*Biochemical Pharmacology*, Vol. 36, No. 3, pp. 323–329, 1987
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00
Pergamon Journals Ltd.

# METABOLIC ACTIVATION OF THE TRICYCLIC ANTIDEPRESSANT AMINEPTINE—I

## CYTOCHROME P-450-MEDIATED *IN VITRO* COVALENT BINDING

JEAN GENEVE, DOMINIQUE LARREY, PHILIPPE LETTERON, VERONIQUE DESCATOIRE, MARINA TINEL, GILLES AMOUYAL and DOMINIQUE PESSAYRE*

Unité de Recherches de Physiopathologie Hépatique (INSERM U-24), Hôpital Beaujon, 92118 Clichy, France

(*Received* 14 *April* 1986; *accepted* 4 *August* 1986)

**Abstract**—Incubation of [$^{14}$C]amineptine (1 mM) with hamster liver microsomes resulted in the irreversible binding of an amineptine metabolite to microsomal proteins. Covalent binding measured in the presence of various concentrations of amineptine (0.0625–1 mM) followed Michaelis–Menten kinetics. Pretreatment with phenobarbital increased not only the $V_{max}$, but also the $K_m$, for this binding. Covalent binding required NADPH and molecular oxygen and was decreased when the incubation was made in the presence of inhibitors of cytochrome P-450 such as piperonyl butoxide (4 mM), SKF 525-A (4 mM) or carbon monoxide (80:20 CO–$O_2$ atmosphere). In contrast, binding was increased when microsomes from untreated hamsters were incubated in the presence of 0.5 mM 1,1,1-trichloropropene 2,3-oxide, an inhibitor of epoxide hydrolase. Metabolic activation also occurred in kidney microsomes. *In vitro* covalent binding to kidney microsomal proteins required NADPH and was decreased by piperonyl butoxide (4 mM) but was not increased by pretreatment with phenobarbital. We conclude that amineptine is activated by hamster liver and kidney microsomes into a chemically reactive metabolite that covalently binds to microsomal proteins.

Amineptine is widely used in France, Greece, Italy, Portugal, Spain and several other European, Asian, African and South American countries. In France, amineptine is the second most prescribed anti-depressant, after clomipramine.

Amineptine differs from other anti-depressant drugs, such as imipramine or amitriptyline, in that it possesses a long acyl chain (Fig. 1), and is partially metabolized by $\beta$-oxidation *in vitro* [1]. Amineptine is also peculiar in its mechanism of action, which is thought to be related to the inhibition of the re-uptake of dopamine [2, 3]. Amineptine resembles, however, several other anti-depressant drugs in having a tricyclic nucleus (Fig. 1). Like other tricyclic antidepressants [4, 5], amineptine may produce hepatitis in some patients [6, 7]. It has been estimated that amineptine may be responsible for 83% of the cases of hepatitis caused by tricyclic anti-depressants in France [8].

The mechanism for the hepatotoxicity of amineptine remains unknown. Several hepatotoxic drugs, including the related tricyclic anti-depressant imipramine [9], are transformed by cytochrome P-450 into reactive metabolites that covalently bind to hepatic macromolecules, mainly proteins [10, 11]. Such metabolites may produce hepatitis in one of two ways. They may be (directly or indirectly) toxic [12, 13], or they may trigger, in a few subjects, an immune response against the metabolite–macromolecule complexes, and produce an allergic type of hepatitis [14–17].

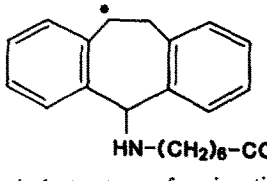


Fig. 1. Chemical structure of amineptine. The asterisk indicates the position of the radiolabel in [11-$^{14}$C]-amineptine.

In this communication, we report that amineptine is transformed by liver and kidney cytochrome P-450 into a reactive metabolite that covalently binds to microsomal proteins *in vitro*. In the next communication [18], we report the protective role of glutathione and the *in vivo* covalent binding of an amineptine metabolite to proteins in various tissues.

### MATERIALS AND METHODS

*Chemicals.* Amineptine hydrochloride and [11-$^{14}$C]amineptine hydrochloride were generously given by Servier laboratories (Neuilly, France). The radiochemical compound (24 mCi/mmol), labeled on the tricyclic ring, was prepared by Comissariat à l'Energie Atomique, Gif-sur-Yvette, France; its radiochemical purity was found to be >99% by HPLC.

