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Activities of hepatic biotransformation enzymes of turkey embryos and turkey

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ABSTRACT

The activities of two enzymes from phase I of biotransformation, aniline hydroxylase and ethylmorphine N-demethylase were investigated in turkey embryos and poults. Measurements were performed five times prior to hatching, at the 18th, 22nd, 24th, 26th and 27th day of embryos' life and five times after hatching, at the 1st, 5th, 9th, 16th and 26th day of turkeys' life. Ten days prior to hatching the enzyme activities in embryo livers amounted to 116.8 ± 1.6 nmol p-aminophenol/g liver/30 min, and 222.8 ± 25.0 nmol formaldehyde/g liver/30 min for aniline hydroxylase and ethylmorphine N-demethylase, respectively. A three- and two-fold increase in aniline hydroxylase and ethylmorphine N-demethylase activities was observed immediately after hatching (1st day) in relation to 27-day-old embryos, respectively. Thereafter activities continued to rise and in 26-day-old poults they amounted to 672.9 ± 10.3 nmol p-aminophenol/g liver/30 min, and 2672.6 ± 27.9 nmol formaldehyde/g liver/30 min. Body masses, as well as absolute and relative liver masses were measured after hatching. The ratio between liver/body mass showed that relative liver mass increased from the 1st to the 9th day of life, followed by a constant slight decrease until the 26th day of life. These results generated in physiological conditions indicate that biotransformation phase I reactions are higher at the end of first month of turkey life than those in turkey embryos and in newly-hatched turkey. Thereby, liver capacity

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for hydroxylation and N-demethylation of xenobiotics and endogenous compounds gradually increase in young turkey.

Key words: turkey embryo, turkey, biotransformation enzymes

Introduction

Biotransformation enzymes of phase I are involved in the synthesis and/or degradation of many endogenous compounds such as steroids, prostaglandins, fatty acids and other biological molecules, and they are also responsible for the majority of oxidation of xenobiotic chemicals such as drugs, pesticides, carcinogens, etc. This is followed by phase II reactions, which involve reactions such as conjugation with UDP-glucuronic acid, sulfate or glutatione. The biotransformation of a foreign chemical may result in either increased or decreased toxicity depending upon its chemical nature and function of the metabolic pathways by which a compound is degraded. Microsomal enzymes have been extensively studied in mammals, i.e. rats, mice, rabbits, cows, sheep, swine, dogs and cats (PATERSON and ROBERTS, 1970; EHRLICH and LARSEN, 1983; SMITH et al., 1984; DALVI et al., 1991; SHORT et al., 1988b; KAWALEK and EL SAID, 1990a; KAWALEK and EL SAID, 1990b), but not so much in birds, which is apparent in the list of P450 genes published in the review by NELSON et al. (1993).

It is known that many endogenous factors, such as species, age, sex, and developmental or hormonal status, regulate the activities of biotransformation enzymes (RONIS and CUNNY, 1994). Although much is known about metabolism in adult organisms, little information exists on the role of cytochrome-P450-dependent enzymes early during their development. The developing organism is remarkably dynamic and many of the enzymes present in adults are not expressed in the foetus (MILLER et al., 1996). JUCHAU et al. (1980) suggest that toxic effects of foreign organic chemicals during prenatal life are dissimilar in different species. There are no references about the activities of aniline hydroxylase and ethylmorphine N-demethylase in turkey embryos, and few studies have been done concerning activities of these two enzymes in the first days and weeks after hatching (BARTLET and KIRINYA, 1976; THABREW et al., 1982; SHORT et al., 1988a). Additionally, hatching is one of the most critical periods in avian life. Accommodation to new environmental conditions and food

after hatching require an enhanced activity of detoxication enzymes in birds. For this purpose we determined the activities of two cytochrome-P450-dependent enzymes: aniline hydroxylase and ethylmorphine N-demethylase that catalyze the enzymatic reactions of phase I of biotransformation in turkey embryos and poults.

