Phenobarbital and 3-Methylcholanthrene Inducible Cytochromes P450 Are not Responsible for Metabolism of Deltamethrin in Rat Liver Microsomes

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INTRODUCTION

The microsomal cytochrome P450 monoxygenases (P450 monooxygenases) are one of the most important metabolic systems known and can be found in virtually all aerobic organisms. Their activity is important in detoxification and activation of xenobiotics, such as pesticides, and the synthesis and breakdown of endogenous compounds. P450s have diverged into a wide variety of isofoms (>400) with varying substrate specificity, and have been grouped into 36 different gene families. An important aspect of the P450 monooxygenase system is its inducibility. Following pretreatment with various compounds the monooxygenase system can show increased levels of metabolism. The amount of induction that can occur is highly variable for different P450s, but is correlated with the half life of the compound. These inductive responses are of great importance to the metabolism of drugs, pharmacokinetics, compound-molecule interactions, the potency of endogenous cell signaling molecules, and the toxicity and carcinogenicity of xenobiotics. In addition, the P450 isofoms responding to well studied inducers, such as phenobarbital (PB) and 3-methylcholanthrene (3-MC), have been well characterized in rat.

Synthetic pyrethroids are the newest class of insecticides. By 1991 they accounted for 21% of worldwide insecticide use and deltamethrin (S-α-cyano-3-phenoxybenzyl (1R, 3R)-3-(2, 2-dibromovinyl)-2, 2-dimethylcyclopropanecarboxylate) is one of the most widely used pyrethroids. The P450 monooxygenases are important for detoxification of pyrethroids such as deltamethrin. Although some of the P450s involved in metabolism of organophosphate insecticides have been identified, there has been very limited work aimed at identification of the P450s involved in pyrethroid metabolism.

Herein, we examine the effects of two classical inducers, PB and 3-MC, on the in vitro metabolism of deltamethrin in rats. The goal of this study was to determine if the major PB and 3-MC inducible P450s (i.e. CYP2B1, 2B2, 1A1, 1A2 and/or 2C6) were involved in the metabolism of pyrethroid insecticides.

MATERIALS AND METHODS

Sodium phenobarbital (PB), ethoxyresorufin, pentoxyresorufin, glucose 6-phosphate, glucose 6-phosphate dehydrogenase, 3-MC, and NADPH were obtained from Sigma (St. Louis, MO, U.S.A.). S-S-S-Tributyl phosphorothriothioate (DEF) and piperonyl butoxide were obtained from Chem Service (West Chester, PA, U.S.A.). Deltamethrin ([14C] acid labeled, 55.6 Ci/mol) was kindly provided by Russel-Uclaf (Paris), and was purified prior to use.

Male Sprague-Dawley rats were certified virus free and obtained from Charles River Labs (Willmington, MA, U.S.A.). Rats weighed between 225-250 g and grew at a rate of approximately 7 g per day while under study. The animals were maintained in an environmental chamber at 21°C, with 60% room humidity, and a 14:10 (light:dark) photoperiod. The animals were fed standard rat chow and given fresh water daily.

Sprague-Dawley rats were weighed and treated intraperitoneally three consecutive days starting one day after animals were received. Controls were administered 0.5 ml of either double distilled water or corn oil. Treated rats were administered either 75 mg PB dissolved in double distilled water per kilogram of body weight or 25 mg 3-MC dissolved in corn oil per kilogram of body weight. Approximately 0.5 ml were given for each injection.

Rats were sacrificed 24 hr after the last injection, the liver removed, weighed, and then rinsed with homogenization buffer. The liver was then flash frozen in liquid nitrogen and stored at -80°C for less than 2 weeks. Livers were thawed on ice and placed in a Wheaton potter glass-teflon homogenizer (Wheaton, Millville, NJ, U.S.A.) containing 25 ml homogenization buffer. The contents were then homogenized for 30 sec and thent strained through two layers of muslin. Homogenates were then centrifuged at 10,000 × g for 30 min at 4°C in a Beckman J-21C centrifuge (Beckman Instruments, Palo Alto, CA, U.S.A.) equipped with a JA-20 rotor. The 10,000 × g supernatant was filtered through two layers of muslin and centrifuged at 100,000 × g for 1 hr at 4°C in a Beckman L8-70M ultracentrifuge using a 70Ti rotor. The microsomal pellet from the 100,000 × g spin was then resuspended in 25 ml homogenization buffer and recentrifuged at 100,000 × g for 1 hr at 4°C. The microsomal pellet from this stage was resuspended by homogenizing in 8 ml of resuspension buffer. The microsomal suspension from each treatment was bubbled under N2 for 10 sec and stored in 1 ml aliquots at -80°C. All of the above procedures were conducted at 0–4°C.