*Animals and treatments.* Male golden Syrian hamsters, weighing 90–110 g, were purchased from Fichot (Ormesson, France). Animals were fed a normal standard diet ($M_{25}$ biscuits, Extra Labo, Provins, France), given *ad libitum*. Some hamsters were given phenobarbital (100 mg/kg i.p. in 0.5 ml of water), or 3-methylcholanthrene (20 mg/kg i.p. in 0.5 ml of corn oil), daily for 3 days, and were killed 24 hr after the last dose of the inducer.

* To whom correspondence should be addressed.

*Preparation of microsomes; microsomal enzymes.* Liver, kidney and lung samples were homogenized in 3 volumes of ice-cold 0.154 M KCl, 0.01 M sodium–potassium phosphate buffer, pH 7.4. The homogenate was centrifuged at 10,000 *g* for 10 min. The 10,000 *g* supernatants were pooled and centrifuged at 100,00 *g* for 60 min. Microsomal pellets were stored at −20° until used, 1–15 days later.

Microsomal protein concentration was determined by the method of Lowry *et al.* [19]. Hepatic microsomal cytochrome P-450 was measured as described by Omura and Sato [20]. Renal microsomal cytochrome P-450 was measured as reported by Jakobsson and Cinti [21].

In vitro *covalent binding studies.* Unless otherwise indicated the standard incubation was performed as follows: [11-$^{14}$C]amineptine (1 μCi/ml, 1 mM), dissolved in 10 μl of 0.1 N NaOH, was added to an incubation mixture containing: NADP (1 mM), glucose-6-phosphate (8 mM), glucose-6-phosphate dehydrogenase (3 enzyme units/ml), $MgCl_2$ (6 mM) and hepatic or renal microsomes (6 mg of microsomal proteins/ml) in a total volume of 1 ml of 0.07 M KCl, 0.13 M sodium–potassium phosphate buffer, pH 7.4. A zero-time sample (250 μl) was removed, and the remaining mixture was incubated under air, with shaking at 37° for 5 min; another sample (250 μl) was then taken.

In some experiments, we varied the incubation time, the source of microsomes, the amount of microsomal proteins, or the concentration of amineptine or NADP. In other experiments, piperonyl butoxide (4 mM), SKF 525-A (4 mM), or 1,1,1-trichloropropene 2,3-oxide (0.5 mM) was added to the incubation mixture. In other experiments, the NADPH-generating system was omitted. In some flasks, a $CO$–$O_2$ (80:20) mixture was bubbled, first in the ice-cold incubation mixture for 10 min, and then for 5 min of incubation at 37°. For experiments in anaerobiosis, an oxygen-consuming system consisting of glucose (13 mM), glucose oxidase (8 U/ml) and catalase (1000 U/ml) was added to the incubation mixture. Nitrogen was bubbled in the ice-cold incubation mixture for 5 min and then, for 5 min of pre-incubation at 37°. [11-$^{14}$C]Amineptine was then added, and the incubation was carried out for 5 min, with nitrogen bubbling in the incubation mixture. The amount of [11-$^{14}$C]amineptine metabolite irreversibly bound to proteins in the zero-time and in the incubated samples was measured as previously reported [22–24]. Briefly, proteins were precipitated on filter paper disks (Durieux 111) which had been previously soaked in 5% trichloroacetic acid and dried at room temperature [22–24]. Proteins entrapped in the filter paper disks were then washed three times with 10% trichloroacetic acid and extracted repeatedly with various solvents of various polarities (methanol, twice; *n*-heptane, once; ether, once) as previously reported [22–24]. The filter paper disks were then dried and placed in the bottom of scintillation vials; 10 ml of scintillation fluid was added and the $^{14}$C remaining irreversibly bound to proteins was counted with background and quench correction [22–24]. By running an identical experiment except for the absence of microsomes in the incubation mixture, we verified that [11-$^{14}$C]amineptine did not bind to the filter paper itself (in the absence of entrapped proteins). After standard incubations, we also verified that radioactivity could not be removed further by introducing additional solvent extractions. We verified that similar $^{14}$C counts were obtained when the proteins were counted while still entrapped into the filter paper disks, or after being first solubilized in 1 N NaOH (followed by subsequent neutralization with HCl). Finally, we verified [18] that *in vivo* covalent binding measured with this filter paper technique gave results essentially identical to those obtained when similar washings and extractions were performed on proteins remaining free in the test tubes.