Materials and methods

Experimental animals. Embryonated eggs originating from Nicholas hybrid line turkeys were incubated at 37 °C. Poults were allocated to cages. They were housed under optimal zoohygienic conditions and fed a balanced commercial diet. Water was given *ad libitum*. Light was maintained 24 h a day and room temperature was controlled at 26 °C. Poults were weighed individually prior to sacrificing by cervical dislocation

Enzyme activities. Livers were removed immediately after exanguination of stunned embryos or poults, and weighed. In order to prepare a liver homogenate, whole embryo livers (n = 10) and samples of tissue from the same area of the poult livers (n = 6), were taken. A 10% w/v liver homogenate was prepared in 0.025 M saccharose in a Teflon homogenizer in an ice bath. A post-mitochondrial supernatant liver fraction was prepared by centrifugation of the homogenate at 9000 x g for 30 min in a high-speed refrigerated centrifuge (Janetzky K23) at 5 °C.

Aniline hydroxylase activity was determined by measuring the amount of p-aminophenol formed from aniline hydrochloride (IMAI et al., 1969).

The activity of ethylmorphine N-demethylase was assayed according to the method of COCHIN and AXELROD (1959) using ethylmorphine chloride as a substrate. The amount of formaldehyde formed during N-demethylation was estimated by the method described by NASH (1953).

Enzyme activities are expressed as nmol metabolite/ g liver/ 30 min.

Statistics. The data were subjected to statistical analysis - Statgraphics, ver. 4.0 and presented as mean ± standard error. The significance of differences was assessed by the Student's *t*-test. Values were considered significant when $P<0.01$.

Results

Body masses of one-day-old poults amounting to 65.5 ± 2.4 g, increased to 801.0 ± 22.7 at the 26th day of life (data not shown). Liver masses ranged from the 1st to the 26th day of life 1.43 ± 13 g and 17.7 ± 0.8 g, respectively (data not shown).

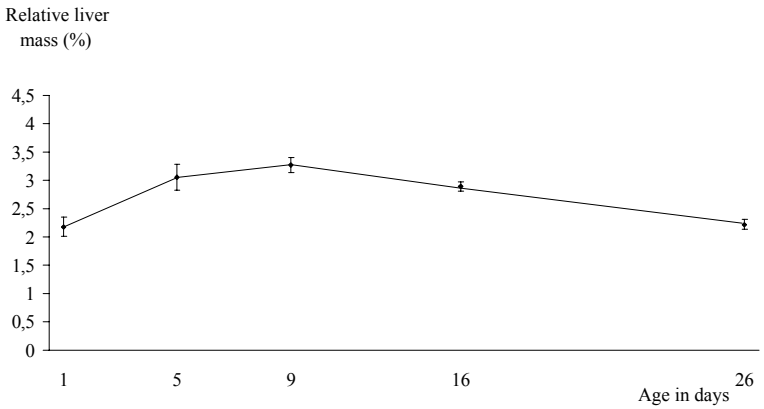

Fig. 1. Relative liver mass (%) in poults aged 1, 5, 9, 16 and 26 days

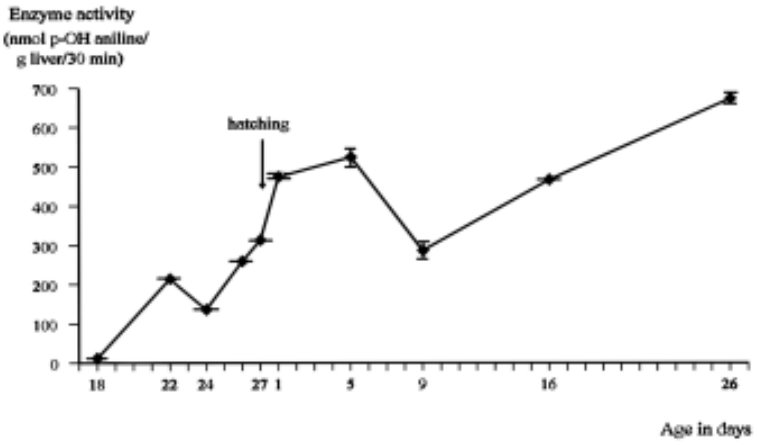

Fig. 2. Activity of aniline hydroxylase in liver of turkey embryos from the 18th to the 27th day of development, and during the first 26 days of turkey life

Relative liver mass increased from the 1st to the 9th day of life, followed by slight decrease until the 26th day of life (Fig. 1).

