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Nicotine Abstinence Syndrome Precipitated by the Competitive Nicotinic Antagonist Dihydro-β-Erythroidine

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MALIN, D. H., J. R. LAKE, T. P. UPCHURCH, M. SHENOI, N. RAJAN AND W. E. SCHWEINLE. *Nicotine abstinence syndrome precipitated by the competitive nicotinic antagonist dihydro-β-erythroidine.* PHARMACOL BIOCHEM BEHAV **60**(3) 609–613, 1998.—Rats infused subcutaneously with 9 mg/kg/day nicotine tartrate for 7 days exhibit behavioral abstinence signs following either termination of nicotine infusion or injection of the noncompetitive nicotinic antagonists mecamylamine (SC) or hexamethonium (ICV). This study examined the abstinence precipitating effects of dihydro-β-erythroidine (DHβE), a competitive nicotinic antagonist. Twenty-four nicotine-dependent rats were injected in the third ventricle with 10, 18, or 25 μg DHβE in 20 μl saline or with saline alone and observed for abstinence signs over a 20-min period. There was a significant positive linear trend of overall abstinence signs as a function of dose, $p < 0.01$. In 12 nondependent rats, the high dose of DHβE did not induce more abstinence-like signs than saline alone. In a second experiment, 18 nicotine-dependent rats were injected SC with 1 or 6 mg/kg of the muscarinic antagonist scopolamine or with saline alone. Few abstinence signs were observed in any group; there was no significant drug effect. The results suggest that nicotine abstinence signs observed in the rat are specific to reduced stimulation of previously overstimulated nicotinic receptors. © 1998 Elsevier Science Inc.

Nicotine dependence Nicotine withdrawal DHβE Nicotinic receptor antagonists Scopolamine Rat

A rat model of nicotine abstinence syndrome based on the emergence of spontaneous behavioral signs was developed by Malin et al. (27). In this model, abstinence signs are induced (27) by termination of chronic subcutaneous nicotine tartrate infusion or precipitated (26,29) by injection of the nicotinic antagonists mecamylamine (SC) and hexamethonium (ICV). The occurrence of both spontaneous and mecamylamine-precipitated nicotine abstinence syndromes in this rat model has been independently confirmed by Hildebrand et al. (22) and Carboni et al. (7). In addition, a number of similar spontaneous abstinence signs has also been observed in mice following termination of chronic nicotine tartrate injections (19).

The rat model of nicotine abstinence (27) has met a number of validity criteria. First, significantly more signs were observed after termination of nicotine infusion than before infusion, during infusion, or after a subsequent recovery period (27). Second, significantly more signs were observed following termination of nicotine infusion than following termination of saline infusion (27). Third, the number of abstinence signs depended on the rate of nicotine infusion. Significantly more signs were observed in rats infused with 9 mg/kg/day nicotine tartrate than in rats infused with 5 or 3 mg/kg/day nicotine tartrate (27,28). Fourth, the signs were potently reversed by a subcutaneous nicotine injection (27). Finally, abstinence signs were promptly precipitated by peripheral injection of mecamylamine (26) or central injection of hexamethonium (29).

The nicotinic antagonists mecamylamine and hexamethonium inactivate the nicotinic receptor complex in a noncompetitive manner, apparently through interference with associated cation channels (2,3,13,25,42). In fact, mecamylamine's actions may not be totally specific to nicotinic receptors because mecamylamine also interferes with NMDA receptors by a similar ion channel mechanism (31). Consequently, there is a need to verify that selective inactivation of nicotinic receptors precipitates an abstinence syndrome in nicotine-dependent rats. The nicotinic antagonist dihydro-β-erythroidine (DHβE) acts competitively and selectively at the nicotine recognition site of the nicotinic receptor complex (9,10,15,17,30,41), al-

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though it may also inhibit 5-HT_3 receptors with a very low affinity (18). DHβE potently reverses a number of behavioral (12,14,39) and physiological (11,14,44) actions of nicotine.

Another issue related to further validation of the rat model of nicotine abstinence concerns the selective role of the nicotinic as opposed to the muscarinic cholinergic system. One would expect nicotinic antagonists, but not muscarinic antagonists, to selectively precipitate nicotine abstinence. On the other hand, involvement of muscarinic receptors in nicotine dependence is conceivable because nicotine may indirectly stimulate muscarinic receptors through increasing acetylcholine release (23), and nicotinic and muscarinic mechanisms interactively modulate EEG and certain behaviors (24,34,35). Scopolamine is a broad spectrum competitive muscarinic cholinergic antagonist (5). It readily crosses the blood–brain barrier (1,4) and blocks both M1 and M2 and both postsynaptic and presynaptic muscarinic receptors (5,38,40).

