Studies on the Time Course and the Effect of Cholinergic and Adrenergic Receptor Blockers on the Stimulus Effect of Nicotine *

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Received April 11, 1974; Final Version July 10, 1974

Abstract. This study investigated the stimulus property of nicotine in the rat. The primary objectives of the study were 1. to determine the time course of the nicotine stimulus and its relationship to brain levels of the drug and 2. to determine whether the nicotine stimulus is dependent upon the integrity of specific neurotransmitter systems. A lever choice discrimination was used. After injection of nicotine, depression of one lever in an operant test chamber resulted in food reinforcement according to a variable interval schedule of 15 sec. When saline was administered, the opposite lever was reinforced. A high degree of discriminated responding was observed when either 400 μg/kg or 200 μg/kg of nicotine was used as a discriminative stimulus. The degree of discrimination decreased as the length of the time period between the injection of nicotine and the test of discrimination was increased. This decline in discrimination was similar to the decline in brain levels of nicotine suggesting that nicotine discrimination is directly related to the concentration of nicotine in the brain. Atropine, mecamylamine, dibenamine, propranolol and α-methyl-para-tyrosine (AMPT) were all tested, in a range of doses, for effects upon nicotine discrimination. Of these, only mecamylamine antagonized the nicotine stimulus. These results indicate that the stimulus effect of nicotine is mediated specifically through nicotinic-cholinergic receptors and not muscarinic-cholinergic or adrenergic receptors.

Key words: Drug Discriminations — Nicotine — Adrenergic — Cholinergic.

Nicotine has no therapeutic use, but is widely self-administered as a constituent of tobacco. There is much evidence which suggests that many people who voluntarily ingest tobacco products do so to attain the pharmacological effects of nicotine. Deneau and Inoki (1967) have been able to train monkeys to self-administer nicotine intravenously. This indicates that nicotine, per se, can have reinforcing properties. In addition, when human subjects receive an intravenous injection of nicotine, they reduce their consumption of cigarettes in a kind of self-titration of nicotine administration (Lucchesi et al., 1967).

Which of the pharmacological actions of nicotine might be sought by the tobacco user is not known, but prominent among nicotine’s central

nervous system actions are effects which are perceptual and subjective in nature. It is not possible to directly assess this kind of drug effect in animals. In humans, these effects are generally measured by directly questioning the subject. However, the observation that certain drugs can serve as controlling or discriminative stimuli (Overton, 1968, 1971; Barry, 1968; Winter, 1973) indicates that these drugs produce effects which animals can distinguish from the non-drug condition. Furthermore, the bulk of the published data on this subject suggests that drug stimuli are highly specific. Kubena and Barry (1969), for example, have reported that the stimulus characteristics of alcohol generalize to appropriate doses of other drugs which are classified as general central nervous system depressants such as pentobarbital and chlordiazepoxide, but not to drugs of other pharmacological classes like chlorpromazine or d-amphetamine. Similarly, the stimulus properties of mescaline are similar to those of lysergic acid diethylamide (LSD-25), but different from those of barbital (Hirschhorn and Winter, 1971a, b).

Morrison and Stephenson (1969) and Schechter and Rosecrans (1971) have demonstrated that nicotine may serve as a discriminative stimulus in the rat. The present study investigated further the stimulus property of nicotine. One segment of the investigation sought to determine the time course of the nicotine stimulus and its relationship to brain levels of the drug. Another segment of the investigation attempted to determine whether the nicotine stimulus is dependent upon the integrity of specific central neurotransmitter systems. This was accomplished by the administration, prior to nicotine, of agents known to compete for cholinergic or adrenergic receptor sites.

**Methods**

**Subjects.** Male Sprague-Dawley rats with no previous drug or experimental experience (Flow Research Animals, Dublin, Va.) were housed in individual home cages and exposed to a 12 h light-dark cycle. Water was freely available in home cages and adjusted amounts of commercial rat chow were offered after each experimental session to maintain the animals at 70—80% of their expected free feeding weight.

**Chemical Methods.** Brain nicotine levels. Nicotine (methyl-\(^{14}\)C)-HCl, with a specific activity of 9.2 mCi/mM, was diluted with cold nicotine hydrogen (±)tartrate to make solutions of 400 \(\mu\)g/ml and 200 \(\mu\)g/ml of nicotine. At various times after the administration of \(^{14}\)C nicotine, rats were sacrificed by decapitation. Four rats were used at each time interval. The brains were quickly removed, dissected into telencephalon, diencephalon, and brainstem, and quickly frozen for future assay.

Nicotine concentrations in each brain area were determined by the methods of Hucker, Gillette, and Brodie (1960). Brain tissue was homogenized in 0.1 N NaOH and nicotine was extracted into 15 ml of heptane containing 1.5% isoamyl alcohol. Extracted \(^{14}\)C nicotine was returned to 0.1 N NaOH. The radioactivity of \(^{14}\)C nicotine levels was determined by the procedures of Weiss (1968). A Nuclear-Chicago