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DIFFERENTIAL EFFECT OF CHRONIC NICOTINE ADMINISTRATION ON BRAIN CYTOCHROME P4501A1/2 and P4502E1*

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The effects of chronic nicotine administration on the enzymatic activities and immunoreactivities of P4501A1/2 and P4502E1 were determined in microsomal fractions from brain regions and liver. Chronic nicotine increased 7-ethoxyresorufin O-deethylase activity and P4501A1/2 immunoreactivity in hippocampus and brain stem, decreased values in cerebral cortex, thalamus, and striatum, and did not affect parameters in other brain regions. In contrast, N-nitrosodimethylamine N-demethylase activity from nicotine-injected rats increased in all brain regions examined, and this increase was associated with increased P4502E1 immunoreactivity. Chronic nicotine did not alter either enzyme activities or immunoreactivities of these isoforms in hepatic microsomes.

Recent studies have indicated that many isoforms of cytochrome P450 (P450; EC1.14.14.1), a superfamily of hemoproteins responsible for the oxidation of a variety of foreign chemicals and endogenous compounds, are present in the brain. Until recently, it was unclear whether the expression and activity of P450s in brain are subject to regulation, as is known for hepatic P450s (for review see 1). However, studies (2-4) have shown that rat brain P450s can be induced by phenobarbital, 3-methylcholanthrene, or ethanol (ETOH), compounds known to induce hepatic P4502B1 and 2B2 (P4502B1/2), P4501A1 and 1A2 (P4501A1/2), and P4502E1, respectively. In addition, the brain P450 monooxygenase system has been shown to be induced by various centrally acting drugs, including phenytoin and tricyclic antidepressants (2,5), and P4502B1/2 and P4501A1/2 from rat brain have been either purified or partially purified to homogeneity (6,7).

Recent studies in our laboratory have shown that the chronic administration of nicotine, a potent psychoactive alkaloid present in tobacco, induces P4502B1/2-mediated benzyloxyresorufin

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Abbreviations:

P450, cytochrome P450; P4501A1/2, P4501A1 and P4501A2; P4502B1/2, P4502B1 and P4502B2; EROD, 7-ethoxyresorufin O-deethylase; NDMA, N-nitrosodimethylamine N-demethylase; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; ot/na, olfactory tubercle/nucleus accumbens; β-NF, β-napthoflavone; ETOH, ethanol.

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and pentoxyresorufin 0-dealkylation in brain, but not liver; these increases were associated with an increase in P4502B1/2 immunoreactivity (8). Because compounds may affect the expression of multiple isoforms of P450 (for review see 9), the objective of the present study was to determine the effects of chronic nicotine administration on P4501A1/2 and P4502E1, isoforms known to be expressed in brain. For comparative purposes, studies were also conducted in liver.

MATERIALS AND METHODS

Male Sprague-Dawley rats (200-250 g, Harlan Industries, Indianapolis, IN) were group housed and maintained on a 12-hr light/dark cycle with food and water available *ad libitum*. All animal use procedures were in accordance with the "NIH Guide for the Care and Use of Laboratory Animals" and were approved by the University of South Florida Animal Care and Use Committee. Nicotine hydrogen bitartrate (1.76 mg (3.6 μmoles)/kg, dissolved in saline) was administered subcutaneously to animals twice daily for 10 days. Control rats received injections of saline.

Animals were killed by decapitation 16 hr following the last injection, and brains and the main lobe of the livers were removed and rinsed in ice-cold buffer (0.1 M Tris HCl, pH 7.4 containing 1.15% (w/v) KCl, 0.2 mM EDTA, 0.1 mM dithiothreitol, 10% (v/v) glycerol, 0.1 mM phenylmethylsulfonyl fluoride, and 20 μM butylated hydroxytoluene). Brains and livers were dissected on ice, weighed, and homogenized in 5 volumes of buffer. Brain regions (frontal cerebral cortex, striatum, cerebellum, thalamus, brain stem, hippocampus, and olfactory tubercle/nucleus accumbens) from 4 animals were pooled. Microsomal fractions were prepared using the calcium precipitation procedure (10), and microsomal protein was measured using bovine serum albumin as the standard (11).

