Biochemical Pharmacology, Vol. 52, pp. 1915–1919, 1996.

ISSN 0006-2952/96/$17.00 + 0.00
PII S0006-2952(96)00522-9

SHORT COMMUNICATION

Induction of Liver and Kidney CYP1A1/1A2 by Caffeine in Rat

*Thierry Goasduff, Yvonne Dréano, Bernard Guillois, Jean-François Ménez and François Berthou**

LABORATOIRES DE BIOCHIMIE-NUTRITION EA-948, FACULTÉ DE MÉDECINE, BP 815-29285 BREST CEDEX, FRANCE

ABSTRACT. Caffeine metabolism by hepatic microsomal P450 enzymes is well documented in experimental animals and humans. However, its induction effect on P450 enzymes has not been thoroughly studied. In a preliminary experiment, the time-dependent incubation of 1 mM caffeine with rat hepatocyte culture resulted in an increase of its own metabolic rate. The dose-dependent expression of rat hepatic and renal cytochromes (CYP) 1A1/1A2 was then investigated after *per os* administration of caffeine. P450 expression was monitored by using specific enzymatic activities and Northern blot analysis. Caffeine caused a dose-dependent elevation of hepatic CYP1A1/1A2 activities in microsomal preparations, which ranged from 1.7- to 6-fold for ethoxyresorufin O-deethylase and 3- to 8.9-fold for methoxy-resorufin O-demethylase according to the dose regimen of 50 and 150 mg caffeine/kg/day for 3 days, respectively. Northern blot analysis demonstrated that caffeine treatment increased liver CYP1A1 and CYP1A2 mRNA levels over the dose regimen of 50–150 mg caffeine/kg/day for 3 days, respectively. The result of this study demonstrates that caffeine increases its own metabolism in a dose-dependent manner and induces CYP1A1/1A2 expression through either transcriptional activation or mRNA stabilization. *Copyright © 1996 Elsevier Science Inc.* BIOCHEM PHARMACOL 52;12:1915–1919, 1996.

KEY WORDS. Caffeine; CYP1A1; CYP1A2; induction mechanism; mRNA, rat; liver; kidney

CAF,† a major constituent of coffee, tea and cola beverages, is consumed worldwide. Moreover, CAF has several pharmacological properties, such as stimulation of the central nervous system and diuresis and biochemical properties based on inhibition of AMPc phosphodiesterase [1].

Numerous studies have demonstrated that the biotransformation of CAF occurs principally in the liver via microsomal CYP monooxygenase enzymes [2]. Although CAF metabolism is complex, the different biotransformation pathways are now well characterized in different species [3]. CYP1A2 is the major P450 isoform involved in its primary *N*-demethylation reactions [3–7], and CYP1A1 plays a minor role when it is expressed. This evidence is based on studies carried out by means of hepatic microsomes [8], cultured hepatocytes [5], liver slices [5, 9] or heterologous expression systems [7, 10].

The effects of CAF on P450 enzymes have up to now not been the object of much research. However, several studies on animals have suggested that this drug is able to induce some P450 isoforms and thus increase its own metabolism [11, 12]. All these studies using low doses of CAF (30–50 mg/kg) showed either induction [13] or inhibition [14]. Recently, induction of CYP1A and CYP2B enzymes by CAF have been demonstrated [15] and validated [16]. It has long been believed that induction of CYP1A1/1A2 involves the binding of planar molecules (such as polycyclic aromatic hydrocarbons such as TCDD, β-NF, and 3MC [17]) to the Ah receptor. However, omeprazole induces both CYP1A1/1A2 in human liver *in vivo* and *in vitro* [18] but without being a ligand for the Ah receptor. CAF itself has been described as an inducer of CYP1A2 in rat liver without association with the Ah receptor [16].

The goal of the present study was to examine the effect of caffeine *in vitro* on rat hepatocyte culture and to investigate the expression of CYP1A enzymes following *in vivo* treatment of rat with increased doses of CAF. Such an investigation should elucidate the mechanism of CYP1A induction by CAF.