The present study sought to answer two questions: 1) will central administration of the competitive nicotinic antagonist DHβE potently precipitate nicotine abstinence syndrome? 2) Will peripheral administration of the muscarinic cholinergic antagonist scopolamine precipitate nicotine abstinence syndrome?

EXPERIMENT 1: CENTRALLY ADMINISTERED DIHYDRO-β-ERYTHROIDINE (DHβE) PRECIPITATES NICOTINE ABSTINENCE SYNDROME

This experiment determined whether third ventricle injection of DHβE dose dependently precipitates nicotine abstinence syndrome. DHβE is commonly administered centrally to avoid some curare-like effects associated with peripheral administration, including respiratory depression (12). The third ventricle is a site where hexamethonium potently precipitated nicotine abstinence syndrome (29). This experiment also determined whether DHβE induces signs more readily in nicotine-dependent rats than in nondependent rats, as would be expected in a true nicotine abstinence syndrome.

Method

The subjects were 36 male Sprague–Dawley rats, weighing 388–460 g, maintained on ad lib food and water and a 12 L:12 D cycle. Twenty-four rats were stereotaxically cannulated in the third ventricle under equithesin anesthesia. The coordinates were 0.2 anterior, 0.0 lateral, and 9.1 mm inferior to skull bregma. Cannula placements were confirmed following the experiment by injection of methylene blue dye and histological examination. Under the same anesthesia, each rat was implanted subcutaneously with an Alzet 2ML1 osmotic minipump and rendered dependent by 7 days continuous infusion of 9 mg/kg/day nicotine tartrate [−]isomer in saline. Rats were housed individually following surgery.

After 7 days of nicotine infusion (approximately 164 h after pump implantation), the 24 rats were randomly divided into four equal groups. Subjects were challenged by third ventricle injection of 10, 18, or 25 μg DHβE (RBI, Natick, MA) in 20 μl saline, or saline alone. These doses were selected on the basis of small pilot experiments. Injections were gradually infused via motorized syringe at the rate of 4 μl/min. The injection location, rate, and volume were consistent with procedures for precipitating nicotine abstinence syndrome by the nicotinic antagonist hexamethonium (29).

Beginning with the onset of the third ventricle injection, each subject was observed for 20 min in a clear plastic rectangular chamber measuring 48 × 38 × 20 cm. All observations were performed under "blind" conditions. Observers tallied each occurrence of the various behavioral signs based on a standard check list of nicotine abstinence signs developed and validated by Malin et al. (27). Categories included gasps/abdominal writhes, teeth chatter/chews, wet shakes/tremors, and miscellaneous less frequent signs (ptosis, seminal ejaculation/genital licks, scratches, yawns, and escape jumps). Ptosis was counted no more than once per minute, and continuous teeth chattering no more than once every 15 s. Each rat's overall abstinence score consisted of abstinence signs cumulated across all categories.

To assess the effect of DHβE on nondependent subjects, 12 additional rats were cannulated in the third ventricle as earlier described, and implanted subcutaneously with an Alzet osmotic minipump filled with saline alone. After 7 days of saline infusion, six rats were challenged by third ventricle injection of 25 μg DHβE in 20 μl saline and six were challenged by saline alone. Each rat was then observed for 20 min and abstinence-like behavioral signs tallied as above.

Results

Figure 1A shows overall abstinence signs precipitated by third ventricle injection of 10, 18, or 25 μg DHβE or saline alone in nicotine dependent rats. One-way ANOVA indicated a significant effect of DHβE dose, $F(3, 20) = 19.29$, $p < 0.01$. Linear trend analysis indicated a significant positive trend of abstinence signs as a function of dose, $F(1, 20) = 57.16$, $p < 0.01$. According to Tukey's HSD test for multiple post hoc comparisons, rats injected with saline alone had significantly fewer abstinence signs than rats injected with 10 μg DHβE, $p < 0.05$, or rats injected with 18 or 25 μg DHβE, $p < 0.01$. In addition, rats injected with the lowest dose of DHβE had significantly fewer abstinence signs those receiving 18 μg DHβE, $p < 0.05$, or 25 μg DHβE, $p < 0.01$.

