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The discriminative stimulus effects of i.v. nicotine in rhesus monkeys: Pharmacokinetics and apparent pA_2 analysis with dihydro-β-erythroidine

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DHβE
dihydro-β-erythroidine
FR
fixed ratio
nAChR
nicotinic acetylcholine receptor
MLA
methyllycaconitine

ABSTRACT

Quantitative analysis of antagonism is infrequently used to identify nAChRs mediating behavioral effects. Here, nicotine (0.032 mg/kg i.v.) was established as a discriminative stimulus in rhesus monkeys responding under a fixed ratio 5 schedule; pharmacokinetics and underlying nAChR mechanism(s) were examined. When measured up to 4 h in venous blood, the training dose resulted in the following mean pharmacokinetic parameters: nicotine C_{max} = 71.7 ng/ml, $t_{1/2}$ = 116 min, and clearance = 6.25 ml/min/kg; cotinine C_{max} = 191 ng/ml; and 3OH-cotinine C_{max} = 63 ng/ml. The ED_{50} value of nicotine to produce discriminative stimulus effects was 0.013 mg/kg. Epibatidine and varenicline increased drug-lever responding to 97% and 95%, respectively (ED_{50} values = 0.00015 and 0.031 mg/kg, respectively), whereas cocaine, midazolam, and morphine produced no more than 28% drug-appropriate responding. Mecamylamine and dihydro-β-erythroidine (DHβE) dose-dependently attenuated the discriminative stimulus effects of the nicotine training dose, whereas methyllycaconitine (MLA) did not. DHβE (0.1 and 0.32) produced rightward shifts of the nicotine and varenicline dose-response functions; Schild plots fitted through individual data resulted in slopes that were not different from unity; the apparent pA_2 calculated for DHβE did not significantly differ in the presence of nicotine (6.58) or varenicline (6.45). Compared to human cigarette smoking, nicotine blood levels after 0.032 mg/kg nicotine i.v. took a similar time to reach maximal concentration, levels at Cmax were similar to smoking 2–3 cigarettes, while average nicotine levels were comparable to smoking 5–6 cigarettes. Apparent pA_2 analysis with DHβE under these conditions is consistent with nicotine and varenicline acting through the same nAChRs to produce discriminative stimulus effects.

1. Introduction

Tobacco use, predominantly in the form of cigarette smoking, remains the largest preventable cause of death in the United States (U.S. Department of Health and Human Services, 2014). Even with the assistance of tobacco cessations products, only 10% of quit attempts result in continuous abstinence for up to one year (U.S. Department of Health and Human Services, 2014). A more thorough understanding of the pharmacology of tobacco cessation products in whole animals is needed to identify new pharmacotherapeutics. The abuse-related effects of nicotine are mediated by α4β2* nAChRs (* denotes the possible presence of additional subunits; Gotti et al., 2010) and current nAChR-based smoking cessation aids (e.g., varenicline) target α4β2* nAChRs (Rollema et al., 2007).

Drug discrimination is used to identify *in vivo* pharmacological mechanism(s) that mediate the effects of centrally acting drugs. Discrimination of abused drugs, in particular, can be used to identify potential drug abuse treatments. For example, drugs that mimic

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or antagonize the discriminative stimulus effects of nicotine are considered possible candidates for the development of tobacco cessation products (Rollema et al., 2007; Smith and Stolerman, 2009). Varenicline has been shown to mimic the discriminative stimulus effects of nicotine in some studies (Rollema et al., 2007; Jutkiewicz et al., 2011) and to antagonize those effects in another study (LeSage et al., 2009). However, the pharmacological mechanism(s) by which varenicline produces discriminative stimulus effects, i.e., whether or not nicotine and varenicline share receptor mechanisms *in vivo*, has not been firmly established using quantitative analysis of antagonism. Rightward shifts in the dose response functions of these compounds with nAChR subtype-specific antagonists can be subjected to *in vivo* apparent pA_2 analysis, providing a measure of antagonist potency and allowing for hypotheses and/or conclusions to be drawn about the involvement of nicotinic receptor subtypes in mediating the discriminative stimulus effects of these drugs. In the current study, a nicotine discrimination assay in rhesus monkeys was developed with the following objectives in mind. First, nicotine was administered i.v. to mimic the rapid delivery of nicotine to the brain associated with inhalation of nicotine from smoking. The feasibility of training i.v. nicotine as a discriminative stimulus has been demonstrated using squirrel monkeys (Takada et al., 1988). Pharmacokinetics were assessed by measuring nicotine and its metabolites, cotinine and 3OH-cotinine, in whole blood at various times after administration of the training dose. Second, a relatively small training dose was selected to increase the selectivity of nicotine for $\alpha4\beta2^*$ nAChRs. The results of previous studies suggest that selectivity for β2-containing nAChRs decreases as nicotine training dose increases (Jutkiewicz et al., 2011). Receptor subtypes were assessed by testing nAChR agonists (epibatidine and varenicline), nAChR antagonists (mecamylamine, dihydro-β-erythroidine [DHβE], and MLA), and drugs whose primary sites of action do not include nAChRs (midazolam, cocaine, and morphine). A third objective was to gain adequate sensitivity and experimental space so that multiple rightward shifts in nicotine dose-response functions could be generated in the presence of an antagonist. The abuse-related effects of nicotine are mediated by $\alpha4\beta2^*$ nAChRs; however, additional subtypes (e.g., $\alpha7$) have been implicated (Brunzell and McIntosh, 2012; Harenza et al., 2014). Quantitative analysis of surmountable antagonism in the presence of two doses of DHβE was used to gain insight into whether or not the discriminative stimulus effects of nicotine and varenicline are mediated by the same nAChR subtypes.