MATERIALS AND METHODS

Incubation of Rat Hepatocytes with CAF

Rat hepatocytes were isolated by the collagenase perfusion of the whole liver from male Wistar rats weighing 200–250 g according to [19]. Hepatocytes were seeded at the density of 2.5×10^6 cells per 28-cm^2 Petri dish and 4 hr after cell seeding were incubated with CAF at 1 mM dissolved in the

* Corresponding author: Dr. F. Berthou, Laboratoires de Biochimie-Nutrition EA-948, Faculté de Médecine, BP 815-29285 Brest Cedex, France; FAX: (33) 02 98016603; E-MAIL: Francois.Berthou@univ-brest.fr.

† *Abbreviations:* CYP, cytochromes P450 (EC 1.14.14.1); MROD, methoxyresorufin O-demethylase; EROD, ethoxyresorufin O-deethylase; 3MC, 3-methylcholanthrene; CAF, caffeine; β-NF, 5,6-benzoflavone; TCDD, tetrachloro-dibenzo-*p*-dioxin; Ah, aromatic hydrocarbons; PCR, polymerase chain reaction; RT, reverse transcriptase.

Received 13 March 1996; accepted 28 June 1996.

culture medium as previously described [20]. CAF was incubated 24 hr for metabolism studies. Cells were cultured for 48 hr and 72 hr with or without presence of CAF, according to scheme shown in Fig. 1. After incubation, culture medium was collected and stored at −80° until analysis. Culture media were saturated by ammonium sulfate and then extracted by 2 volumes of chloroform-isopropanol mixture (85/15; v/v) using a mechanical shaker for 2 hr. After centrifugation at 2500 rpm for 5 min, the organic phase was removed, dried at 40°C under nitrogen stream and redissolved into HPLC mobile phase. CAF metabolites were analyzed as previously described [5].

In vivo *Induction Experiments*

ANIMALS AND CAF TREATMENT. Male Wistar rats (160–180 g) were treated by CAF that was administered *per os* in two aliquots per day at 9 a.m. and at 4 p.m. at the doses of 50 (CAF 50), 100 (CAF 100) and 150 (CAF 150) mg/kg/day for 3 days. CAF was given orally in 1 mL of 0.9% sodium chloride. The control group was given only a saline solution. Each group consisted of 5–6 animals. Rats were fasted 12 hr before death. Liver and renal microsomes were prepared as previously described [5] and stored at −80°C until use.

MONOOXYGENASE ACTIVITIES. MROD and EROD activities were determined by spectrofluorimetry according to [15, 21].

ISOLATION OF TOTAL RNA. Total RNA was isolated by the acid guanidium thiocyanate phenol chloroform extraction method [22] from 500 mg of liver or kidney. The final RNA pellet was washed with 80% ethanol, vacuum dried and dissolved in 400 μL (liver samples) or 200 μL (kidney samples) of diethylpyrocarbonate-treated water and stored at −80°. The concentration of RNA was estimated by absorbance at 260 nm and the purity determined from the 260/280 nm ratio, which ranged from 1.6 to 1.8.