The activity of 7-ethoxyresorufin O-deethylase (EROD), an index of P4501A1/2, was measured fluorometrically (12). The reaction was initiated by the addition of 20 μM substrate to 200 μg microsomal protein. Samples were incubated for 15 min at 37°C in a shaking water bath, the reaction was terminated by the addition of 1 ml ice-cold methanol, and the product, resorufin, was measured fluorometrically using a Farrand Spectrofluorometer (Farrand Optical Corp, Inc., Valhalla, NY) with excitation and emission wavelengths of 528 nm and 590 nm, respectively. Authentic resorufin was used as the standard. The activity of N-nitrosodimethylamine N-demethylase (NDMA), an index of P4502E1, was measured by a modification of the procedure of Tu and Yang (13). The assay mixture (total volume 0.5 ml) contained 50 mM Tris HCl buffer (pH 7.4), 20 mM $MgCl_2$, an NADPH-generating system (10 mM glucose-6-phosphate, 0.8 mM NADP, and 0.4 units/ml glucose-6-phosphate dehydrogenase), 200 μg microsomal protein, and 4 mM N-nitrosodimethylamine. The tubes were preincubated for 3 min in a shaking water bath at 37°C and the reaction initiated by the addition of the NADPH generating system. Samples were incubated for 20 min, the reaction was terminated by the addition of 250 μl 10% (w/v) trichloroacetic acid, and samples were centrifuged (500 x g for 10 min at 4°C). The product, formaldehyde, was measured fluorometrically using 420 nm and 514 nm as the excitation and emission wavelengths, respectively (14). Formaldehyde was used as the standard.

For immunoblots, microsomal proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), electrophoretically transferred to a nitrocellulose membrane, and immunostained as previously described (15) using a polyclonal antibody that recognizes P4501A1/2 (generously provided by Dr. P. Sinclair, VA Hospital, White River Junction, VT) or a polyclonal antibody to P4502E1 (Oxygene, Dallas, TX.). Hepatic microsomal protein from rats treated with β-napthoflavone (β-NF) or ETOH were obtained from Oxygene, and were run in parallel with samples for the detection of P4501A1/2 and P4502E1, respectively. Immunoblots were scanned using a BIORAD Video Densitometer (model 620, Bio-Rad, Rockville Center, NY) to obtain the relative optical densities of the immunoreactive bands.

Data were analyzed on a Macintosh IIci using StatView II (Abacus Concepts, Berkeley CA) by the Student's unpaired two-tailed t-test. A level of $p < 0.05$ was accepted as evidence of a statistically significant effect.

RESULTS AND DISCUSSION

The effect of chronic nicotine administration on EROD activity in brain and liver is shown in Fig. 1. EROD activity was present in all brain regions, and the activity in whole brain was