Figure 2 shows the occurrence of various categories of abstinence signs. Linear trend analysis revealed significant positive linear trends of shakes/tremors, $F(1, 20) = 14.70$, $p <$

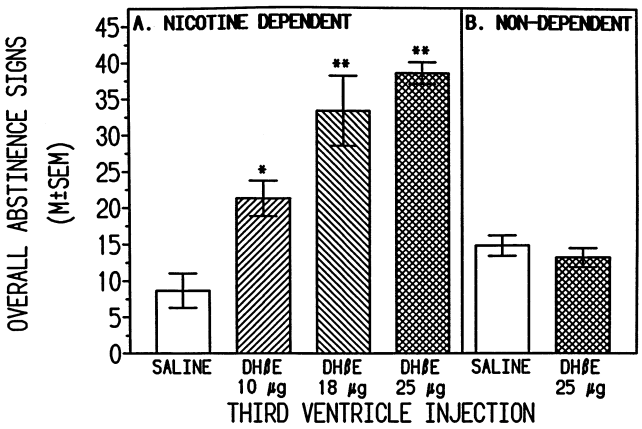

FIG. 1. (A) Overall abstinence signs (mean ± SEM), cumulated across all categories, in nicotine-dependent rats precipitated by third-ventricle injection of saline alone or 10, 18, or 25 μg DHβE. *$p < 0.05$ vs. saline alone and the 18 μg group, and $p < 0.01$ vs. the 25 μg group; **$p < 0.01$ vs. saline alone (Tukey's HSD). There was also a positive linear trend of signs as a function of dose, $p < 0.01$. (B) Overall abstinence signs in nondependent rats injected in the third ventricle with saline alone or with 25 μg DHβE.

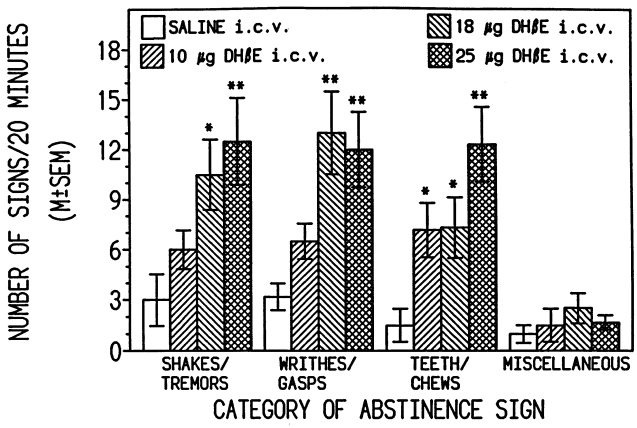

FIG. 2. Individual categories of nicotine abstinence signs precipitated by third-ventricle injection of saline alone or 10, 18, or 25 μg DHBE. *$p < 0.05$, **$p < 0.01$ vs. saline (Dunnett's Test). There were also positive linear trends for shakes/tremors, writhes/gasps and teeth/chews, $p < 0.01$.

0.01, chews/teeth chatter, $F(1, 20) = 18.08$, $p < 0.01$, and gasps/writhes, $F(1, 20) = 17.66$, $p < 0.01$. There was no significant linear trend of miscellaneous less frequent signs, $F(1, 20) = 0.96$, NS. According to Dunnett's procedure for multiple post hoc comparisons to a single control group, the 10 μg dose group differed significantly, $p < 0.05$, from saline controls only on chews/teeth chatter. The 18 μg dose group differed significantly from saline controls on shakes/tremors and chews/teeth chatter, $p < 0.05$, as well as on gasps/writhes, $p < 0.01$. The 25 μg dose group differed significantly, $p < 0.01$, from saline controls on the same three categories of abstinence signs as the 18 μg dose group.

As shown in Fig. 1B, the nondependent rats challenged with 25 μg DHβE actually had no more abstinence-like signs than the nondependent rats challenged with saline alone. This difference was not significant, $t(10) = 0.86$, NS.

EXPERIMENT 2: SCOPOLAMINE FAILS TO PRECIPITATE NICOTINE ABSTINENCE SYNDROME

This experiment determined whether nicotine abstinence syndrome would be precipitated by subcutaneous injection of the muscarinic antagonist scopolamine. Peripheral injection was employed since scopolamine readily crosses the blood–brain barrier (1,4).

Method

The subjects were 18 male Sprague–Dawley rats, weighing 442–464 g. Animals were implanted subcutaneously under halothane anesthesia with one Alzet 2ML1 osmotic minipump and rendered dependent by 7 days continuous infusion of 9 mg/kg/day nicotine tartrate [−]isomer in saline. On day 7 of infusion, subjects were randomly divided into three groups consisting of six rats each. Subjects were challenged with 1 or 6 mg/kg scopolamine HCl (Sigma, St. Louis, MO) SC or saline alone. Doses of scopolamine ranging from 1 to 6 mg/kg have frequently been used to evaluate the role of muscarinic receptors in various behaviors (16,32,37).

