Noncompetitive Functional Inhibition at Diverse, Human Nicotinic Acetylcholine Receptor Subtypes by Bupropion, Phencyclidine, and Ibogaine

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ABSTRACT
Nicotinic acetylcholine receptors (nAChR) are diverse members of the neurotransmitter-gated ion channel superfamily and play critical roles in chemical signaling throughout the nervous system. The present study establishes the acute functional effects of bupropion, phencyclidine, and ibogaine on two human nAChR subtypes. Function of muscle-type nAChR (α1β2γδ) in TE671/RD cells or of ganglionic nAChR (α3β4γ5-β2) in SH-SY5Y neuroblastoma cells was measured with 86Rb efflux assays. Functional blockade of human muscle-type and ganglionic nAChR is produced by each of the drugs in the low to intermediate micromolar range. Functional blockade is insurmountable by increasing agonist concentrations in TE671/RD and SH-SY5Y cells for each of these drugs, suggesting noncompetitive inhibition of nAChR function. Based on these findings, we hypothesize that nAChR are targets of diverse substances of abuse and agents used in antiaddiction/smoking cessation strategies. We also hypothesize that nAChR play heretofore underappreciated roles in depression and as targets for clinically useful antidepressants.

Nicotinic acetylcholine receptors (nAChR) are diverse members of the neurotransmitter-gated ion channel superfamily (see reviews, Lukas and Bencherif, 1992; Galzi and Changeux, 1994; Lindstrom, 1996; Lukas, 1998). nAChR are found throughout the nervous system, where they play critical and novel roles in physiology. nAChR are composed of multiple and diverse subtypes encoded by at least 16 distinct genes (α1-α9, β1-β4, γ, δ, and ε). Muscle-type nAChR are composed as pentamers of two α1 and one each of β1, δ, and either γ (fetal) or ε (adult) subunits. One form of ganglionic nAChR contains α3, β4, and α5 with or without β2 subunits (Lukas et al., 1993; Conroy and Berg, 1995).

Bupropion (Wellbutrin; Zyban; Glaxo Welcome, Research Triangle Park, NC) has been well established as an antidepressant and has recently been shown to act as an aid to smoking cessation (Hurt et al., 1997). However, mechanisms of these actions of bupropion are still unclear. The current, presumed mechanism involves modulation of noradrenergic and dopaminergic systems implicated in addiction (Ascher et al., 1995).

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ABBREVIATIONS: nAChR, nicotinic acetylcholine receptors; PCP, phencyclidine; carb, carbamylcholine; IC50, concentration that inhibits response by 50%.
Materials and Methods

Materials. Bupropion HCl was purchased from Research Biochemicals International (Natick, MA). Ibogaine HCl was kindly provided by Dr. Henry Sershen (Nathan S. Kline Institute, Orangeburg, NY). PCP, carbamylcholine (carb), and common salts were purchased from Sigma (St. Louis, MO). Dulbecco's modified Eagle's medium, trypsin, penicillin/streptomycin solution, amphotericin B, and horse sera were obtained from Irvine Scientific (Santa Ana, CA), and fetal calf sera was obtained from Hyclone (Logan, UT).

Model Cell Lines and Cell Culture. The human clonal cell line TE671/RD expresses muscle-type nAChR containing α1, β1, γ, and δ subunits. TE671/1RD nAChR function is detectable with 86Rb+ efflux assays (Lukas et al., 1986, 1989). The human neuroblastoma cell line SH-SY5Y expresses ganglionic nAChR containing α3, β4, and α5 with or without β2 subunits. SH-SY5Y cell nAChR function is also detectable with 86Rb+ efflux assays (Lukas et al., 1993). SH-SY5Y cells also express nAChR containing α7 subunits that have high-affinity binding sites for α-bungarotoxin, but these α7-nAChR do not contribute to 86Rb+ functional responses under the conditions used here (Lukas et al., 1993; Puchacz et al., 1994).

Assays of nAChR Function. 86Rb+ efflux assays with intact SH-SY5Y or TE671/1RD cultured on 24-well plates were performed according to Bencherif et al. (1995). Levels of nonspecific ion flux were comparable (and unaffected by ibogaine, PCP, or bupropion) whether defined with samples containing agonist (carb) plus 100 μM d-tubocurarine or with blank samples that contained no agonist.

Specific nAChR function was defined as total, experimentally determined ion flux in the presence of agonist with or without test drugs, minus nonspecific ion flux. Typical values for specific and nonspecific 86Rb+ efflux are 40,000 and 5000 cpm, respectively, for TE671/1RD cell samples (−50 μg of protein) loaded with ~100,000 of 350,000 cpm of 86Rb+ applied (quantified by Cerenkov counting at 40% efficiency). Typical values for specific and nonspecific 86Rb+ efflux are 6000 and 2000 cpm, respectively, for SH-SY5Y cell samples (−50 μg of protein) loaded with ~60,000 of 350,000 cpm of 86Rb+ applied (quantified by Cerenkov counting at 40% efficiency).