*Statistical analysis.* Student's *t*-test for independent data was used to assess the significance of differences between means when only one comparison had been performed in the experimental protocol. Analysis of variance and Dunnett's test were used whenever a single control group had been compared to several other groups in the experimental protocol.

## RESULTS

### *Liver microsomes*

Incubation of [11-$^{14}$C]amineptine (1 mM), under air, for 5 min, with an NADPH-generating system (1 mM NADP) and hepatic microsomes from control hamsters (6 mg of microsomal protein/ml) resulted in the irreversible binding of $^{14}$C to microsomal proteins (Fig. 2). Covalent binding was three times higher with microsomes from phenobarbital-pretreated hamsters, but was unchanged with microsomes from hamsters pretreated with 3-methylcholanthrene (Fig. 2). Hepatic microsomal

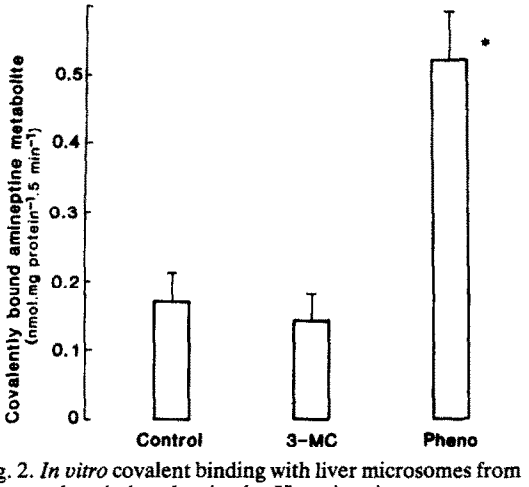


Fig. 2. *In vitro* covalent binding with liver microsomes from untreated or induced animals. Hepatic microsomes were prepared from untreated hamsters ("control") or from hamsters pretreated for 3 days with 3-methylcholanthrene ("3-MC") or phenobarbital ("Pheno"). The incubation mixture (1 ml) contained [11-$^{14}$C]amineptine (1 μCi/ml, 1 mM), an NADPH-generating system (1 mM NADP), and pooled hepatic microsomes (6 mg of microsomal protein/ml). After 5 min of incubation, under air, at 37°, the amount of $^{14}$C irreversibly bound to microsomal proteins was determined. Results are means ± SEM for six determinations. The asterisk indicates a significant difference from value in non-pretreated hamsters, $P < 0.01$.

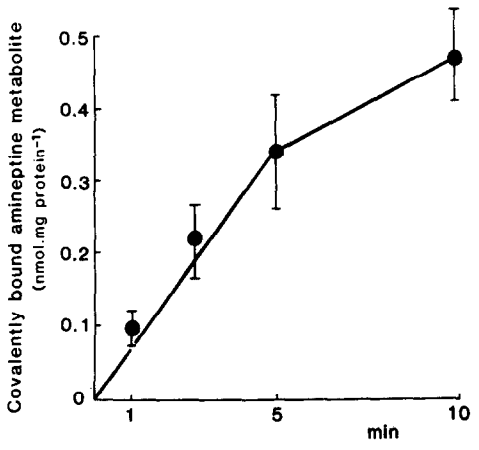


Fig. 3. Time course of *in vitro* covalent binding. The incubation mixture (5 ml) contained [11-$^{14}$C]amineptine (1 $\mu$Ci/ml, 1 mM), an NADPH-generating system (1 mM NADP) and pooled liver microsomes (6 mg of microsomal protein/ml) from phenobarbital-pretreated hamsters. At various times, aliquots were taken and the amount of $^{14}$C irreversibly bound to microsomal proteins was determined. Results are means ± SEM for eight determinations.