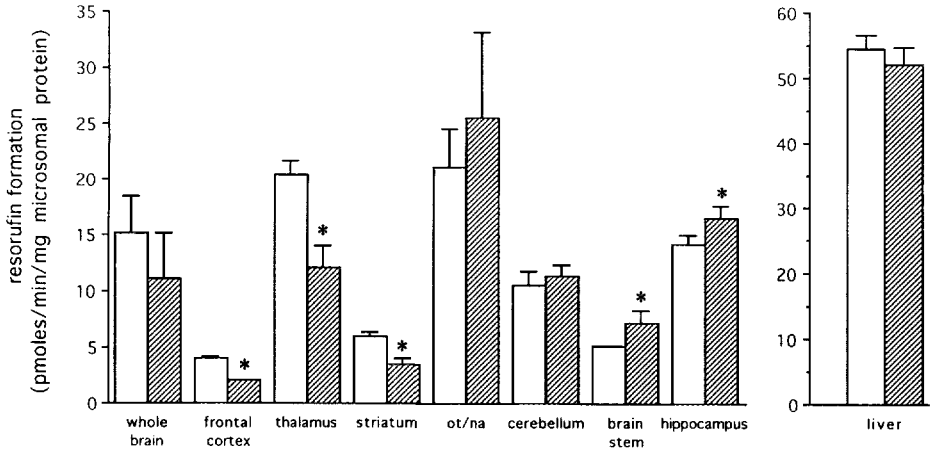

Fig. 1. Effect of chronic nicotine administration on the O-deethylation of 7-ethoxyresorufin (EROD activity) in brain and liver. Male Sprague-Dawley rats received injections of saline (open bars) or nicotine hydrogen bitartrate (1.76 mg/kg, s.c.; hatched bars) twice daily for 10 days. Whole brain, brain regions and liver were isolated, microsomal fractions prepared, and enzyme activity determined as described in the text. Each value is the mean ± S.D. of determinations from 3 experiments. Abbreviation: ot/na, olfactory tubercle/nucleus accumbens. The asterisks denote significant differences as compared to corresponding saline group values determined by the Student's t-test, $p < 0.05$.

approximately 28% of that in liver. The highest constituitive activity was present in the olfactory tubercle/nucleus accumbens (ot/na) and thalamus, followed by hippocampus and cerebellum; low activity was noted in the frontal cortex, striatum, and brain stem. Chronic nicotine administration affected EROD activity differently, depending on the brain region. In frontal cortex, thalamus, and

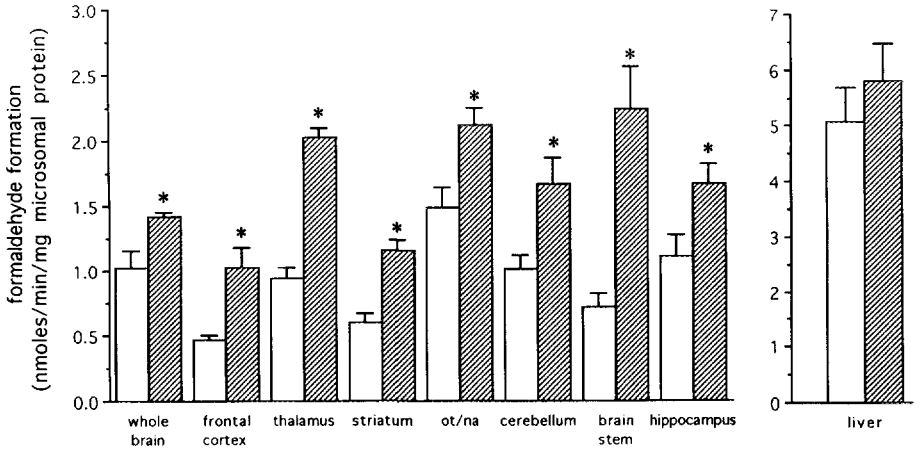

Fig. 2. Effect of chronic nicotine administration on the N-demethylation of N-nitrosodimethylamine (NDMA activity) in brain and liver. Procedures were as described in Fig. 1. Each value is the mean ± S.D. of determinations from 3 experiments. Abbreviation: ot/na, olfactory tubercle/nucleus accumbens. The asterisks denote significant differences as compared to corresponding saline group values determined by the Student's t-test, $p < 0.05$.

