AHH activities in the chick embryo liver is substantially different from that reported for the mammalian species studied. Mammalian fetal AHH activity is typically 1–5% that of adults during late development [9, 20–24] and is inducible by xenobiotics to 2–3 times control levels [9, 20, 25–29]. The high level of AHH activity in the early chick embryo correlates with its ability to activate promutagens/carcinogens, as shown by sister chromatid exchange [14, 15, 30], and the onset of inducibility between 3 and 6 DI is reflected in a change in mutagenic response during this period [14].

Pulmonary AHH activity in the chick embryo and neonate also differs substantially from that of mammals. Mammalian lung generally has the highest AHH activity of the non-hepatic tissues, and this activity is inducible by xenobiotics over a similar dose range as in liver [7–12]. Typically, basal AHH activity in mammalian lung microsomes is 1–5% that of liver microsomes from the same individual or strain, while induced AHH of lung microsomes shows a slightly greater percent increase over controls than induced liver microsomal AHH [7–12]. In the chick embryo, basal pulmonary AHH activity was substantially higher than basal hepatic AHH activity at 14–16 DI, but it decreased to approximately half that of liver by hatching (Table 1, Fig. 1b). However, AHH activity in the lung was not inducible by TCB for the majority of the developmental

* Abbreviations: AHH, aryl hydrocarbon hydroxylase; DI, days of incubation; PH, post-hatching; TCB, 3,4,3′4′-tetrachlorobiphenyl; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; BaP, benzo[*a*]pyrene; and MFO, mixed-function oxidase.

Table 1. Development of basal and inducible aryl hydrocarbon (benzo[*a*]pyrene) hydroxylase (AHH) activities in chick embryo liver and lung.

Day of development	AHH activity (nmoles benzo[*a*]pyrene metabolized/min/mg microsomal protein)			
	Liver		Lung	
	Control	Induced*	Control	Induced*
14 DI†	0.48 ± 0.04‡	14.13 ± 0.48§	0.68 ± 0.05	0.80 ± 0.44
15 DI	0.39 ± 0.05	13.37 ± 1.21§	0.99 ± 0.33	0.97 ± 0.16
16 DI	0.35 ± 0.02	10.82 ± 0.39§	0.65 ± 0.33	0.52 ± 0.07
17 DI	0.37 ± 0.04	9.97 ± 0.15§	0.59 ± 0.08	0.62 ± 0.13
18 DI	0.31 ± 0.03	8.06 ± 0.86§	0.38 ± 0.04	0.36 ± 0.11
19 DI	0.35 ± 0.06	8.50 ± 0.83§	0.21 ± 0.02	0.23 ± 0.01
20 DI	0.33 ± 0.01	13.03 ± 0.85§	0.26 ± 0.08	0.54 ± 0.01§
21 DI‖	0.98 ± 0.02	14.50 ± 1.23§	0.19 ± 0.06	0.68 ± 0.03§
1 DPH¶	1.63 ± 0.10	15.13 ± 0.45§	0.18 ± 0.03	0.44 ± 0.08§
2 DPH	1.01 ± 0.03	15.04 ± 0.10§	0.16 ± 0.01	0.31 ± 0.17§
3 DPH	0.99 ± 0.11	8.71 ± 0.18§	0.20 ± 0.02	0.25 ± 0.08
4 DPH	0.99 ± 0.03	9.08 ± 1.67§	0.18 ± 0.01	0.25 ± 0.14
10 DPH	0.39 ± 0.06	9.55 ± 0.73§	0.19 ± 0.03	0.23 ± 0.05
Adult	0.43 ± 0.07	9.01 ± o.42§	0.10 ± 0.01	0.15 ± 0.04

* Induced animals received 5×10^{-6} moles/kg 3,4,3',4'-tetrachlorobiphenyl in 10 μl acetone 24 hr prior to being killed.
† Days of embryonic incubation.
‡ Values are means ± S.D. for replicate samples.
§ Significantly different from controls at $P < 0.01$.
‖ Hatching.
¶ Days post-hatching.

