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Short communication

Induction of pulmonary cytochrome *P*4501A1: interactive effects of nicotine and mecamylamine

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Abstract

The effect of the nicotinic receptor antagonist mecamylamine on nicotine-mediated convulsions and induction of pulmonary cytochrome *P*4501A1 (CYP1A1) was examined in the rat. Mecamylamine blocked the convulsions and inhibited CYP1A1 induction by nicotine at the level of CYP1A1 activity (93%) and protein (97%), but independently induced the enzyme also at the level of activity and protein. The results show that mecamylamine antagonizes both the CYP1A1 induction and convulsions by nicotine but, independently, is an inducer of the enzyme. The results indicate that CYP1A1 induction is not a consequence of the convulsant effects of nicotine.

Keywords: Nicotine; Mecamylamine; Pulmonary cytochrome *P*4501A1; Induction: Antagonism

1. Introduction

Nicotine, the major alkaloid in tobacco (International Agency for Research on Cancer, 1986) accounts for the addictive property as well as other pharmacological and deleterious effects of smoking, including cardiovascular, endocrinological and neurological responses (US Public Health Service, 1988). The drug is currently available in various formulations for the controlled cessation of the smoking habit (Benowitz, 1996).

Nicotine also upregulates cytochrome *P*4501A1 (CYP1A1) expression in the rat in a lung-preferential and transient manner following a single, low s.c. dose (15.4 μmol (or 2.5 mg)/kg) in rats (Iba et al., 1998). Pulmonary CYP1A1 is of toxicological significance because its inducibility (Nakachi et al., 1991; Kellerman et al., 1977) as well as abundance (McLemore et al., 1990; Antilla et al., 1991) correlates highly with increased lung cancer susceptibility from tobacco smoking. These relationships stem from the established role of CYP1A1 in the metabolic bioactivation of polyaromatic hydrocarbons in cigarette smoke (e.g., benzo[*a*]pyrene) (Gautier et al., 1996) to electrophiles capable of reacting with genomic DNA to cause oncogenic mutations (Denissenko et al., 1996). Because tobacco smoke (Welch et al., 1968; McLemore et al., 1990) and nicotine (Iba et al. 1998, 1999) are potent inducers of pulmonary CYP1A1, the alkaloid may contribute to the CYP1A1 inducing property of tobacco smoke. Previous studies have emphasized polyaromatic hydrocarbons as the constituents responsible for CYP1A1 induction by tobacco smoke (Akin et al., 1975).

Induction of CYP1A1 expression is regulated transcriptionally by the ligand-activated transcription factor aryl hydrocarbon receptor (Okey et al., 1994), and previous studies have implicated aryl hydrocarbon receptor-mediated transcriptional mechanisms in CYP1A1 induction by nicotine (Iba et al., 1998). However, aryl hydrocarbon receptor binding by nicotine is weak (Iba et al., 1998) compared to the high level of CYP1A1 induction by the compound (Iba et al., 1998, 1999). This disparity suggested the involvement of, perhaps, indirect mechanisms in the induction by nicotine. Such indirect mechanisms could result from the motor effects of the compound, which include tremors and convulsions (Clarke and Kumar, 1983), and are elicited at the CYP1A1-inducing parenteral doses of the compound (Iba et al., 1998).

However, CYP1A1 induction by nicotine may not be related to the motor effects of the compound because the

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induction is observed in nicotine-fed animals, in which the motor effects are not observed (Iba et al., 1999). The nicotine feeding regimen requires extended duration and is labor-intensive (Iba et al., 1999), rendering the feeding protocol unsuitable for acute CYP1A1 induction studies with the compound. These limitations of the feeding regimen prompted us to attempt pharmacological dissociation of the motor and CYP1A1-inducing effects of the chemical that would allow us to parenterally administer the drug without its convulsive effects.