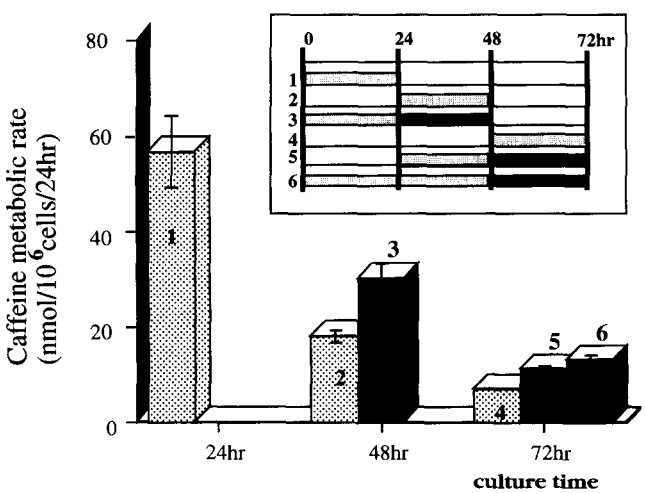

FIG. 1. Effect of culture time in presence of CAF on its biotransformation rate by rat hepatocyte cultures. Hepatocytes were treated by 1 mM CAF for 24 hr (light gray) and 48 hr (dark gray) before the 24-hr final incubation with 1 mM CAF to measure its biotransformation rate. For comparison, CAF was incubated at 1 mM for 24 hr after 24-hr and 48-hr culture times (dotted bar) without pretreatment. (Results are expressed as means ± SD of four dishes). Inset: Treatment of rat hepatocyte by CAF; the last 24 hr always consisted of incubation of caffeine to determine its metabolic rate. The culture medium was renewed every 24 hr.

DIGOXIGENIN-LABELED DNA PROBE PREPARATION. The DNA probes for CYP1A1 [23,25] and 1A2 [24,26] were obtained from RT-PCR products of hepatic RNA isolated from rat treated by 3MC and using primer sequences specific for these isoforms (Table 1). Briefly, 1 μg of total liver RNA was used for reverse transcription (first-strand cDNA synthesis kit, Pharmacia) in a total volume of 15 μL. The cDNA templates were diluted 1/4 for the first PCR. PCR consisted of 1 μL of this diluted solution, 1 μM of each of the required primers, 0.2 mM dNTP, 0.5 units of Taq polyerase (Eurotaq, Eurogentec, France) and 5 μL of 10X PCR buffer (100 mM Tris HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$, 0.1% Tween 20) in a total volume of 50 μL. PCRs were performed in a personal thermocycler (Biometra, Kontron, France) for 35 cycles, and each cycle consisted of a 1-min denaturation step at 95°C, a 1 min hybridization step at 50°C and a 2 min elongation step at 72°C. The PCR products were purified with a Microcon-100 microconcentrator (Amicon, Beverly, MA, USA). An aliquot of the purified PCR products was used to synthesize the digoxigenin-labeled DNA probe. These probes were synthesized by a second PCR by using a reaction mixture that contained 0.2 mM each of dNTP (dATP, dCTP and dGTP), 0.19 mM of dTTP, 0.01 of mM digoxigenin-11-dUTP (Boehringer, Mannheim, Germany) and the same components as those used for the first PCR. PCRs were performed for 30 cycles. An aliquot of the first PCR reaction was digested by different restriction enzymes for 2 hr at 37°C to confirm PCR products. These digested fragments and the labeled PCR fragment were run in 1.5% agarose gel stained with ethidium bromide (data not shown).

NORTHERN HYBRIDIZATION. The samples were denatured by mixing 5 μL (20 μg total RNA) of aliquot with 15 μL of a denaturing solution (70% formaldehyde, 30% deionized formamide, v/v) at 70°C for 5 min and then adding 3 μL of 10X loading dye (50% glycerol, 1 mM EDTA, pH 8.0, 0.4% bromophenol blue, 0.4% xylene cyanol). These samples were subjected to denaturing electrophoresis on formaldehyde agarose gel. Electrophoresis was at 8 V/cm in 1X MOPS buffer. RNA was then transferred directly to a nylon membrane (Hybond N+, Amersham, UK) under slight vacuum (50 mbar) for 1 hr and fixed by ultraviolet light on the membrane. The membranes were prehybridized in Northern prehybridization buffer containing 7% SDS, 50 mM sodium phosphate, pH 7.0, 50% formamide, 2% blocking reagent (Boehringer), 5X SSC and 0.1% lauryl sarkosine for 2 hr at 50°C. The hybridization