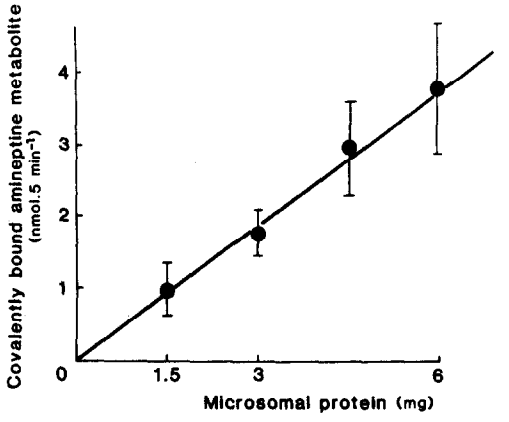


Fig. 4. *In vitro* covalent binding as a function of the amount of hamster liver microsomes. The incubation mixture (1 ml) contained [11-$^{14}$C]amineptine (1 $\mu$Ci/ml, 1 mM), an NADPH-generating system (1 mM NADP) and pooled liver microsomes from phenobarbital-pretreated hamsters (1.5–6 mg of microsomal protein per ml). After 5 min of incubation, under air, at 37°, the amount of $^{14}$C irreversibly bound to microsomal proteins was determined. Results are means ± SEM for six determinations.

cytochrome P-450 (mean ± SEM for six hamsters) was 1.03 ± 0.05 nmol/mg of microsomal protein in control hamsters, 1.6 ± 0.1 in hamsters pretreated with phenobarbital, and 1.70 ± 0.05 in hamsters pretreated with 3-methylcholanthrene. Unless otherwise indicated, microsomes from phenobarbital-pretreated hamsters were used in subsequent experiments.

*In vitro* covalent binding was almost linear with time for the first 5 min of incubation, and then increased less rapidly (Fig. 3). An incubation time of 5 min was selected for further experiments. Covalent binding increased linearly with the amount of microsomal proteins added in the incubation mixture (Fig. 4), up to 6 mg of microsomal protein per ml, which was selected for further studies. When measured in the presence of various concentrations of amineptine (0.0625–1 mM), *in vitro* covalent binding followed Michaelis–Menten kinetics (Fig. 5). Both $V_{max}$ and $K_m$ were higher with microsomes from phenobarbital-induced animals than with control microsomes (Fig. 5). In all other experiments, the con-

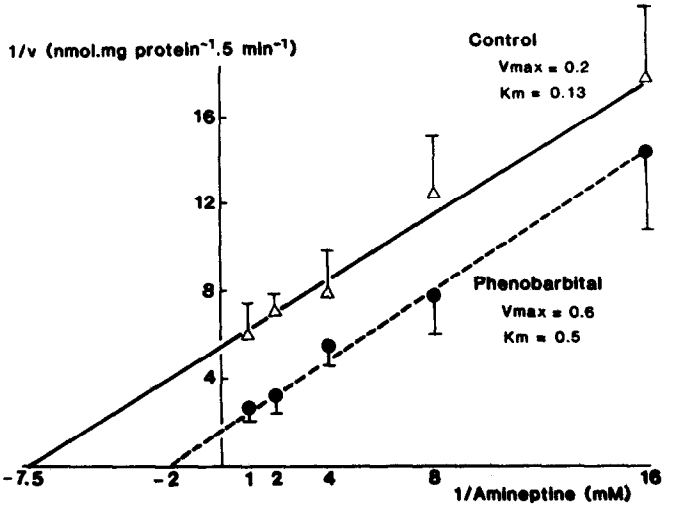


Fig. 5. Double reciprocal plot of *in vitro* covalent binding to hamster liver microsomes in the presence of various concentrations of amineptine. The incubation mixture (1 ml) contained [11-$^{14}$C] amineptine (1 $\mu$Ci/ml; 0.062–1 mM), an NADPH-generating system (1 mM NADP) and pooled liver microsomes (6 mg of microsomal protein/ml) from either control hamsters or hamsters pretreated with phenobarbital. After 5 min of incubation, under air, at 37°, the amount of $^{14}$C irreversibly bound to microsomal proteins was determined. Results are means ± SEM for eight determinations. Values for $V_{max}$ are in nmol bound/mg microsomal protein/5 min. Values for $K_m$ are in mM.