The objective of the study reported here was to assess the extent to which CYP1A1 induction by nicotine may be contributed by the convulsive effect of the alkaloid. We accomplished this by examining the effect of the nicotinic receptor antagonist mecamylamine, which blocks the convulsive and other motor effects of nicotine (Clarke and Kumar, 1983), on the nicotine-mediated induction of CYP1A1.

2. Materials and methods

2.1. Animals and treatment

Female Sprague–Dawley rats (200–225 g, from Taconic, Germantown, NY), were separated into three groups of eight, four, and four animals per group. Animals in the group of eight rats were injected s.c. with mecamylamine hydrochloride (from Sigma, St. Louis, MO) at a dose of 15.4 μmol/kg. The second group of four rats received (*S*)-nicotine (free base, from Sigma), also at a dose of 15.4 μmol/kg, whereas the third group of four rats was injected s.c. with saline (1 ml/kg) only. Fifteen minutes following mecamylamine administration, four of the mecamylamine-treated rats were administered nicotine (15.4 μmol/kg) and all of the remaining rats were administered saline (1 ml/kg) only. Protocols for the animal studies were approved by the Rutgers University Institutional Review Board for the Use and Care of Animals.

2.2. Preparation of microsomes and western blot analysis of CYP1A1

Twelve hours after treatment, the animals were decapitated and the lungs isolated for the preparation of washed microsomes by differential centrifugation as described previously (Iba et al., 1993). Microsomal CYP1A1 protein was determined by western blotting, followed by quantification of the blots by densitometry, as described previously (Iba et al., 1998).

2.3. Assay of ethoxyresorufin O-deethylase activity

Ethoxyresorufin *O*-deethylase activity, a CYP1A1-preferential activity (Yang et al., 1988), was determined in microsomes fluorometrically as described previously (Iba et al., 1998).

2.4. Other assays and data analysis

Protein was determined by the method of Lowry et al. (1961). Differences between group means in ethoxyresorufin *O*-deethylase activity were analyzed by one-way analysis of variance and paired multiple comparisons, using the Student–Newman–Keuls test, with the level of significance set at $P < 0.05$.

3. Results

Nicotine caused convulsions and tremors in the animals as reported previously (Iba et al., 1998), and these stimulant effects were completely prevented by prior mecamylamine administration (data not shown), as reported by other investigators (Clarke and Kumar, 1983; Creasy et al., 1996). Mecamylamine alone elicited no observable pharmacological effects in the animals (data not shown), also as reported by others (Clarke and Kumar, 1983).

Nicotine administration induced ethoxyresorufin *O*-deethylase activity (Fig. 1), an induction (25-fold) more

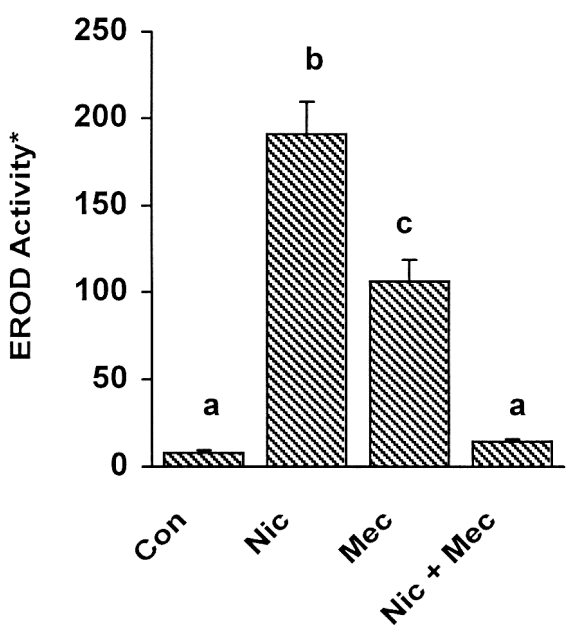

Fig. 1. Ethoxyresorufin *O*-deethylase activity in pulmonary microsomes from control and treated rats. Rats were treated with either saline (Con), nicotine (Nic), mecamylamine (Mec), or a combination of nicotine and mecamylamine (Nic + Mec), as described in the Materials and methods section. *Ethoxyresorufin *O*-deethylase activity (pmol resorufin formed/mg microsomal protein/min) was determined as described in the Materials and methods section. Each value is the mean (±SE) of determinations in four rats from two separate experiments. Values not bearing identical letters (a,b,c) are significantly different from each other ($P < 0.05$).