TABLE 1. PCR primer sequences specific for rat CYP1A1 and CYP1A2. The PCR products were used to detect the corresponding mRNA by Northern blot (NB) analysis, as described in Materials and Methods

P450 gene		Primers	Product size (bp)	Reference	cDNA sequence	NB (kb)
CYP1A1	Sense	TGA CCT CTT TGG AGC T	1050	23	25	2.9
	Antisense	TTG AGC CTC AGC AGA T				
CYP1A2	Sense	CTA CAA AGA CAA CGG TGG TCT	664	24	26	2.0
	Antisense	CTT GGA GAA GCG TGG CCA GG				

buffer consisted of a 10-mL Northern prehybridization solution containing the heat-denatured labeled probe. Hybridization was performed for 18–20 hr at 52°C (CYP1A1) and 53°C (CYP1A2). The blot was washed twice with 2X SSC, 0.1% SDS for 5 min at room temperature and twice with 0.1X SSC, 0.1% SDS for 5 min at 55°C. The probe detection was performed by using the anti-DIG alkaline phosphate antibody (at 1/10,000 dilution) and a chemiluminescent alkaline phosphatase substrate (CSPD) as described by the manufacturer's protocol (Boehringer). Autoradiography was conducted with hyperfilm ECL Film (Amersham) by using intensifying screens for 2–3 hr.

RESULTS AND DISCUSSION

Rat Hepatocyte Culture

CAF biotransformation increased when rat hepatocytes were cultured for 24 and 48 hr in presence of 1 mM CAF previous to the final 24-hr incubation with CAF (Fig. 1). The overall biotransformation rate increased 1.5- and 2.0-fold for 48- and 72-hr cultures as compared with a single 24-hr incubation time in the presence of CAF at the same time of culture. As has previously been reported [20], the metabolic rates decreased when CAF was incubated for 24 hr with hepatocytes cultured for 24, 48 and 72 hr after cell seeding (4.26%, 1.27% and 0.44% of CAF metabolization, respectively). Thus, this CAF-stimulating effect on its own metabolism could involve the maintenance of hepatocyte functions and the protection of P450 enzymes from degradation rather than the induction of CAF-metabolizing enzymes, in particular CYP1A enzymes. To show that these observations were not pitfalls due to hepatocyte model, experiments on CAF induction were done *in vivo*.

In vivo *Induction Experiments*

Rat weights after CAF treatment were significantly different from those of controls: 170 ± 8 (CAF 50), 168 ± 8 (CAF 100) and 171 ± 6 (CAF 150) vs. 196 ± 3 g for control rats. CAF-treated rats probably reduced their food consumption. However, starvation status was not observed because CYP2E1, inducible by starvation, was not modified [15]. The CAF blood concentration was 3.1 ± 0.7 μM, 29.3 ± 7.7 μM and 63.3 ± 18 μM for rats treated with CAF 50, CAF 100 and CAF 150, respectively.

DOSE-DEPENDENT EFFECTS OF CAF TREATMENT ON CYP1A ENZYME ACTIVITIES IN LIVER AND KIDNEY OF RAT. Metabolic activities specific to CYP1A1 (EROD) and CYP1A2 (MROD) were measured (Table 2). These enzymatic activities in hepatic microsomes of treated rats increased in a dose-dependent manner. EROD activity was elevated 1.7-, 3.8- and 6.0-fold, whereas MROD activity increased by 3.1-, 4.3- and 8.9-fold vs. control for treatment with CAF 50, CAF 100 and CAF 150, respectively. In renal microsomes, EROD activity increased by 2.2-, 3.2- and 5.9-fold, but MROD activity increased only by 1.5-, 1.8- and 2.7-fold for treatment with CAF 50, CAF 100 and CAF 150, respectively (Table 2).