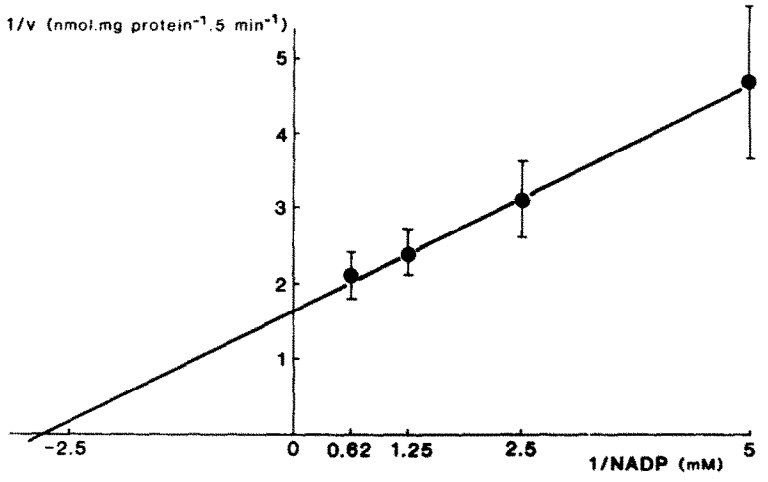


Fig. 6. Double reciprocal plot of *in vitro* covalent binding to hamster liver microsomes in the presence of various concentrations of NADP(H). The incubation mixture (1 ml) contained [11-$^{14}$C]amineptine (1 $\mu$Ci/ml, 1 mM), pooled liver microsomes from phenobarbital-pretreated hamsters (6 mg of microsomal protein/ml) and an NADPH-generating system made up with various concentrations of NADP (0.2–1.6 mM). After 5 min of incubation, under air at 37°, the amount of $^{14}$C irreversibly bound to microsomal proteins was determined. Results are means ± SEM for six determinations.

centration of amineptine was kept at 1 mM. *In vitro* covalent binding also followed Michaelis–Menten kinetics with respect to the concentration of NADP(H) in the incubation mixture (Fig. 6). Other experiments were carried out in the presence of 1 mM NADP(H). Covalent binding was almost absent when the incubation was carried out under a nitrogen atmosphere (Fig. 7). Covalent binding was decreased markedly when the NADPH-generating system was omitted (Fig. 7). Covalent binding was also decreased markedly when the incubation was made in the presence of piperonyl butoxide (4 mM), SKF 525-A (4 mM) or under a CO–$O_2$ (80:20) atmosphere (Fig. 8).

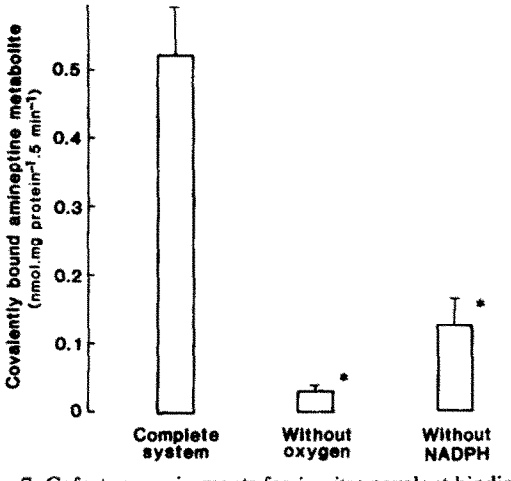


Fig. 7. Cofactor requirements for *in vitro* covalent binding to liver microsomes. In the "complete system", the incubation mixture (1 ml) contained [11-$^{14}$C]amineptine (1 $\mu$Ci/ml, 1 mM), an NADPH-generating system (1 mM NADP), and pooled hepatic microsomes from phenobarbital-pretreated hamsters (6 mg of microsomal protein/ml). In some flasks, the NADPH-generating systems was omitted. Other flasks were incubated under nitrogen, in the presence of an oxygen-depleting system (see Materials and Methods). After 5 min of incubation at 37°, the amount of $^{14}$C irreversibly bound to microsomal proteins was determined. Results are means ± SEM for four determinations. The asterisks indicate significant differences from the value in the complete system, $P < 0.05$.

Similar observations were made with liver microsomes from untreated hamsters. Indeed, after 5 min of incubation with [11-$^{14}$C]amineptine (1 $\mu$Ci/ml, 1 mM), an NADPH-generating system (1 mM NADP) and pooled hepatic microsomes from untreated hamsters (6 mg of microsomal protein per ml), the amount of covalently bound material was 0.17 ± 0.04 nmol/mg of microsomal protein/5 min (mean ± SEM for six determinations) in the complete system but only 0.5 ± 0.02 when the NADPH-generating system was omitted and 0.04 ± 0.02, 0.05 ± 0.02 and 0.10 ± 0.02, respectively when the incubation was made in the presence of piperonyl butoxide (4 mM), SKF 525-A (4 mM) or a CO–$O_2$ (80:20) atmosphere. In contrast, *in vitro* covalent binding to liver microsomes from untreated hamsters was almost doubled when the incubation was made in the presence of 0.5 mM 1,1,1-trichloropropene 2,3-oxide (Fig. 9).