Protein was determined by the method of Bradford using bovine serum albumin as the standard. Cytochrome P450 content was quantitatively analyzed in triplicate by the method of Omura & Sato. O-Dealkylase activities were...
determined using ethoxyresorufin and pentoxyresorufin as substrates as described by Lee & Scott.20) These substrates have previously been shown to be reliable indicators of 3-MC (ethoxyresorufin) and PB (pentoxyresorufin) induction.21, 22) Both reactions were measured in triplicate and done under atmospheric conditions in an Aminco SPF-500 spectrofluorometer (American Instrument Company, Silver Spring, MD, U.S.A.).

Metabolism experiments were carried out as described previously23, 24) with minor modifications as described below. Metabolism was carried out in a 25 ml Erlenmeyer flask containing approximately 200,000 dpm of [14C]deltamethrin, microsomes (0 or 1.4 nM cytochrome P450s), piperonyl butoxide and DEF (0 or 10^-4 M), NADPH (0 or 1 mM) and a NADPH regenerating system25) in 0.1 M phosphate buffer (pH 7.4)(2.6 ml final volume). Reactions were incubated in a shaking water bath (Labline Instruments, Inc., Melrose Park, IL, U.S.A.) at 37°C for 30 min. All reactions were stopped with the addition of 1 g ammonium sulfate and 0.2 ml IN HCl. Contents of all flasks were then transferred to graduated centrifuge tubes along with 0.4 ml of phosphate buffer. Each tube was then extracted three times with ethyl ether. The ether extracts were combined and evaporated under N2 to 0.5 ml. Triplicate 10 µl aliquots were taken from both the organic and aqueous phases of all tubes and measured by liquid scintillation counting in Liquiscint at an efficiency of 89% on a Beckman LS 5801 liquid scintillation counter. Another triplicate set of 10 µl aliquots from the organic phase were spotted on prebaked (110°C for 30 min) 60F-254 Kiesegel (Merck) thin layer chromatography (TLC) plates. Plates were developed once in a 6:1 benzene: ethyl acetate solvent system and dried overnight. Plates were exposed to X-ray film for 5 days in order to locate parent compound. Spots were scraped off and measured by liquid scintillation counting. Reactions using microsomes from each rat liver were carried out in duplicate.

RESULTS AND DISCUSSION

The rats in this study showed an increase in three key microsomal properties following the administration of PB and 3-MC. PB induced enzymatic activity, as measured by pentoxyresorufin O-dealkylation (PROD) activity, was 13-fold higher than control values (Table 1). 3-MC induced enzymatic activity, as measured by ethoxyresorufin O-dealkylation (EROD) activity, was 14-fold higher than control values (Table 1). These results suggest that the experimental animals responded typically to induction by PB and 3-MC.

PB administration causes increases in CYP2B1, 2B2 and 2C6, as well as some other isoforms.8'9) As the relative levels of these enzymes increase, so does the capacity to metabolize their various specific substrates. In this study, PB induction caused no significant difference in deltamethrin metabolism from that of water controls (Table 1). Since PB induction was demonstrated (increased PROD activity), this suggests that deltamethrin is not readily metabolized by CYP2B1, 2B2, 2C6 or other PB inducible P450s.

3-MC administration activates the Ah receptor and induces an elevation of P450 1A1 and 1A2 following treatment8'26) which can be monitored as an increase in EROD activity. In this study, 3-MC induction caused no change in deltamethrin metabolism, relative to corn oil controls (Table 1) suggesting that CYP1A1 and 1A2 are not responsible for metabolism of deltamethrin.