Thus, the effect of CAF on these monooxygenase activities appears to be different in liver and kidney, as assessed by the EROD/MROD ratio that reflects the relative con-

TABLE 2. Enzymatic activities of hepatic and renal microsomal preparations from untreated (0) and treated rats by CAF 50, CAF 100 and CAF 150 for 3 days

	CAF treatment (mg/kg/day)			
	0	50	100	150
EROD liver	772 ± 146	1283 ± 75*	2959 ± 982*	4645 ± 1088*
MROD liver	505 ± 84	1565 ± 160*	2182 ± 369*	4490 ± 456*
EROD kidney	40 ± 14	89 ± 43**	130 ± 42**	238 ± 86***
MROD kidney	20 ± 1	30 ± 6**	36 ± 9**	55 ± 12**

Results are expressed as pmol/mn/mg microsomal proteins (mean ± SD, n = 5).

* Significantly different from control using unpaired Student's *t*-test at $p < 0.001$.

** Significantly different from control by using unpaired Student's *t*-test at $p < 0.05$ and *** at $p < 0.005$.

tribution of CYP1A1/1A2 isoforms. In the liver microsomes, the EROD/MROD ratio was approximately 1.0 for the three treatments, but in the kidney microsomes, this ratio increased with the dose of CAF administered (2.9, 3.6 and 4.3 for treatment of CAF 50, CAF 100 and CAF 150, respectively), suggesting that CYP1A2 was not induced in kidney at the same level as in liver.

DOSE-DEPENDENT EFFECTS OF CAF TREATMENT ON LIVER AND KIDNEY CYP1A1/1A2 mRNA. Northern blot analysis of hepatic CYP1A1/1A2 mRNA following CAF induction was performed by using digoxigenin-labeled RT-PCR product probes to examine the induction mechanism. The CYP1A1 digoxigenin-labeled probe (1050 bp) and CYP1A2 digoxigenin-labeled probe (664 bp) recognized mRNA with a molecular size of approximately 2.9 kb and 2.0 kb, respectively (Fig. 2). The Northern blot analysis showed a dose-dependent change in hepatic CYP1A1/1A2 mRNA levels following CAF treatment.

The Northern blot analysis revealed that renal CYP1A1 mRNA was not detectable in controls but that its level increased with the administered CAF doses (data not shown). Quantification of blot areas showed an increase of approximately 2-fold by CAF 150 treatment vs. CAF 100 treatment. CYP1A2 mRNA in kidney was not detectable by this method. This result suggests that, although CYP1A1/1A2 are induced by transcriptional mechanism, the activation of CYP1A2 by CAF is regulated in a tissue-specific manner.

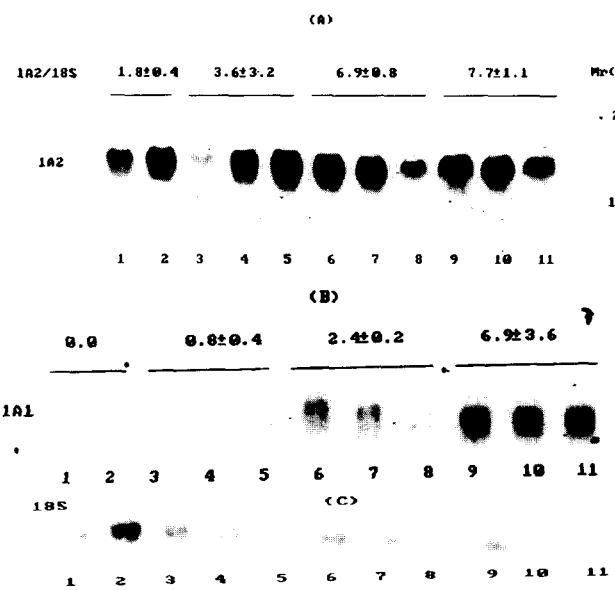

FIG. 2. Northern blot hybridization analysis of RNA (20 μg/lane) obtained from rat liver using a digoxigenin-labeled PCR probe (664 bp) for CYP1A2 mRNA (A) and a digoxigenin-labeled PCR probe (1050 bp) for CYP1A1 mRNA (B). Lanes 1 and 2: RNA obtained from untreated rat liver; lanes 3–5: RNA obtained from rat treated by CAF 50; lanes 6–8: RNA obtained from rat treated by CAF 100; lanes 9–11: RNA obtained from rat treated by CAF 150. C shows the 18S rRNA content after methylene blue coloration. Each lane was normalized to contrast with the corresponding 18S rRNA content; values represent the ratio of blot intensities relative to 18S.