Activities of aniline hydroxylase and ethylmorphine N-demethylase were relatively low during the embryos' development (Fig. 2 and 3). However, at the 26th day of embryo life and one day prior to hatching, concentrations of both metabolites increased two- and three-fold in relation to the 24th day. Further, a significant increase of activity in investigated enzymes was observed at hatching (between the 27th day of embryo life and the 1st day of turkey life). Activity of ethylmorphine N-demethylase in the liver of 26-day-old poults was about two-fold higher than in liver of one-day-old poults (Fig. 3). Simultaneously, activity of aniline hydroxylase increased by about 1.5 times (Fig. 2).

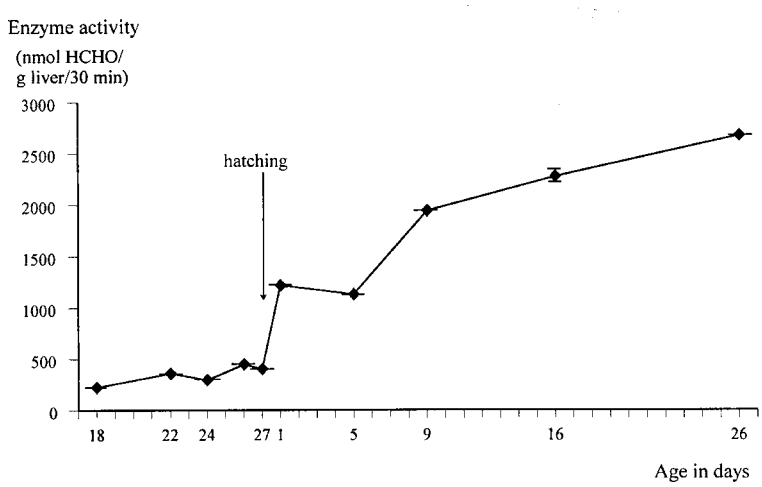

Fig. 3. Activity of ethylmorphine N-demethylase in liver of turkey embryos from the 18th to the 27th day of development, and during the first 26 days of turkey life

Discussion

A number of enzymes in animal organisms are capable of biotransforming lipidsoluble xenobiotics in such a way as to render them more water soluble. These enzymic reactions are of two types: phase I

reactions, which involve oxidation, reduction, and hydrolysis, and phase II reactions, which consist of conjugation or synthetic reactions. A prime function of phase I reaction is to add or expose functional groups, e.g., -OH, -SH, -NH_2, -COOH. These functional groups then permit the compound to undergo phase II reactions. The enzymes or enzyme systems that catalyze the biotransformation are localized mainly in the liver. Cytochrome P-450 enzymes play a central role in phase I reactions because they catalyze many steps of sterol biosynthesis, the oxidative metabolism of fatty acids, sterols and another endogenous substrates, as well as the physiological oxidation of the vast majority of drugs and other xenobiotics. In this study we investigated two cytochrome P-450-dependent enzymes: aniline hydroxylase and ethylmorphine N-demethylase, during the development of turkey embryos and in the first 26 days after hatching.

Our results show that livers in 18-day-old embryos were sufficiently developed to be explored for biochemical investigations. During the development period of embryos, enzyme activities tend to increase as the time required for hatching is shorter, and are higher than those in chick embryos (POWIS et al., 1976; SAKAR, 1984). Corresponding assessment show that hepatic enzyme activities among avian species are seldom comparable (SHORT et al., 1988a; WALKER and RONIS, 1989; GAWAI et al., 1992; AMSALLEM-HOLTZMAN and BEN-ZVI, 1997). Geese, chickens and turkeys showed similar aniline hydroxylase activity, while it was markedly lower in quail and ducks (DALVI et al., 1987). The O-demthylation of p-aminophenol, ester hydrolysis of procaine and glucuronidation of p-nitrophenol studied in the birds showed that within the avian subset, chicken and turkey were usually the most similar species (SHORT et al., 1988b).