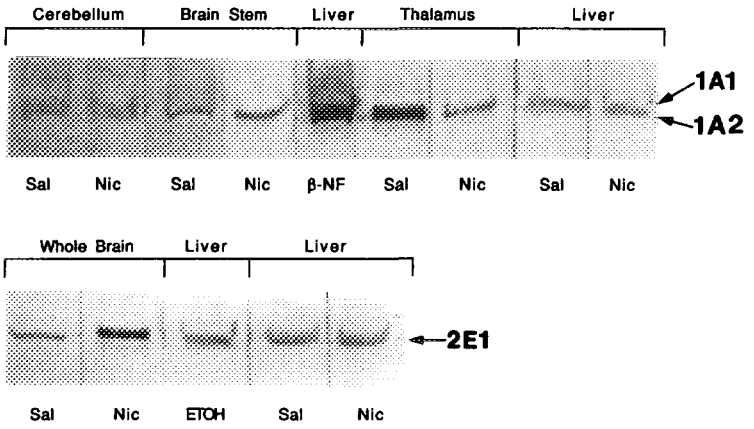

Fig. 3. Representative Western immunoblots for P4501A1/2 and P4502E1. Brain and liver microsomal protein, 100 μg and 25 μg, respectively, from rats injected with saline (Sal) or nicotine hydrogen bitartrate (Nic) were separated by SDS-PAGE, transferred electrophoretically to nitrocellulose membranes, and immunostained with antibodies to P4501A1/2 or P4502E1. For P4501A1/2, the reference standard was 2 μg hepatic microsomal protein from a rat treated with β-napthoflavone (β-NF); for P4502E1, the standard was 2 μg hepatic microsomal protein from a rat treated with ethanol (ETOH).

striatum, EROD activity decreased significantly to approximately 50% of control, whereas in the brain stem and hippocampus, activity increased significantly to 117% and 131% of control values, respectively. EROD activity in the ot/na, cerebellum, and liver was unaffected by chronic nicotine administration.

The effect of chronic nicotine administration on NDMA activity in microsomal fractions obtained from whole brain, various brain regions, and liver is shown in Fig. 2. NDMA activity was present in each of the brain regions studied, and activity in whole brain was approximately 20% of that in liver. The relative rank order of activity in brain regions from saline-injected rats was ot/na > hippocampus ≥ cerebellum ≥ thalamus > brain stem ≥ striatum; the lowest activity was in frontal cerebral cortex. In general, the relative rank order of NDMA activity in brain regions from saline-injected rats was similar to that for EROD activity. Chronic nicotine administration increased significantly NDMA activity in all brain regions with the greatest increase in brain stem (317% of control) and the lowest increase in the ot/na (142% of control). Similar to our previous results indicating no effect of chronic nicotine administration on hepatic P4502B1/2 enzymatic activities (8), hepatic NDMA activity was unaffected.

Western immunoblots of microsomal proteins from brain and liver of saline- and nicotine-injected rats indicated that immunoreactivity corresponding to P4501A1/2 and P4502E1 was present in all samples (Fig. 3). The immunoblots show that the mobility of the P450 isoforms was identical to that of hepatic microsomal protein from β-NF or ETOH treated rats, corresponding to P4501A1/2 and P4502E1, respectively. For P4501A1/2, cerebellum, brain stem, and thalamus were chosen as representative samples because these brain regions from nicotine-injected rats exhibited no change, an increase, or a decrease in EROD activity, respectively. For P4502E1, whole brain was used because all brain regions from nicotine-injected rats exhibited increased NDMA activity. Densitometric analyses indicated that P4501A1/2 immunoreactivity increased in those brain regions from nicotine-injected rats that exhibited increased EROD activity, decreased in