This study demonstrates that CAF selectively induced CYP1A1 and 1A2 in liver and induced only CYP1A1 in kidney of rat. The doses of CAF administered were high. However, the lowest concentration employed, CAF 50, is equivalent to 3.5 g of CAF per day for a man weighing 70 kg. The daily CAF intake of heavy coffee drinkers has been estimated to be more than 1 g/day, even up to 3 g/day [27, 28]. In addition, CAF elimination was faster in rat (half-life; 0.8 hr) than in man (half-life: 4.2 hr), resulting in a higher CAF plasma level for man [29]. Thus, the daily CAF consumption of heavy coffee drinkers was comparable to the lowest dose administered to rats.

In a previous study, caffeine increased the O-deethylation of ethoxyresorufin and O-demethylation of methoxyresorufin, enzymatic activities associated with CYP1A1 and CYP1A2, respectively. This finding was confirmed by immunoblot analysis, resulting in CYP1A1 and CYP1A2 induction [15]. Increase in the O-depentylation of pentoxyresorufin and CYP2B level was also found. This result was confirmed by Ayalogu *et al.* [16]. In the present study, the induction by CAF was shown to be dose-dependent for hepatic and renal catalytic activities specific to CYP1A. The EROD/MROD ratio was, however, different in the two tissues studied, suggesting a more efficient induction of CYP1A1 than of CYP1A2 in kidneys. CYP1A1 is considered mainly as an extrahepatic enzyme. The time-dependent increase in CAF metabolism by rat hepatocyte culture incubated with CAF was probably due to induction (or maintenance) of CYP1A enzymes. The Northern blot analysis revealed a dose-dependent increase in CYP1A1 and CYP1A2 mRNA levels in liver by CAF treatment but only the CYP1A1 mRNA level in kidney.

These data suggest that CAF induces CYP1A isoforms through either transcriptional activation or mRNA stabilization. CYP1A enzymes can be regulated by transcriptional activation but not by mRNA stabilization [17]. The inducer must be a planar molecule binding to a cytosolic protein, the Aryl hydrocarbon receptor (Ah receptor). The complex formed could interact with a DNA-specific sequence called XRE (xenobiotic responsive element) upstream from the promotor following association with an Ah receptor nuclear translocator and phosphorylation. This specific DNA binding could activate the transcription of CYP1A subfamily [30]. If induction by CAF occurred by this mechanism, this compound would have to interact with the Ah receptor. Conversely, CAF does not bind to the Ah receptor [16]. Interestingly, the benzimidazole derivatives (omeprazole, lansoprazole, oxfendazole and thiabendazole) can induce CYP1A without association with the Ah receptor [31]. The induction mechanism for these drugs has yet to be elucidated. However, a ligand-independent activation of the Ah receptor by benzimidazole derivatives has been proposed [31]. Such a mechanism could occur by a membrane receptor binding, stimulating intracellular phosphorylation [25]. Such a hypothesis could be made for CAF induction because CAF is an antagonist

of AMPc phosphodiesterase. Furthermore, CYP1A2 may be regulated in a tissue-specific manner because it was not significantly induced in kidney as opposed to liver.

Because CAF is widely consumed in the population, its effect on human hepatic CYP1A enzymes remains to be demonstrated. In heavy coffee drinkers, however, CAF may induce CYP1A enzymes, resulting in interindividual variations of their catalytic activities and, thus, metabolism of drugs and xenobiotics.