### *Kidney and lung microsomes*

Covalent binding also occurred when [11-$^{14}$C]amineptine was incubated with an NADPH-generating system and renal microsomes from untreated hamsters (Fig. 10). Covalent binding to kidney microsomal proteins required NADPH and was inhibited by piperonyl butoxide (Fig. 10), but was not higher with microsomes from phenobarbital-pretreated hamsters (Fig. 10). Renal microsomal cytochrome P-450 (mean ± SEM for four hamsters) was 0.14 ± 0.01 nmol/mg of microsomal protein in control hamsters and 0.15 ± 0.01 in hamsters pretreated with phenobarbital.

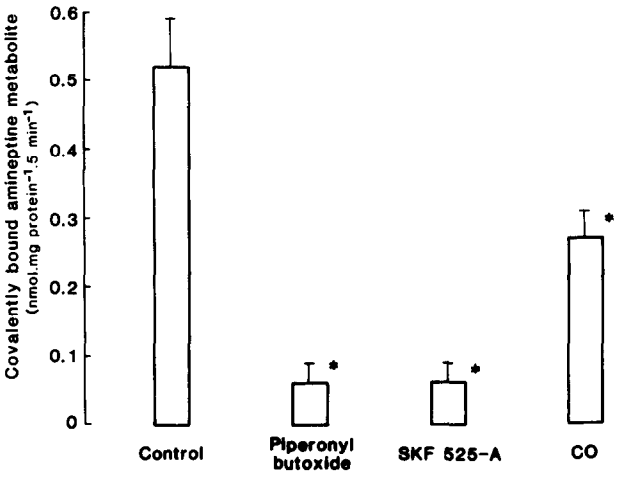


Fig. 8. Effects of cytochrome P-450 inhibitors on *in vitro* covalent binding in liver microsomes. The control incubation mixture (1 ml) contained [11-$^{14}$C]amineptine (1 $\mu$Ci/ml, 1 mM), an NADPH-generating system (1 mM NADP), and pooled hepatic microsomes from phenobarbital-pretreated hamsters (6 mg of microsomal proteins/ml). In some experiments, SKF 525-A (4 mM) or piperonyl butoxide (4 mM) were added, or the incubation was made under a CO–$O_2$ (80:20) atmosphere. After 5 min of incubation at 37°, the amount of $^{14}$C irreversibly bound to microsomal proteins was determined. Results are means ± SEM for four determinations. The asterisks indicate significant differences from the value in the control incubation, $P < 0.05$.

*In vitro* covalent binding could not be detected in hamster lung microsomes (not shown).

## DISCUSSION

Our results show that amineptine is transformed by hamster liver microsomes into a reactive metabolite which covalently binds to microsomal proteins *in vitro*. In such a system, the rate-limiting step is the enzymatic formation of the metabolite, followed by its spontaneous reaction with nucleophilic sites of proteins. Indeed, *in vitro* covalent binding followed Michaelis–Menten kinetics with respect to the concentrations of amineptine or those of NADP(H) (Figs 5, 6). Metabolic activation was mediated by cytochrome P-450, since it required NADPH and oxygen (Fig. 7) and was decreased by carbon monoxide and other inhibitors of cytochrome P-450 (Fig. 8).

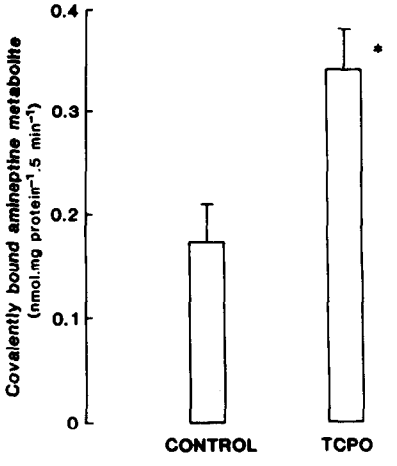


Fig. 9. Effect of an inhibitor of epoxide hydrolase on *in vitro* covalent binding. The control incubation mixture (1 ml) contained [11-$^{14}$C]amineptine (1 $\mu$Ci/ml, 1 mM), an NADPH-generating system (1 mM NADP), and pooled liver microsomes from untreated hamsters (6 mg of microsomal protein per ml). In some flasks, 0.5 mM 1,1,1-trichloropropene 2,3-oxide (TCPO) was added to the incubation mixture. After 5 min of incubation at 37°, the amount of $^{14}$C irreversibly bound to microsomal proteins was determined. Results are means ± SEM for six experiments. The asterisk indicates a significant difference from the value in the control incubation, $P < 0.01$.