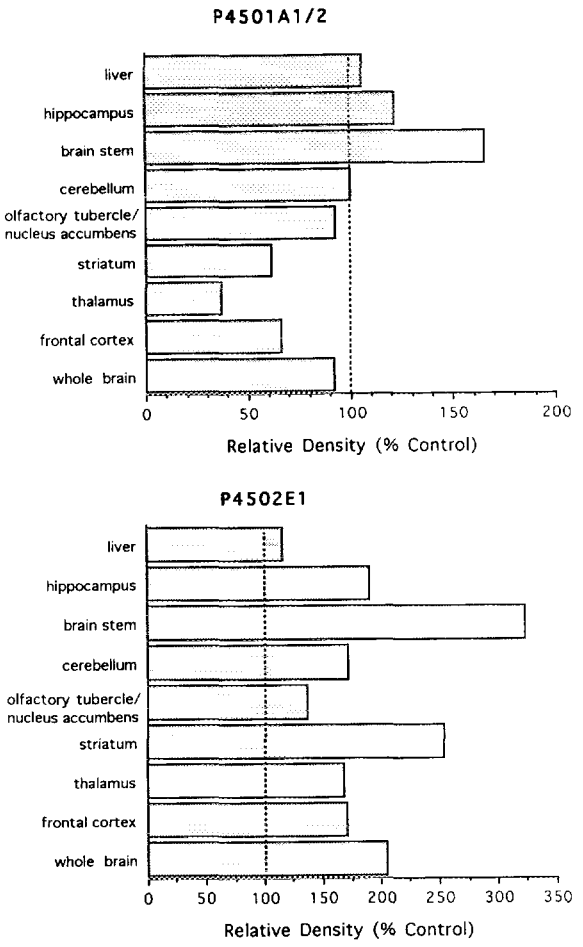

Fig. 4. Densitometric analysis of Western immunoblots for P4501A1/2 and P4502E1. Relative densitometric values obtained from two immunoblots were averaged; values for samples from nicotine-injected animals are expressed as % control (saline-injected). Mean densitometric values (O.D. x mm) for P4501A1/2 (top graph) and P4502E1 (bottom graph), respectively, from saline-injected animals were: whole brain: 0.07, 0.095; frontal cortex: 0.06, 0.035; thalamus: 0.12, 0.125; striatum: 0.065, 0.055; olfactory tubercle/nucleus accumbens: 0.075, 0.16; cerebellum: 0.065, 0.09; brain stem: 0.03, 0.065; hippocampus: 0.045, 0.105; and liver: 0.08, 0.09.

those regions that exhibited decreased EROD activity, and was unaltered in regions with unchanged EROD activity (Fig. 4 top). Similarly, an increased intensity of P4502E1 immunoreactivity was present in microsomal fractions from all brain regions from nicotine-injected rats (Fig. 4 bottom). The levels of P4501A1/2 and P4502E1 immunoreactive proteins in hepatic microsomes were unaffected by nicotine administration. These results, in concert with our previous observation that chronic nicotine increases the activity and immunoreactivity of P4502B1/2 in brain (8), indicate that chronic nicotine administration affects multiple isoforms of P450 in brain.

Studies on the regulation of hepatic P450 have shown that inducing agents may differentially affect the expression of various P450s, resulting in an increase, decrease, or no change in the levels of specific P450 isoforms (for review see 9). For example, although the major isoforms induced by phenobarbital in liver are P4502B1/2, phenobarbital also increases P4503A.

In addition, despite induction of these isoforms, phenobarbital decreases hepatic P4501A1/2. Another well-known enzyme inducer, ETOH, induces P4502E1, while the polycyclic aromatic hydrocarbons are classical inducers of hepatic P4501A1/2. Thus, in some brain regions, the effects of chronic nicotine are similar to the effects of both phenobarbital and ETOH in the liver, i.e., P4502B1/2 and P4502E1 increase, while P4501A1/2 decrease. In other brain regions, however, the effects of nicotine resemble that of the polycyclic aromatic hydrocarbons to increase P4501A1/2. Furthermore, because nicotine does not increase NADPH-P450 reductase activity (8), its actions are similar to the polycyclic aromatic hydrocarbons, and not phenobarbital which increases the activity of this enzyme. Thus, the effects of nicotine on P450 isoforms in brain are complex, and differ from known inducers, depending on the brain region examined.

The consequences of the ability of chronic nicotine administration to alter P450s in brain have not been examined. Brain is a target organ for a number of centrally acting compounds. The presence of P450 isoforms and its regulation in brain by nicotine and other centrally acting compounds may be of importance to the pharmacological and toxicological effects of many centrally active agents including environmental toxins and psychoactive drugs (1,3,16). The finding that chronic nicotine administration alters brain P450 without affecting hepatic P450 suggests that: a) chronic exposure to nicotine may influence the cerebral disposition and possible pharmacodynamic effects of centrally acting agents; and b) chronic nicotine may alter the central metabolism of nicotine itself. Studies have shown the involvement of both the P4502B and P4502E subfamilies in the hepatic metabolism of nicotine (17,18). Since these isoforms in the brain are induced by nicotine, one may expect an enhanced cerebral metabolism of nicotine upon chronic exposure. Indeed, the chronic administration of nicotine produces tolerance to many of its centrally-mediated actions, but the cellular mechanisms involved have not yet been determined. A role for pharmacokinetic tolerance as a consequence of alterations in cerebral nicotine metabolism has not been investigated. Studies to determine the molecular mechanisms responsible for the nicotine-induced effect on P450 isoforms and their role in the metabolism of nicotine are currently being pursued.

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