This work was supported in part by the Ligue contre le cancer-comité du Finistère (F.B.) and the Ministère de l'Enseignement Supérieur et de la Recherche (EA-948). T.G. is a fellowship student supported by the Région Bretagne through a grant. We acknowledge Thirion Nathalie for her careful typing of the manuscript.

References

1. Rall TW, Central nervous system stimulants. In: *Pharmacologicals Basis of Therapeutics* (Eds. Goodman Gilman A, Rall TW, Nies AS and Taylors P), Pergamon, New York, pp. 618–637. 1990.
2. Arnaud MJ, Products of metabolism of caffeine. In: *Caffeine, Perspectives from Recent Research* (Ed. Dews PB), pp. 3–38. Springer-Verlag, Berlin, 1984.
3. Bonati M, Latini R, Togononi G, Young JF and Garatini S, Interspecies comparison of *in vivo* caffeine pharmacokinetics in man, monkey, rabbit, rat and mouse. *Drug Metab Rev* **15:** 1355–1383, 1985.
4. Butler MA, Iwasaki M, Guengerich FP and Kadlubar FF, Human cytochrome P450PA (P450 IA2), the phenacetin O-deethylase, is primarily responsible for the hepatic 3-demethylation of caffeine and N-oxidation of carcinogenic arylamine. *Proc Natl Acad Sci USA* **86:** 7696–7700, 1989.
5. Berthou F, Ratanasavanh D, Riché C, Picart D, Voirin T and Guillouzo A, Comparison of caffeine metabolism by slices, microsomes and hepatocyte culture from adult human liver. *Xenobiotica* **19:** 401–427, 1989.
6. Berthou F, Guillois D, Riché C, Dréano Y, Jacqz-Aigrain V and Beaune PH, Interspecies variations in caffeine metabolism related to cytochrome P4501A enzymes. *Xenobiotica* **22:** 671–680, 1992.
7. Tassaneeyakul W, Mohamed Z, Birkett DJ, McManus ME, Veronese ME, Tukey RH, Quattrochi LC, Gonzalez FJ and Miners JO, Caffeine as a probe for human cytochromes P450: Validation using cDNA expression, immuno-inhibition and microsomal kinetic and inhibitor techniques. *Pharmacogenetics* **2:** 173–183, 1992.
8. Bonati M, Celardo A, Galleti F, Latini R, Tursi F and Belvedere G, Kinetics of caffeine metabolism in control and 3-methylcholanthrene induced rat liver microsomes. *Toxicol Lett* **21:** 53–58, 1984.
9. Warszawski D, Ben-Zvi Z and Gorodischer R, Caffeine metabolism in liver slices during postnatal development in the rat. *Biochem Pharmacol* **30:** 3145–3150, 1981.
10. Fuhr U, Doehmer J, Battula N, Wolfel C, Kudla C, Keita Y and Staib AH, Biotransformation of caffeine and theophylline in mammalian cell lines genetically engineered for expression of single cytochrome P450 isoforms. *Biochem Pharmacol* **43:** 225–235, 1992.
11. Mitoma C, Lombroso L, Le Valley SA and Dehn F, Nature of the effect of caffeine on the drug metabolizing enzymes. *Arch Biochem Biophys* **134:** 434–441, 1969.
12. Aeschbacher HU and Würzner HP, Effect of methylxanthines on hepatic microsomal enzymes in the rat. *Toxicol Applied Pharmacol* **3:** 575–581, 1975.
13. Ahokas JT, Pelkonen O, Ravenscroft PJ, Emmerson BT, Effects of theophylline and caffeine on liver microsomal drug metabolizing mono-oxygenase in genetically Ah responsive and non responsive mice. *Res Commun Subst Abuse* **2:** 277–290, 1981.