The chemical structure of the reactive metabolite(s) of amineptine remains unknown. In rats, dogs and humans, amineptine is metabolized by $\beta$-oxidation of the acyl chain, 10- or 11-hydroxylation on the central dimethylene bridge of the tricyclic nucleus, and to a lesser extent, by lactame formation; in rats only, there is a slight formation of a 10–11 double bond (Servier Laboratories, personal communication). Up to now, no oxidation of the benzene rings has been detected. Our findings that the *in vitro* covalent binding was almost doubled in the presence of 0.5 mM 1,1,1-trichloropropene 2,3-oxide, a potent inhibitor of microsomal epoxide hydrolase [25], may indicate the possible formation of an epoxide as a minor metabolic pathway. Similarly, the *in vitro* covalent binding of a reactive imipramine metabolite has been found to be doubled in the presence of 1,1,1-trichloropropene 2,3-oxide and a reactive epoxide has been suggested as the probable structure of the reactive metabolite of imipramine [9]. Further studies are required, however, to delineate better the chemical structure of these reactive metabolites.

It is noteworthy that pretreatment with phenobarbital increased not only the $V_{max}$ (3-fold) but also the $K_m$ (4-fold) for the *in vitro* covalent binding of a

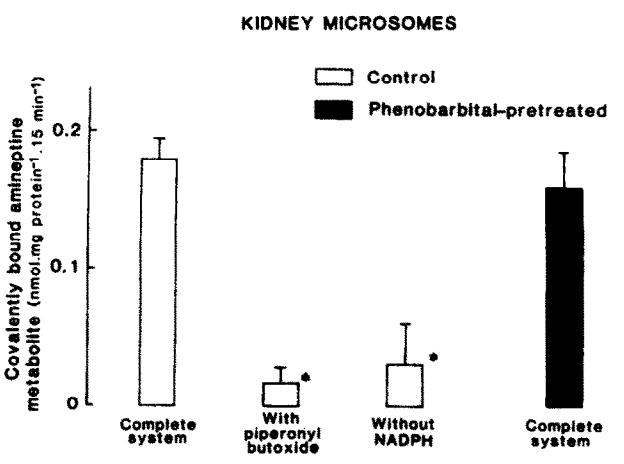


Fig. 10. *In vitro* covalent binding with kidney microsomes. In the "complete system", the incubation mixture (1 ml) contained [11-$^{14}$C]amineptine (1 $\mu$Ci/ml, 1 mM), an NADPH-generating system (1 mM NADP) and renal microsomes (6 mg of microsomal protein) from either untreated, or phenobarbital-pretreated, hamsters. In some flasks, the NADPH-generating system was omitted. In other flasks, piperonyl butoxide (4 mM) was added. The mixtures were incubated, under air, at 37° for 15 min, and the amount of $^{14}$C irreversibly bound to microsomal proteins was determined. Results are means ± SEM for six determinations. The asterisks indicate a significant difference from the incubation made with microsomes from untreated hamsters in the presence of the "complete system", $P < 0.01$.

reactive amineptine metabolite to microsomal proteins (Fig. 5). This observation may indicate that different cytochrome P-450 isozymes may participate in the metabolic activation of amineptine. Some isozyme(s), present in low amounts in untreated animals, may exhibit a high apparent affinity for amineptine, while other isozyme(s), induced in high amounts by phenobarbital, may exhibit a lower apparent affinity for amineptine.

Interestingly, amineptine also underwent metabolic activation in kidney microsomes (Fig. 10). As in liver, metabolic activation was probably mediated by cytochrome P-450 since it required NADPH and was inhibited by piperonyl butoxide (Fig. 10). Unlike hepatic cytochrome P-450, however, renal cytochrome P-450 is not inducible (or poorly inducible) by phenobarbital in rats [26], mice [15] and hamsters (this study), explaining the failure of phenobarbital pretreatment to increase the *in vitro* covalent binding of a reactive amineptine metabolite to kidney microsomal proteins (Fig. 10).

We conclude that hamster liver and kidney microsomal cytochrome P-450 transforms amineptine into a chemically reactive metabolite. The protective role of glutathione against *in vitro* and *in vivo* covalent binding is reported in the next communication [18].