A significant rise in enzyme activities in turkey, obtained immediately prior to, as well as at hatching time, seems to be comparable with that observed in chicken embryos (POWIS et al., 1976). Dynamic enzyme activity might be a consequence of a significant increase in concentration of plasma corticosterone in embryos (SCOTT et al., 1981) accompanying decreased binding of the corticosterone on plasma albumin at hatch (SIEGEL and GOULD, 1976; BARTLET et al., 1990; TAKAHASHI et al., 1993). Also, resorption of liposoluble substances from the rest of egg yolk (POWIS et al., 1976) could be one of possible reasons for the enhanced enzyme activity during hatching

time. This period is one of most critical times in a bird's life, characterized by adaptation the new environmental and feeding conditions, so that a sharp increase in the activity of detoxication enzymes may be a compensatory mechanism in newly-hatched turkey.

Activity of aniline hydroxylase gradually increases up to day 5 after hatching, which then transiently decreases (54%), before reaching maximal increase at day 26 (Fig. 2).

In contrast, the activity of ethylmorphine N-demethylase mildly decreases during the first 5 days after hatching, and then drastically increased (58%) through the study (Fig. 3).

If we compare the dynamics of enzyme activities in turkeys and chickens during the first month of life, there are some similar trends, although the values are generally higher in turkeys (POWIS et al., 1976; SAKAR et al., 1991; SAKAR et al., 1992a).

Based on data obtained in this study our initial hypothesis that overall sensitivity of newly-hatched turkey would be accompanied with impaired activities of their biotransformation enzymes needs to be rejected. These results from studies performed in physiological conditions would be a good basis for further investigations of turkey biotransformation system activity against variety types of xenobiotics.

Values of relative liver masses, which increased from the 1st to the 9th day of life, followed by a slight decrease until the 26th day of life (Fig. 1) correspond well with the results obtained in chickens (SAKAR et al., 1992a; SAKAR et al., 1992b). This is in agreement with reports supporting the physiological similarity between turkey and chickens.

The results provide some novel information to add to the sparse knowledge about biotransformation enzymes in turkey embryos and poults. The results showed that biotransformation phase I reactions are higher at the end of the first month of turkey life than those in turkey embryos and in newly-hatched turkey, as a result of which liver capacity for hydroxylation and N-demethylation of both endogenous compounds and xenobiotics seems to gradually increase.

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PREVENDAR CRNIĆ, A., D. SAKAR, J. POMPE-GOTAL, Z. BIĐIN, B. POKRIĆ: Aktivnost biotransformacijskih enzima u jetri puranskih zametaka i purića.Vet. arhiv 73, 211-220, 2003.

SAŽETAK

U puranskih zametaka i purića istražene su aktivnosti dva enzima iz I. faze biotransformacije: anilin hidroksilaze i etilmorfin N-demetilaze. Mjerenja su provedena pet puta prije leženja, i to 18., 22., 24., 26. i 27. dana razvitka zametka, te pet puta poslije leženja, 1., 5., 9., 16. i 26. dana života purića. Aktivnost jetrene anilin hidroksilaze u osamnaestodnevnih zametaka bila je 116.8 ± 1.6 nmol p-aminofenola/g jetre/30 min, a aktivnost etilmorfin N-demetilaze 222.8 ± 25.0 nmol formaldehida/g jetre/30 min. Dvostruko odnosno trostruko povećanje aktivnosti tih enzima izmjereno je neposredno nakon leženja u odnosu na 27 dana stare zametke. Nakon toga, aktivnosti enzima uglavnom su i dalje rasle, a najviše su bile zadnjeg dana mjerenja, tj. 26. dana života: 672.9 ± 10.3 nmol p-aminofenola/g jetre/30 min i 2672.6 ± 27.9 nmol formaldehida/g jetre/30 min. Tjelesne mase purića te apsolutne i relativne mase jetre mjerene su nakon leženja, neposredno prije uzorkovanja jetre za enzimske analize. Vrijednosti relativne mase jetre u purića uvećavale su se od 1. do 9. dana života, nakon čega su se smanjivale do kraja promatranog razdoblja. Rezultati istraživanja aktivnosti navedenih enzima, iz I. faze biološkog prijetvora u fiziološkim uvjetima pokazuju da su njihove vrijednosti više u purića u dobi od mjesec dana nego u netom izleženih purića i puranskih zametaka, što je znak da se postupno uvećava kapacitet jetre za hidroksilaciju i N-demetilaciju endogenih i organizmu stranih tvari.

Ključne riječi: puranski zametci, purići, biotransformacijski enzimi