14. Cornish HH, Wilson CE, Abar EL, Effect of foreign compounds on liver microsomal enzymes. *Am Ind Hygen Assoc J* **31:** 605–608, 1970.
15. Berthou F, Goasduff T, Dréano Y and Ménez JF, Caffeine increases its own metabolism through cytochrome P4501A induction in rats. *Life Sci* **57:** 541–549, 1995.
16. Ayalogu EO, Snelling J, Lewis DFY, Talwar S, Clifford MN, and Ionnides C. Induction of heptic CYP1A2 by the oral administration of caffeine to rats: Lack of association with the Ah locus. *Biochem Biophys Acta* **1272:** 89–94, 1995.
17. Gonzalez FJ, Liu SY and Yano M, Regulation of cytochrome P450 genes: Molecular mechanisms. *Pharmacogenetics* **3:** 51–57, 1993.
18. Daujat M, Peryt B, Lesca P, Fourtanier G, Domergue J and Maurel D, Omeprazole, an inducer of human CYP1A1 and 1A2 is not a ligand for the Ah receptor. *Biochem Biophys Res Commun* **188:** 1–9, 1992.
19. Guguen C, Guillouzo A, Boisnard M, Le Cam A and Bourel M, Etude ultrastructurale de monocouche d'hépatocytes de rat adulte cultivés en présence d'hémisuccinate d'hydrocortisone. *Biol Gastroenterol* **8:** 223–231, 1975.
20. Berthou F, Ratanasavanh D, Alix D, Carlhant D, Riché C and Guillouzo A. Caffeine and theophylline metabolism in newborn and adult human hepatocytes: Comparison with adult rat hepatocytes. *Biochem Pharmacol* **37:** 3691–3700, 1989.
21. Burke MD, Thompson S, Weaver RJ, Wolf CR and Mayer RT, Cytochrome P450 specificities of alkoxyresorufin O-dealkylation in human and rat liver. *Biochem Pharmacol* **48:** 923–936, 1994.
22. Chomczynski P and Sacchi N, Single step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal Biochem* **162:** 156–159, 1987.
23. Giachelli CM and Omiecinski CJ, Developmental regulation of cytochrome P450 genes in the rat. *Mol Pharmacol* **31:** 477–484, 1987.
24. Geng J and Strobel H, Identification of cytochrome P4501A2, 2A1, 2C7, 2E1 in rat glioma C6 cell line by RT-PCR and specific restriction enzyme digestion. *Biochem Biophys Res Commun* **197:** 1179–1184, 1993.
25. Yabusaki Y, Shimizu M, Murakami H, Nakamura K, Oeda K and Ohkawa H, Nucleotide sequence of a full length cDNA coding for 3-methylcholanthrene inducible in rat liver cytochrome P450 MC. *Nucleic Acid Res* **12:** 2929–2938, 1984.
26. Kawajiri K, Gotoh O, Sogawa K, Tagashira Y, Muramatsu M and Fujii-Kuriyama Y, Coding nucleotide sequence of 3-methylcholanthrene inducible cytochrome P450d cDNA from rat liver. *Proc Natl Acad Sci USA* **81:** 1649–1653, 1984.
27. Stavric B, Methylxanthines: Toxicity to humans: Caffeine. *Fed Chem Toxicol* **26:** 645–662, 1988.
28. Barone JJ and Roberts HR, Caffeine consumption. *Fed Chem Toxicol* **34:** 119–129, 1996.
29. Arnaud MJ, Metabolism of caffeine and other components of coffee. In: *Caffeine, Coffee and Health* (Ed. Garattini S), pp. 43–98, Raven Press, New York, 1993.
30. Nebert DW and Gonzalez FJ, P450 genes: Structures, evolution and regulation. *Annu Rev Biochem* **56:** 945–993, 1987.
31. Lesca P, Peryt B, Larrieu G, Alvinerie M, Galtier P, Daujat M, Maurel P and Hoogenbaum L, Evidence for the ligand-independent activation of the Ah receptor. *Biochem Biophys Res Commun* **2:** 474–482, 1995.