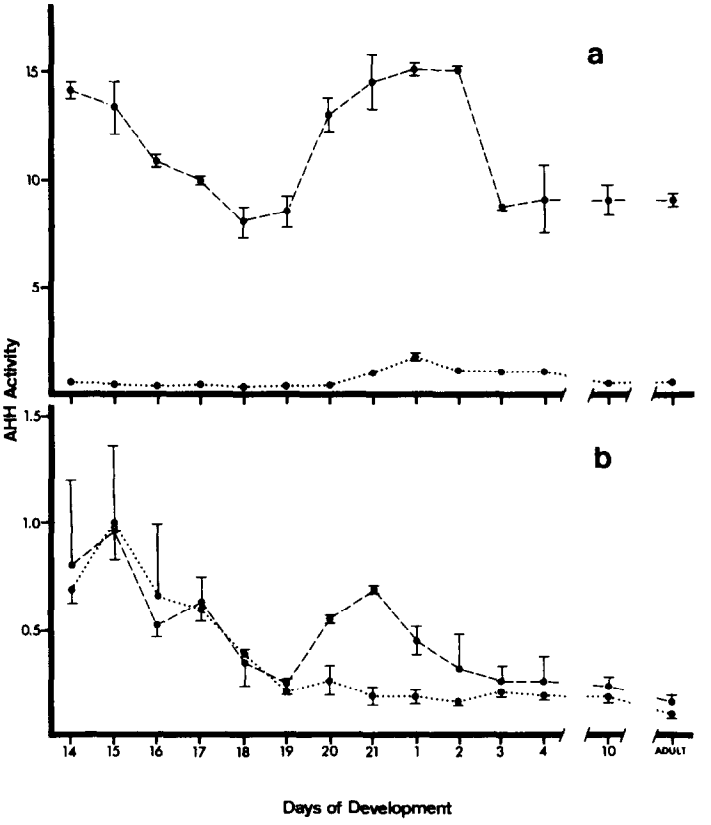

Fig. 1. Development of basal, (dotted line) and maximum TCB-induced (24 hr) (dashed line) AHH activities in the chick embryo from 14 DI through 4 days PH, at 10 days PH, and at maturity in microsomes from (a) liver and (b) lung. AHH activity is expressed as nmoles benzo[*a*]pyrene metabolized per min per mg microsomal protein. Each point represents the average ± S.D. for replicate samples.

period studied or in adults. There was a transient period of induction from 20 DI to 1 day PH ($P < 0.01$), with a maximum at 21 DI (hatching) of 3 times control.

The developmental patterns of basal and inducible AHH activity in chick embryo liver and lung had interesting similarities and differences. The full pattern of development in the liver showed several dramatic changes in both basal and induced levels. Of particular interest was the onset of inducibility between 5 and 7 DI [1, 14], the peaks in both basal and induced activity at 12–14 DI and 21 DI–1 day PH, and the continued high basal activity for several days PH. While the lung also had a peak of basal activity at 14–15 DI, this was not accompanied by an increase in inducibility. Similarly, the moderate inducibility of lung AHH around hatching was not accompanied by a parallel change in basal activity. Basal lung AHH decreased steadily toward hatching and stayed at that level for the remainder of neonatal development. The changes in basal hepatic AHH from 19 DI through 10 days PH have been shown to be correlated with parallel changes in levels of NADPH–cytochrome P-450 reductase [6], while the mechanism(s) involved in the change in inducibility at hatching is unknown. A comparison of the developmental pattern of basal and induced activity in liver and lung suggests that the changes in induced AHH activity are independent of the changes in basal AHH activity.

The developmental profile of basal and maximum TCB-induced AHH activity has been reported for liver and lung in the chick embryo from 14 DI through 10 days PH and at maturity. Activities in both tissues differed markedly from those reported for mammalian liver and lung. Hepatic AHH activity was equal to or greater than adult activity throughout late embryonic and neonatal development and was inducible to levels equal to or greater than maximum induction in the adult. Pulmonary AHH activity was considerably higher in relation to hepatic AHH activity in the chick embryo than in mammals, ranging from twice hepatic levels at 15 DI to about half hepatic AHH after hatching. AHH activity in lung was not inducible over controls by TCB for the majority of late embryonic and neonatal development or at maturity, and it was only slightly inducible for a 3-day period around hatching. Differences between basal and induced profiles in both liver and lung and differences between the two tissues suggest that the mechanisms involved in the changes in basal AHH activity and induced AHH activity are independent of each other.