Data Analysis. Dose-response curves were fit to data points by the general equation \[ Y = b + \frac{[a-b]}{(1+e^{[cX]})} n \] where Y is the observed specific 86Rb+ efflux response (in control, X is the experimental concentration of the drug, b is the lowest value of observed ion flux (typically equal to nonspecific flux), a is the maximum value of observed ion flux, c is the EC50 value for antagonist dose-response profiles or the IC50 value for antagonist dose-response profiles at fixed agonist concentration, and n is the Hill coefficient (<0 for antagonist dose-response profiles; >0 for agonist dose-response profiles). Best fit, nonlinear regression least-squares curves were determined by an iterative process, and values of a, b, c, and n were derived for each experiment, normalizing a and b values to percent of control flux. Results from replicate experiments were plotted as averages (±S.E.M.) and fit again to the logistic equation to derive the parameters reported below.

Results

Acute Effects of Drugs on nAChR Function. 86Rb+ efflux assays with cell lines TE671/1RD (muscle-type α1- nAChR) or SH-SY5Y (ganglionic α3β4- nAChR) were used to evaluate acute effects of bupropion, ibogaine, or PCP on nAChR function. In these studies, cells were exposed simultaneously to test concentrations of drug and 1 mM carb. All drugs tested produced similar dose-dependent inhibition of muscle-type nAChR function in TE671/1RD cells (Fig. 1), indicating half-maximal block at 10.5 μM bupropion, 17.6 μM PCP, and 22.3 μM ibogaine (see Table 1). Each of the drugs also produced similar dose-dependent inhibition of ganglionic nAChR function in SH-SY5Y cells in the low micromolar range (Fig. 2). Ibogaine was the most potent of these drugs, producing half-maximal inhibition at 1.1 μM, followed by bupropion (1.4 μM) and PCP (5.9 μM; see Table 1).

Mechanisms of nAChR Functional Block. Carb dose-response profiles were obtained either alone or in the presence of bupropion, PCP, or ibogaine at concentrations near their respective IC50 values for each nAChR subtype to illuminate mechanisms of inhibition. For each of the drugs tested, the functional block produced near the IC50 value was insurmountable by increasing concentration of carb in both TE671/1RD (Fig. 3) and SH-SY5Y (Fig. 4) cells, suggesting that these drugs act noncompetitively to inhibit nAChR function.

Discussion

The primary findings of this study are that bupropion, PCP, and ibogaine are potent functional inhibitors of human muscle-type and ganglionic nAChR subtypes, and that this
and ganglionic nAChR with IC50 values of 17.6 and 5.86.

Hill coefficients and 0.71

Specific 86Rb

vindicated concentrations (abscissa, molar log scale) of bupropion, for bupropion, PCP, and ibogaine are 3.22

The rank order potency for muscle-type nAChR is bupropion, PCP, and ibogaine are 3.22

Our findings show that PCP inhibits human muscle-type and ganglionic nAChR with IC50 values of 17.6 and 5.86 M, respectively. Yamamoto et al. (1992) report that 10 M PCP fully blocks a slowly evolving nicotine-stimulated K+ flux from nerve growth factor-differentiated rat PC12 cells expressing ganglionic nAChR. Although this value for PCP IC50 is in close agreement with our finding, the pharmacological profile for the K+ efflux response reported by Yamamoto et al. (1992) does not match that of ganglionic nAChR in PC12 cells (Lukas, 1989; compare d-tubocurarine and hexamethonium sensitivities). Perhaps there are two PCP-sensitive receptors/channels in PC12 cells; nAChR and a receptor/channel with comparable PCP sensitivity mediating a slower K+ efflux response. Blood serum levels of 1.6 M PCP have been reported with corresponding cerebrospinal fluid concentrations as high as 6 M after high-dose intoxication (Donaldson and Baselt, 1979). Thus, inhibition of nAChR function in vivo may contribute to effects of PCP exposure and psychosis induced by this highly addictive drug.

The results of this study show that ibogaine inhibits human muscle-type and ganglionic nAChR with IC50 values of 22.3 and 1.06 M, respectively. Previous reports have shown that low micromolar concentrations of ibogaine inhibit nicotinic receptor-mediated catecholamine release (Schneider et al., 1996). Recent reports have also shown that ibogaine inhibits 22Na+ influx through human muscle-type nAChR in TE671/RD cells and rat ganglionic nAChR in PC12 cells with IC50 values of 2.0 M and 20 M, respectively (Badio et al., 1997). By comparison, our findings indicate substantially lower affinities of nAChR for ibogaine. It is possible that human α3β4-nAChR investigated in our study have lower affinity for ibogaine than do rat α3β4-nAChR from PC12 cells (Badio et al., 1997). Nevertheless, tissue distribution of ibogaine after i.p. or s.c. administration in rats is 109 ng/ml (314 M) in plasma and up to 11 M (314 ng/ml) in fat (Hough et al., 1996). Thus, actions of ibogaine at nAChR would be predicted to occur in vivo, even if human nAChR have comparably lower affinities for the compound like those shown in our study.

Here we present the first evidence that bupropion inhibits function of nAChR. Bupropion blocks function of human muscle-type and ganglionic nAChR with IC50 values of 10.5 M and 1.44 M, respectively. Studies have found that peak plasma levels of bupropion in humans can reach a maximum of 0.52 M (Hsyu et al., 1997). Other studies have found that plasma levels of its major metabolite, hydroxybupropion, reach doses of 4 M (Golden et al., 1988). Given the apparent clinical activity of hydroxybupropion as well as the extremely long half-life of bupropion and its metabolites (see
For example, for IC50 values at human muscle-type nAChR in vivo are likely to be modest.

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References


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