Twenty-five minutes after the SC injection, each subject was observed for 20 min under "blind" conditions as in Experiment 1. The postinjection interval was based on previously observed latencies of scopolamine induced behavioral and EEG effects (5,33,36,37).

Results

Figure 3 shows the overall nicotine abstinence signs precipitated by 1 or 6 mg/kg scopolamine SC or saline alone. There were few signs in any of the three treatment groups. In fact, there were fewer signs in the scopolamine-treated subjects than in the saline-injected controls. One-way analysis of variance (ANOVA) indicated no significant effect of scopolamine dose, $F(2, 15) = 0.84$, NS.

GENERAL DISCUSSION

The data support the validity of the rat model of nicotine abstinence syndrome (27), in that the syndrome was selectively precipitated by interference with nicotinic receptors. The involvement of muscarinic receptors was not indicated because the muscarinic antagonist scopolamine failed to precipitate any more abstinence signs than saline alone in nicotine-dependent rats. In contrast, the competitive nicotinic antagonist DHβE precipitated abstinence signs in a dose-dependent manner. The highest and most effective abstinence-precipitating dose of DHβE in nicotine-dependent rats failed to induce any more abstinence signs than saline alone in nondependent rats. It remains possible that a still higher dose might cause a quasi-nicotine abstinence syndrome in nondependent rats, but clearly DHβE precipitates abstinence signs more readily in nicotine-dependent rats.

The nicotine abstinence signs precipitated by third ventricle injection of DHβE resemble those precipitated by third ventricle injection of hexamethonium (29) and subcutaneous injection of mecamylamine (26). Not only did the same signs occur, but the categories of signs (cumulated over all doses) had the same rank order of occurrence (writhes/gasps > shakes/tremors > teeth chatter/chews > miscellaneous signs) in all three precipitated abstinence syndromes. The rank order of categories was the same in spontaneous abstinence syn-

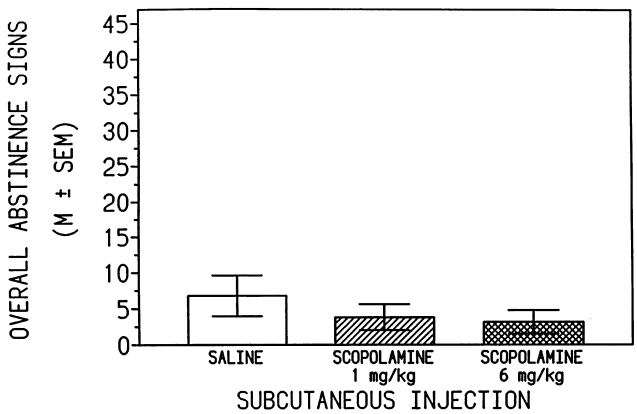

FIG. 3. Overall abstinence signs (mean ± SEM) cumulated across all categories in nicotine-dependent rats injected SC with saline alone or with 1 or 6 mg/kg scopolamine.

drome, except that the occurrence of teeth chatter/chews exceeded that of shakes/tremors (27).

Although centrally administered DHβE and hexamethonium precipitated similar abstinence syndromes, hexamethonium did so at doses lower by a factor of roughly 1000 (29). Differences in selectivity for various nicotinic receptor subtypes might account for this variation in potency. Hexamethonium and DHβE display contrasting profiles of inhibitory potency over a range of α and β combinations. For example, hexamethonium is particularly potent in blocking $\alpha_3\beta_4$ receptors (6), while DHβE has low potency at $\alpha_3\beta_4$ receptors but high potency at $\alpha_4\beta_2$ and $\alpha_4\beta_4$ receptors (8,11,20,21). When directly compared to DHβE, hexamethonium is far more potent in inactivating $\alpha_3\beta_4$ neuronal nicotinic receptors (43). In view of the extremely high nicotine abstinence precipitating potency of hexamethonium, the role of α_3 and β_4 subunits in nicotine dependence should be further investigated.

In summary, similar abstinence syndromes occur in nicotine-infused rats abruptly deprived of nicotine, challenged with a competitive nicotinic antagonist (DHβE), or challenged with two different noncompetitive nicotinic antagonists (mecamylamine and hexamethonium) that bind to totally distinct allosteric sites (13). This pattern of results supports the validity of the rat model of nicotine dependence and abstinence proposed by Malin et al. (27); the simplest interpretation is that the observed abstinence syndromes all result from reduced or impaired stimulation of nicotinic receptors following a period of chronic overstimulation of these receptors.

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