2. Methods

2.1. Subjects

Four adult rhesus monkeys (*Macaca mulatta*), consisting of two males and two females, discriminated 0.032 mg/kg nicotine i.v. from saline. Monkeys were experimentally and pharmacologically naïve prior to the current study. Monkeys weighed 7.5–10.8 kg and were fed primate chow (Harlan Teklad High Protein Monkey Diet; Madison, WI), fresh fruit and peanuts. They were housed individually in stainless steel cages and maintained under controlled humidity and temperature on a 14/10 h light-dark cycle with continuous access to water in the home cage. The maintenance and experimental use of animals was carried out in accordance with the 2011 Guide for Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 2011). All experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at The University of Texas Health Science Center at San Antonio.

2.2. Surgery

Monkeys were anesthetized with ketamine hydrochloride (10 mg/kg i.m.; Butler Pharmaceuticals, Dublin, OH), followed by isoflurane inhaled via face mask (1.5–3.0%). A catheter (heparin-coated polyurethane i.d. = 1.02 mm, o.d. = 1.68 mm; Instech Laboratories, Plymouth Meeting, PA) was inserted into a femoral, jugular or subclavian vein. Suture silk (coated vicryl; Ethicon Inc., Somerville, NJ) was used to secure the catheter to the vessel. The catheter extended from the vessel and was attached to a vascular access port (Mida-cbas-c50; Instech Laboratories) located subcutaneously at the midscapular region of the back.

2.3. Apparatus

When outside of the home cages, monkeys were seated in commercially available chairs (Model R001; Primate Products, Miami, FL) which provided restraint at the neck and arms. Feet were restrained by a pair of shoes mounted on the front of the chair. Shoes were fitted with brass electrodes able to deliver a brief electric shock (3 mA, 250 ms) from an a/c generator (Coulbourn Instruments, Allentown, PA). Training and testing were conducted in sound-attenuating, ventilated operant chambers containing an operant panel below which was mounted a food receptacle. The operant panel consisted of a row of three lights and a second row of two levers; levers were mounted beneath the leftmost and rightmost lights. All operant behavior was controlled and recorded by a computer connected to a commercially available interface and Med-PC software (MedAssociates, Inc., St. Albans, VT).

2.4. Discrimination training

Monkeys discriminated 0.032 mg/kg nicotine from saline i.v. administered via chronic indwelling catheter during experimental sessions conducted once per day at 7:00 a.m., 7 days per week. For two monkeys the left lever was correct after nicotine and the right lever was correct after saline; in the other two monkeys, the lever assignments were reversed. Two training conditions were possible: when monkeys received an injection of the training dose of nicotine, only responses on the drug lever (i.e., correct lever) were reinforced; when monkeys received an injection of saline, only responses on the saline lever (i.e., correct lever) were reinforced. The method of reinforcement was stimulus-shock termination (SST) under a fixed ratio 5 (FR5) schedule. Stimulus-shock termination was used in the current study to be consistent with previously published studies examining the acute and chronic effects of nicotine, including nicotine withdrawal, in rhesus monkeys (Cunningham et al, 2014). Stimulus-shock termination has the advantage of being more resistant than food presentation to the disruptive effects of drugs (McMahon and France, 2002). Following injection was a one min pretreatment interval spent in the operant chamber during which no lights were illuminated and pressing the levers had no scheduled consequences. Following the pretreatment interval, red lights were illuminated above each lever and shock was scheduled to occur every 10 s. Completion of the FR on the correct lever caused the lights to extinguish and postponed the shock schedule for 30 s. Responses on the incorrect lever reset the FR requirement. During the 30 s timeout, lever pressing had no scheduled consequences. After the timeout, the red lights were re-illuminated, the levers became active, and the shock schedule resumed (i.e., a shock scheduled to occur every 10 s). The duration of SST responding was 10 min initially; it was shortened to 5 min once discrimination time course data became available. If four shocks were delivered in a session, then the session was immediately terminated. Nicotine and saline training alternated daily or