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REFERENCES

1. J. W. Hamilton, M. S. Denison and S. E. Bloom, *Proc. natn. Acad. Sci. U.S.A.*, **80**, 3372 (1983).
2. A. Poland and E. Glover, *Molec. Pharmac.* **13**, 924 (1977).
3. A. Poland and E. Glover, *Molec. Pharmac.* **9**, 736 (1973).
4. A. B. Rifkind, P. N. Gillette, C. S. Song and A. Kappas, *J. Pharmac. exp. Ther.* **185**, 214 (1973).
5. A. B. Rifkind, M. Troeger and T. Petschke, *Biochem. Pharmac.* **28**, 1681 (1979).
6. G. Powis, A. H. Drummond, D. E. McIntyre and W. R. Jondorf, *Xenobiotica* **6**, 69 (1976).
7. R. E. Kouri, T. Rude, P. E. Thomas and C. E. Whitmire, *Chem. Biol. Interact.* **13**, 317 (1976).
8. F. J. Weibel, J. C. Leutz and H. V. Gelboin, *Archs Biochem. Biophys.* **154**, 292 (1973).
9. K. Burki, A. G. Liebelt and E. Bresnick, *Archs Biochem. Biophys.* **158**, 641 (1973).
10. B. G. Lake, R. Hopkins, J. Chakraborty, J. W. Bridges and D. V. Parke, *Drug Metab. Dispos.* **1**, 342 (1973).
11. B. G. Lake, M. A. Collins, R. A. Harris and S. D. Gangolli, *Xenobiotica* **9**, 723 (1979).
12. P. M. Warren and G. D. Bellward, *Biochem. Pharmac.* **27**, 2537 (1978).
13. A. O. Banjo and A. M. Nemeth, *J. Cell Biol.* **70**, 319 (1976).
14. L. A. Todd and S. E. Bloom, *Environ. Mutagen.* **2**, 435 (1980).
15. S. E. Bloom, in *Current Progress in Cytogenetics: Sister Chromatid Exchange* (Ed. A. A. Sandberg), p. 249. Alan Liss, New York (1982).
16. R. K. Cole and F. B. Hutt, *Anim. Breed. Abstr.* **41**, 103 (1973).
17. M. S. Denison, M. Murray and C. F. Wilkinson, *Analyt. Lett.*, **16**, 381 (1983).
18. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
19. C. S. Yang and L. P. Kicha, *Analyt. Biochem.* **84**, 154 (1978).
20. R. M. Welch, A. Loh and A. H. Conney, *Life Sci.* **10**, 215 (1971).
21. O. Pelkonen, P. Jouppila and N. T. Karki, *Toxic. appl. Pharmac.* **23**, 399 (1973).
22. W. Kuenzig, J. J. Kamm, M. Boublik, F. Jenkins and J. J. Burns, *J. Pharmac, Exp. Ther.* **191**, 32 (1974).
23. J. R. Gillette and B. Stripp, *Fedn. Proc.* **34**, 172 (1975).
24. O. Pelkonen, in *The Induction of Drug Metabolism* (Eds. R. W. Estabrook and E. Lindelaub), p. 507. F. K. Schattauer, Stuttgart (1978).
25. O. Pelkonen, P. Korhonen, P. Jouppila and N. T. Karki, in *Basic and Therapeutic Aspects of Perinatal Pharmacology* (Eds. P. L. Morselli, S. Gerattini and F. Sereni), p. 65. Raven Press, New York (1975).
26. D. W. Nebert, F. M. Goujon and J. E. Gielen, *Nature, Lond.* **236**, 107 (1972).
27. C. B. Sehgal and J. J. Hutton, *Mutation Res.* **46**, 325 (1977).
28. S. M. Galloway, P. E. Perry, J. Meneses, D. W. Nebert and R. A. Pederson, *Proc. natn. Acad. Sci. U.S.A.* **77**, 3524 (1980).
29. G. F. Kahl, D. E. Frederici, S. W. Bigelow, A. B. Okey and D. W. Nebert, *Dev. Pharmac. Ther.* **1**, 137 (1980).
30. S. E. Bloom, in *Cytogenetic Assays of Environmental Mutagens* (Ed. T. C. Hsu), p. 137. Allenheld, Osmun & Co., Montclair, NJ (1982).