pronounced than observed previously in male rats, but in agreement with our reported higher sensitivity of female than male rats to the induction response (Iba et al., 1998). The nicotine-mediated induction of ethoxyresorufin *O*-deethylase activity was almost completely blocked (93%) by prior mecamylamine administration (Fig. 1). Mecamylamine alone, however, significantly induced the activity, but the induction (14-fold) was about half that by nicotine (Fig.1).

Similar to ethoxyresorufin *O*-deethylase activity, CYP1A1 protein level was significantly elevated (from barely detectable levels in untreated rats) by nicotine treatment (Fig. 2), in agreement with previous findings (Iba et al., 1998). The nicotine-induced elevated CYP1A1 protein abundance was inhibited (97%) by prior mecamylamine treatment (Fig. 2). Similar to ethoxyresorufin *O*-deethylase activity, CYP1A1 protein abundance was elevated by mecamylamine alone but to a lower magnitude than did nicotine (Fig. 2).

4. Discussion

Our results show that mecamylamine antagonizes the stimulant effects, as well as the CYP1A1-inducing effect, of nicotine. However, mecamylamine, which lacked any observable pharmacological effect was, independently, a potent inducer of the enzyme. We interpret the results to mean that CYP1A1 induction by nicotine is unrelated to the convulsive effects of the alkaloid, and that CYP1A1 induction and convulsions by the compound are mediated by different mechanisms. This interpretation is supported by our observation that orally administered nicotine, which lacks the convulsive and other toxic effects of bolus, parenterally administered nicotine (Porchet et al., 1987), caused a dose-dependent induction of the enzyme.

Our observed induction of CYP1A1 on the one hand, and abrogation of the induction by nicotine on the other, were unexpected, and can not be explained readily at this time. Speculatively, however, the complex effect is at-

