INDUCTION OF CYTOCHROME P-450 ACTIVITIES BY NICOTINE IN THE TOBACCO HORNWORM, *Manduca sexta*

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Abstract—The induction by dietary nicotine of a series of cytochrome P-450 enzyme activities was investigated in early fifth-instar *Manduca sexta* larvae. At a low nicotine concentration in the diet (0.1%), three of 12 midgut microsomal enzyme activities were significantly increased. At a higher concentration (0.75%) commonly found in plants of the genus *Nicotiana*, nine of 12 activities were induced by 1.4- to 10.0-fold. Total cytochrome P-450, P-450 reductase activity, and midgut microsomal metabolism of nicotine were also increased by feeding 0.75% nicotine. Nicotine was metabolized by midgut microsomes to nicotine-1-N-oxide and cotinine-N-oxide. Fat body microsomal nicotine metabolism was low and unaffected by dietary nicotine. Isolated nerve cords were able to metabolize nicotine in vitro but this metabolism was not inducible by dietary nicotine. Nicotine-fed fifth-instar *M. sexta* larvae showed an increased tolerance to subsequent nicotine injection when compared to larvae fed a control diet. These results support the idea that induction of midgut cytochrome P-450-related metabolism is an adaptation of *Manduca sexta* to dietary nicotine.

Key Words—Cytochrome P-450, induction, nicotine, *Manduca sexta*, Lepidoptera, Sphingidae.

INTRODUCTION

Microsomal cytochrome P-450 monooxygenases are involved in the metabolism of endogenous and exogenous chemicals in all organisms thus far examined.

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Since the work of Krieger et al. (1971), the involvement of insect cytochrome P-450 enzymes in the detoxification of plant secondary compounds has received considerable attention (Hodgson, 1985, for review). In Lepidoptera, the midgut is generally recognized as a major site of microsomal P-450 activities induced in response to plant chemicals, although the fat body, Malpighian tubules, and other tissues also show induced activities in certain species (Hodgson, 1985).

Induction of microsomal cytochrome P-450 activities is thought to protect insects against subsequent plant allelochemical exposures (Hodgson, 1985, Yu, 1986). Midgut P-450 activities are low around the time of ecdisis and increase when larvae are actively feeding (Tate et al., 1982; Yu and Ing, 1984; Feyer-eisen and Farnsworth, 1985). Induction of P-450 enzyme activities is dependent on either the host plant or the inducer added to an artificial diet (Brattsten et al., 1977; Yu, 1982, 1984, 1987; Yu and Ing, 1984; Brattsten, 1987; Rose et al., 1991). Little is presently known about the levels and number of different cytochrome P-450 enzymes induced by endogenous and exogenous chemicals in insects. Cohen et al. (1990) showed that at least two different P-450 proteins were induced by xanthotoxin in the black swallowtail, *Papilio polyxenes*. Xanthotoxin tolerance in this species (Ivie et al., 1983) is linked to the induction of at least one identified P-450, CYP6B1 (Cohen et al., 1992).

Interest in nicotine as a possible inducer of insect P-450 enzymes arose from the observation that this chemical is tolerated by tobacco-feeding insects such as *Manduca sexta* at concentrations that are toxic to non-tobacco feeders (Hodgson, 1985). Nicotine tolerance by *M. sexta* was initially thought to result from rapid excretion of large amounts of unmetabolized nicotine (Self et al., 1964). Brattsten et al. (1977) showed that feeding α-pinene (a known P-450 inducer) to the southern armyworm increased its tolerance to subsequent dietary nicotine exposure. Dietary piperonyl butoxide resulted in greater nicotine toxicity, thus implicating P-450 in nicotine metabolism in this insect. Recently, Rose et al. (1991) showed that nicotine was a good inducer of midgut P-450 activities in the tobacco budworm, *Heliothis virescens*.

In this paper, we describe the induction of midgut cytochrome P-450 enzyme activities by dietary nicotine in fifth instar larvae of *M. sexta*. Metabolism of nicotine by midgut and fat body microsomes was also examined. Induction of P-450 activities was correlated with an increased capacity to cope with a subsequent nicotine challenge.

**METHODS AND MATERIALS**

*Insects.* Fourth-instar *Manduca sexta* larvae, in the latter stages of head capsule slippage, were selected from a colony reared by established protocols (Prasad et al., 1986). They were maintained (26°C with a 16:8 hr light–dark