Many of the major PB and 3-MC inducible P450s are involved in organophosphate insecticide metabolism. Phenobarbital has been shown to increase monooxygenase-mediated metabolism of parathion,13, 14, 27-29) phorate30) azinphos-methyl31) and fenitrothion14) in vitro. β-Naphthoflavone (a 3-MC type inducer)2) has been shown to increase the metabolism of fenitrothion and parathion.14) These results suggest that there are several P450 isoforms in rat liver microsomes that are induced by PB and β-naphthoflavone and are capable of metabolizing phosphorothionate insecticides (i.e. CYP2B1, 2B2, 2C6, 1A1 and 1A2). In this study we observed good induction by PB and 3-MC, yet no increase in deltamethrin metabolism was observed for either PB or 3-MC induced samples. Thus, metabolism of

| Table 1 | Metabolism of deltamethrin in control and induced rat liver microsomes. |
|---------|---------------------------------|---------------------------------|---------------------------------|---------------------|---------------------|
| Rat     | Water                           | PB                              | Corn oil                        | 3-MC                |
|         | %DMa)                          | PRODb)                          | %DMa)                          | ERODa)             | %DMa)                          | ERODa)             |
| 1       | 18.6                           | 0.21                            | 7.50                            | 1.60                | 19.4                           | 0.21                            |
| 2       | 21.9                           | 0.11                            | 12.6                            | 2.60                | 18.1                           | 0.19                            |
| 3       | 8.97                           | 0.42                            | 20.7                            | 7.45                | 22.0                           | 0.13                            |
| 4       | 10.4                           | 0.68                            | 12.0                            | 7.22                | 23.4                           | 0.14                            |
| 5       | 16.1                           | 0.69                            | 17.4                            | 14.8                | 16.8                           | 2.1                             |
| 6       | 16.3                           | 1.01                            | 12.9                            | 7.07                | 16.4                           | 1.6                             |
| Average | 15.4 ± 4.9                     | 0.52 ± 0.34                     | 13.9 ± 4.6                      | 6.79 ± 4.67         | 20.7 ± 2.4                     | 0.17 ± 0.03                     |
|         | (± S.D.)                       |                                 |                                 |                     | 16.4 ± 1.6                     | 2.4 ± 0.5                       |

a) % [14C]deltamethrin metabolized (NADPH dependent).

b) nmol product min^-1 mg protein^-1. PROD : pentoxyresorufin O-dealkylation, EROD : ethoxyresorufin O-dealkylation. Values represent the average of at least two determinations per animal.
deltamethrin appears to involve P450 isoforms that are different than those involved in organophosphate metabolism.

This apparent difference in the P450s involved in pyrethroid and organophosphate metabolism in rats is in contrast to house flies where it has been shown that cytochrome P450<sub>1pr</sub> (CYP6D1,23) a house fly specific P450<sup>33</sup> can carry out the metabolism of deltamethrin<sup>15</sup> and an organophosphate (phosphorothionate) insecticide (chlorpyrifos).<sup>34</sup> This suggests that rats may have evolved a more specialized group of P450s compared to house fly, as has been generally hypothesized to occur.<sup>35</sup>

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要約

フェノバルパルバートールと3-メチルコラントレン誘導のラット肝ミクロソームチトクロームP450によるデルタメトリシンの代謝

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ミクロソームに存在するcytochrome P450 monoxygenaseはピレスロイド剤や有機リン剤などの殺虫剤の代謝に重要な役割を果たしている。有機リン剤の代謝に関与するいくつかのcytochrome P450のアイソフォームが同定されているのに、ピレスロイド剤の代謝に係わるP450についての知見は少ない。そこで、私たちはphenobarbital (PB)処理、3-methylcholanthrene (3-MC)処理および無処理のラットの肝ミクロソームを用いて<sup>14</sup>C標識のdeltamethrinの代謝を比較した。PBまたは3-MCの処理によってpentoxyresorufinに対するO-脱アルキルとethoxyresorufinに対するO-脱アルキルの活性上昇が誘導された。しかし、<sup>14</sup>C-deltamethrinの代謝はPBまたは3-MCの処理によって影響を受けなかった。この結果は、PBおよび3-MCによって誘導されるcytochrome P450がdeltamethrinの代謝に関与していないことを示唆している。ラットの有機リン剤とピレスロイド剤の代謝に関与するP450アイソフォームの明らかな相違についても検討した。