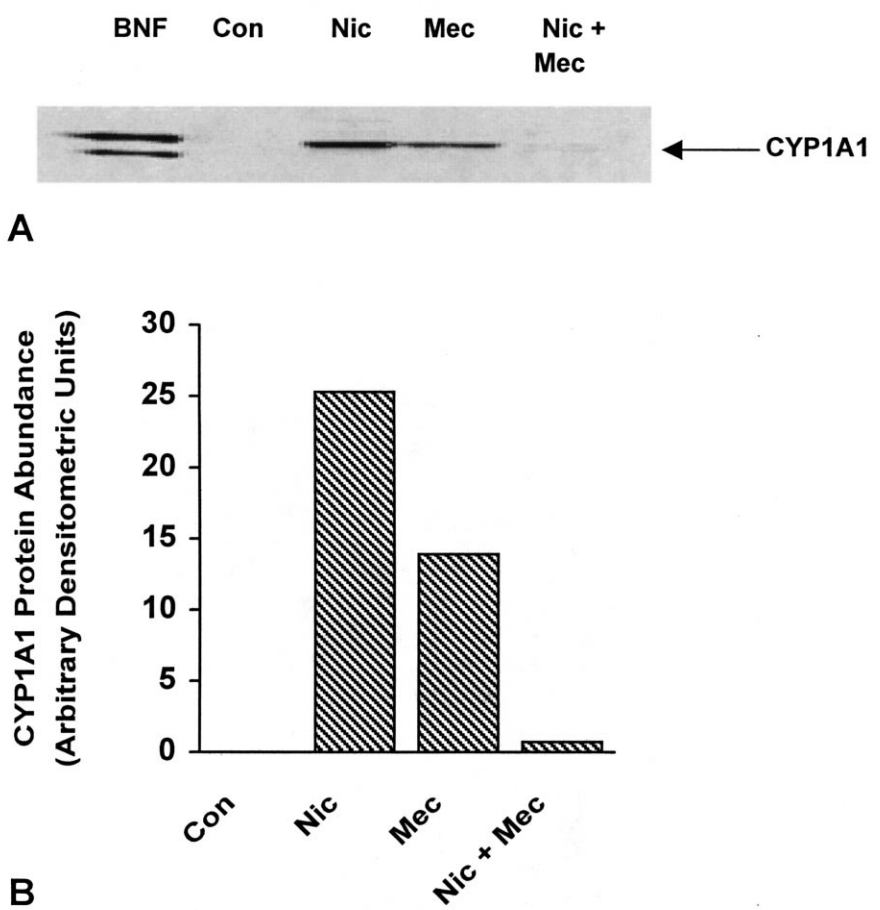

Fig. 2. Upper panel (A) Western blot analysis of pulmonary microsomal CYP1A1 from control and treated rats. The animals were treated as described in the legend to Fig. 1. Each lane represents pooled lung microsomes (50 μg protein) from two rats, except the lane marked BNF, which represents liver microsomes (0.25 μg protein) from β-naphthoflavone-treated rats (BNF) as a positive control for CYP1A1. The lower band in BNF microsomes represents CYP1A2. Lower panel (B) Densitometric analysis of the western blot data in the upper panel.

tributable to pharmacokinetic mechanisms. This is possible if metabolites of nicotine and mecamylamine contribute significantly to the induction by the two compounds, and metabolic formation of the active nicotine metabolite(s) is inhibited by mecamylamine, as might the formation of the active metabolite(s) of mecamylamine by nicotine. Support for this speculation includes the suggested involvement of metabolites in CYP1A1 induction by nicotine (Iba et al., 1999).

Alternatively, mechanisms, possibly nicotinic receptor-mediated but unrelated to those mediating the convulsive effects of nicotine, could contribute to the induction by nicotine and mecamylamine. Of relevance to this speculation is the suggested involvement of the γ-aminobutyric acid receptor-gated ion channel in the regulation of CYP1A1 expression (Sadar et al., 1996). Furthermore, nicotine stimulates nicotinic receptor-dependent and mecamylamine-inhibitable release of γ-aminobutyric acid (Lu et al., 1998). A receptor-based mechanism would accommodate the observed mixed effects of mecamylamine (induction of CYP1A1 on the one hand and abrogation of the induction by nicotine on the other). The mechanism is also consistent with the reported complex effects of nicotine and mecamylamine at the nicotinic receptor (Creasy et al., 1996). It should be pointed out that while the data and the nature of the compounds examined implicate nicotine receptors in our observed effects, involvement of other modulators, including the aryl hydrocarbon receptor, can not be ruled out at this time. Verification of these speculations will, of course, require further studies.

The relative roles of transcriptional and non-transcriptional mechanisms in CYP1A1 induction by mecamylamine, and in the abrogation by the compound of the induction by nicotine, remain to be established. The enhancement and antagonism of CYP1A1 induction by mecamylamine observed in the present study suggests a dose-dependence of the effects, which could be agonism predominantly at low doses and antagonism predominantly at high doses. Based on these considerations, CYP1A1 induction by mecamylamine may even be more pronounced at lower doses of mecamylamine than the 14-fold induction observed with the 15.4 μmol/kg dose of the compound in the current study. A similar dose-dependent dual effect has been reported for α-naphthoflavone, which antagonizes upregulation of the enzyme by other compounds but acts as an inducer at low concentrations (Santostefano et al., 1993). Dose–response studies will be necessary to determine the doses of the compound at which repression rather than induction of CYP1A1 expression predominates. Such inhibitory doses of the compound may be useful for the pharmacological suppression of nicotine-induced CYP1A1 expression if nicotine replacement therapy for smoking cessation in humans is found to be associated with CYP1A1 induction. It remains to be determined, of course, whether mecamylamine induces CYP1A1 in humans.

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