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Nitric oxide mediates mecamylamine- and naloxone-precipitated nicotine withdrawal

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Abstract

A nitric oxide synthase inhibitor blocked nicotine abstinence signs and increased weight loss in male, nicotine-dependent rats during withdrawal precipitated by the nicotinic receptor antagonist mecamylamine or the opioid receptor antagonist naloxone. These results indicate that nitric oxide systems mediate important aspects of the expression of nicotine physical dependence and suggest the potential use of nitric oxide synthase inhibitors as aids in tobacco smoking cessation. © 1998 Elsevier Science B.V.

Keywords: Nitric oxide (NO); Nicotine dependence

A rat model of the nicotine abstinence syndrome has been developed to help study problems of tobacco smoking cessation (Malin et al., 1992). The syndrome includes increases in chewing/teeth-chattering, writhes/gasps, ptosis, tremors/shakes, yawns, and weight gain. Very similar abstinence syndromes are precipitated by treating nicotine-dependent rats with the nicotinic receptor antagonist mecamylamine (Malin et al., 1994) or the opioid receptor antagonist naloxone (Malin et al., 1993).

An important biological messenger, nitric oxide (NO) (Moncada et al., 1991), mediates some aspects of the naloxone-precipitated opioid abstinence syndrome (Adams et al., 1993; Vaupel et al., 1995), and naloxone-induced nicotine withdrawal is similar in some ways to naloxone-induced opioid withdrawal (Malin et al., 1993). Those results suggested the hypothesis that NO mediates some aspects of the naloxone- or mecamylamine-precipitated nicotine abstinence syndromes.

This hypothesis was tested by s.c. implantation of male, adult (60–90 days of age), Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, IN) with Alzet® model 2ML1 osmotic minipumps (Alza Scientific Products, Palo Alto, CA) delivering saline or nicotine tartrate at a rate of 9 mg kg^{-1} day^{-1} (3.15 mg kg^{-1} day^{-1} nicotine base, Malin et al., 1992, 1993, 1994). Seven days after implantation, food and water were removed, the rats were weighed, and each rat was injected with saline, the NO synthase inhibitor N^G-nitro-L-arginine methyl ester 100 mg kg^{-1}, or its relatively inactive stereoisomer N^G-nitro-D-arginine methyl ester 100 mg kg^{-1} (Moncada et al., 1991). Thirty minutes later each rat was injected with saline, the nicotinic receptor antagonist mecamylamine 3 mg kg^{-1} (Malin et al., 1994), or the opioid receptor antagonist naloxone 4.5 mg kg^{-1} (Malin et al., 1993). All drugs were obtained from Sigma (St. Louis, MO) and were injected s.c. in 1 ml kg^{-1} volumes.

Immediately after the mecamylamine, naloxone, or saline injections, the rats were placed individually into clear cages and the following abstinence signs (based on pilot studies and the work of Malin et al., 1992, 1993, 1994) were counted for 30 min: chewing/teeth chattering episodes, writhes/gasps, wet-dog/paw shakes, ptosis (1 count for each minute of ptosis), penis licking episodes, yawns, and head bobbing episodes. All rats were weighed again 60 min after the abstinence syndrome started. Since food and water were removed before the 1.5-h pretreatment and observation periods, the rats could not gain weight through food and water consumption.

Nicotine infusion at this dose over the 7-day period before withdrawal did not alter weight gain compared to controls. The total number of abstinence signs and the % of body weight lost in the two control groups (saline- or nicotine-infused rats without other drug treatments) during withdrawal were the same (Table 1). Mecamylamine- and

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Table 1
The effects of N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-nitro-D-arginine methyl ester (D-NAME) pretreatments on mecamylamine- and naloxone-precipitated nicotine abstinence signs

Treatments	Total signs	% Weight loss
Infusion/pretreatment/withdrawal		
Saline/saline/saline (control)	34 ± 3	1.0 ± 0.1
Nicotine/saline/saline (control)	34 ± 4	1.1 ± 0.2
Nicotine/saline/mecamylamine	84 ± 10 *	0.8 ± 0.1
Nicotine/L-NAME/mecamylamine	26 ± 5	1.9 ± 0.2 *
Nicotine/D-NAME/mecamylamine	88 ± 16 *	1.1 ± 0.1
Nicotine/saline/naloxone	70 ± 7 *	1.1 ± 0.1
Nicotine/L-NAME/naloxone	29 ± 5	1.8 ± 0.2 *
Nicotine/D-NAME/naloxone	69 ± 8 *	0.7 ± 0.1

Rats were infused continuously for 7 days with saline or nicotine tartrate 9 mg kg^{-1} day^{-1} followed by pretreatment injections of saline, L-NAME 100 mg kg^{-1}, or D-NAME 100 mg kg^{-1}; then saline, mecamylamine 3 mg kg^{-1}, or naloxone 4.5 mg kg^{-1} to precipitate nicotine withdrawal. The results are means ± SEM ($n = 8$).
* $P < 0.05$, compared to both control groups that were not treated with mecamylamine or naloxone, Fisher's protected least significant difference test after analysis of variance.

naloxone-precipitated withdrawal substantially increased the total number of abstinence behaviors without altering the amount of weight lost during the withdrawal period. N^G-Nitro-L-arginine methyl ester pretreatment totally blocked these precipitated abstinence behaviors and significantly increased the percentage of body weight that was lost during this short abstinence period. The less active stereoisomer of N^G-nitro-L-arginine methyl ester, N^G-nitro-D-arginine methyl ester, had no significant effects on abstinence signs or weight loss compared to both control groups. In all groups, the chewing behaviors and writhes/gasps were the most prominent signs, and both were substantially decreased by the L-stereoisomer of the nitric oxide synthase inhibitor, but not the D-stereoisomer.

These results indicate that NO inhibition can alleviate nicotine withdrawal symptoms in rats that are physically dependent on nicotine, and suggest that NO mediates important aspects of the nicotine abstinence syndrome. The behavioral effects are important because nicotine withdrawal symptoms contribute to relapse in smoking cessation (Malin et al., 1992). The body weight effects also have important implications. It is well known that nicotine abstinence increases body weight in humans and animals due to metabolic and appetite effects (Benowitz, 1996; Malin et al., 1992). Nitric oxide synthase inhibition can decrease food intake in mice (Morley and Flood, 1991), and, in our studies, decreased abstinence-induced chewing behavior and increased weight loss. Since these effects of the NO synthase inhibition would counteract nicotine withdrawal-induced increases in food consumption and weight gain our results indicate that NO synthase inhibition might decrease appetite and weight gain during nicotine abstinence.

The results suggest that nitric oxide systems represent an important therapeutic target for the development of agents to alleviate the symptoms of nicotine abstinence and facilitate tobacco smoking cessation.

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