*Acknowledgement*—We are grateful to Servier Laboratories for granting a two-year fellowship to one of us, and for providing [11-$^{14}$C]amineptine.

## REFERENCES

1. C. Sbarra, M. G. Castelli, A. Moseda and R. Fanelli, *Eur. J. Drug Metab. Pharmacokin.* **6**, 123 (1981).
2. R. Samanin, A. Jori, S. Bernasconi, E. Morpugo and S. Garattini, *J. Pharm. Pharmac.* **29**, 555 (1977).
3. J. Offermeier, B. Potgieter, H. G. Dupreez and P. J. Meiring, *S. Afr. med. J.* **51**, 62 (1977).
4. H. J. Zimmerman. *Hepatotoxicity. The Adverse Effects of Drugs and Other Chemicals on the Liver.* Appleton-Century-Crofts, New York (1978).
5. B. H. Ch. Stricker and P. Spoelstra. *Drug-Induced Hepatic Injury. A Comprehensive Survey of the Literature on Adverse Drug Reactions up to January 1985.* Elsevier, Amsterdam (1985).
6. P. Bories, G. Pomier-Layrargues, J. P. Chotard, D. Citron, D. Capron-Chivrac, J. P. Capron and H. Michel, *Nouv. Presse med.*, **9**, 3689 (1980).
7. J. Andrieu, J. Doll and C. Coffinier, *Gastroenterol. clin. Biol.* **6**, 915 (1982).
8. B. Lefebure, A. Castot, G. Danan, J. Elmalem and M. J. Efthymiou, *Thérapie* **39**, 509 (1984).
9. J. Kappus and H. Remmer, *Biochem. Pharmac.* **24**, 1079 (1975).
10. J. R. Mitchell and D. J. Jollow, *Gastroenterology* **68**, 392 (1975).
11. P. Mazel and D. Pessayre, in *Advances in Modern Toxicology. New Concepts in Safety Evaluation* (Eds M. A. Mehlman, R. E. Shapiro and H. Blumenthal), p. 307. Hemisphere, Washington, DC (1976).
12. L. F. Prescott. *Drugs* **25**, 290 (1983).
13. D. Pessayre, M. Bentata, C. Degott, O. Nouel, J. P. Miguet, B. Rueff and J. P. Benhamou, *Gastroenterology* **72**, 284 (1977).
14. H. Fouin-Fortunet, P. Lettéron, M. Tinel, C. Degott, J. F. Fléjou and D. Pessayre, *J. Pharmac. exp. Ther.* **229**, 851 (1984).
15. P. Lettéron, H. Fouin-Fortunet, M. Tinel, G. Danan, J. Belghiti and D. Pessayre, *J. Pharmac. exp. Ther.* **229**, 845 (1984).
16. J. Neuberger, J. G. Kenna, K. Nouri Aria and R. Williams, *Gut* **26**, 1233 (1985).
17. D. Vergani, G. Mieli-Vergani, A. Alberti, J. Neuberger, A. L. W. F. Eddelston, M. Davis and R. Williams, *N. Engl. J. Med*, **303**, 66 (1980).
18. J. Genève, C. Degott, P. Lettéron, M. Tinel, V. Descatoire, D. Larrey, G. Amouyal and D. Pessayre, *Biochem. Pharmac.* **36**, 331 (1987).
19. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).

20. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
21. S. V. Jakobsson and D. L. Cinti, *J. Pharmac. exp. Ther.* **185**, 226 (1973).
22. H. Allemand, D. Pessayre, V. Descatoire, C. Degott, G. Feldmann and J. P. Benhamou, *J. Pharmac. exp. Ther.* **204**, 714 (1978).
23. D. Pessayre, J. C. Wandscheer, V. Descatoire, A. Dolder, C. Degott and J. P. Benhamou, *Biochem. Pharmac.* **29**, 1041 (1980).
24. H. Uehleke, K. H. Hellmer and S. Tabarelli-Poplawski, *Naunyn Schmiedeberg's Arch. Pharmac.* **279**, 39 (1973).
25. F. Oesch, *Xenobiotica* **3**, 305 (1972).
26. G. Babany, V. Descatoire, M. Corbic, S. Gendre, C. Degott, D. Larrey, P. Letteron, J. C. Wandscheer, C. Funck-Brentano and D. Pessayre, *Biochem. Pharmac.* **34**, 311 (1985).