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Inhibition of monoamine oxidase isoforms modulates nicotine withdrawal syndrome in the rat

D.H. Malin *, W.D. Moon, P. Goyarzu, E. Barclay, N. Magallanes, A.J. Vela, A.P. Negrete, H. Mathews, B. Stephens, W.R. Mills

University of Houston-Clear Lake, Houston, TX, USA

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ABSTRACT

Aims: There have been many reports of monoamine oxidase (MAO) inhibition by non-nicotine ingredients in tobacco smoke, persisting for days after smoking cessation. This study determined the effect of inhibiting MAO and its isoforms on nicotine withdrawal syndrome.
Main methods: Rats were rendered nicotine-dependent by seven days of subcutaneous (s.c.) 9 mg/kg/day infusion of nicotine bitartrate. Twenty-two hours after termination of infusion, they were observed over 20 min for somatically expressed nicotine withdrawal signs. Three hours before observation, rats were injected intraperitoneally (i.p.) with 4 mg/kg each of the MAO A antagonist clorgyline and the MAO B antagonist deprenyl, or with saline alone. A similar experiment was performed with non-dependent, saline-infused rats. Another experiment compared nicotine-dependent rats that received injections of either saline or 4 mg/kg clorgyline alone. A further experiment compared rats receiving either saline or 4 mg/kg deprenyl alone.
Key findings: Combined treatment with both MAO inhibitors markedly and significantly exacerbated somatically expressed nicotine withdrawal signs in nicotine infused rats, while having no significant effects in saline-infused rats. Rats injected s.c. with 4 mg/kg clorgyline alone had significantly more withdrawal signs than saline-injected rats, while deprenyl-injected rats had significantly fewer signs than saline controls. Assays confirmed that clorgyline thoroughly reduced MAO A enzymatic activity and deprenyl thoroughly reduced MAO B activity.
Significance: The results suggest that inhibition of MAO A may contribute to the intensity of withdrawal syndrome in smoking cessation.

Introduction

It is generally agreed that nicotine is the main substance responsible for tobacco dependence and addiction. However, there are many other ingredients in tobacco that might interact with nicotine in modulating these phenomena. Monoamine oxidase (MAO) down-regulates the levels of biogenic amine transmitters (dopamine, norepinephrine, serotonin). Tobacco smoke inhibits enzymatic activity of both MAO A and B isoforms (Bacher et al., 2011; Berlin et al., 2000; Carr and Basham, 1991; Castagnoli et al., 2002; Fowler et al., 1996, 1998, 2003; Leroy et al., 2009; Oreland et al., 1981; Volkow et al., 1999). A variety of non-nicotine tobacco compounds may be responsible for this (Arib et al., 2010; Bacher et al., 2011; Castagnoli et al., 2002, 2003; Dixon Clarke and Ramsay, 2011; Hauptmann and Shih, 2001; Herraiz and Chaparro, 2005; Khalil et al., 2006; Mendez-Alvarez et al., 1997; Rommelspacher et al., 2002; Talhout et al., 2007). MAO inhibition or knockout synergistically enhances nicotine actions, such as self-administration, discriminative stimulus properties, locomotor activation and sensitization (Agatsuma et al., 2006; Guillem et al., 2005, 2006; Lanteri et al., 2009; Villégier et al., 2003, 2006, 2007, 2010; Wooters and Bardo, 2007). MAO B inhibition can persist for at least a week after smoking cessation (Rose et al., 2001). This raised the question: how would largely complete (at least 90%) elimination of MAO activity affect nicotine withdrawal syndrome?

The present study addressed this question with a rodent model of nicotine physical dependence and withdrawal that has been validated and widely replicated (Malin, 2001; Malin and Goyarzu, 2009; Malin et al., 1992). Prior to observation of nicotine withdrawal signs, MAO A inhibition was induced by injection of clorgyline and MAO B inhibition was induced by deprenyl (also known as selegiline). Enzymatic assays were performed to confirm the thoroughness of enzyme inhibition and specificity of the two drugs to inhibition of the respective isoforms.

Materials and methods

Subjects

54 male Sprague–Dawley rats, 300–380 g.

* Corresponding author at: University of Houston-Clear Lake, 2700 Bay Area Blvd. (mail code 265), Houston, TX 77058, USA. Tel.: +1 713 826 5914; fax: +1 281 283 3406.
E-mail address: malin@uhcl.edu (D.H. Malin).

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Compounds

Nicotine bitartrate, clorgyline and deprenyl were obtained from Sigma-Aldrich, St. Louis, Missouri.

Nicotine dependence induction

Under isoflurane anesthesia, rats were Implanted in the scapular region with Alzet 2ML1 osmotic minipumps. Dependent rats were infused for seven days with 3.15 mg/kg/day nicotine bitartrate, expressed as the base. Non-dependent rats were treated identically except that their osmotic pumps contained saline alone.

Nicotine withdrawal

On day seven, pumps were removed under isoflurane anesthesia. Rats were placed in a large transparent tub and observed over 20 min at 22 h post-pump removal. Blinded observers recorded occurrences of withdrawal signs: writhes, gasps, shakes, tremors, vacuous chewing, teeth chattering, ptosis and miscellaneous less-frequent signs (hind-foot scratching, spontaneous ejaculation and backing up).

Expt. 1 procedure: The effects of MAO A and B inhibition in nicotine-dependent rats

Fourteen rats were nicotine-infused. At 19 h following pump removal, rats were injected i.p. with saline or with 4 mg/kg of clorgyline (MAO A inhibitor) plus 4 mg/kg of deprenyl (MAO B inhibitor). This combined dose was roughly similar to that previously used to intensify nicotine actions (Villégier et al., 2006). Rats were observed 3 h later at 22 h following nicotine withdrawal.

Expt. 2 procedure: The effects of MAO A and B inhibition in saline-infused rats

Eleven rats were infused with saline alone. At 19 h following pump removal, six were injected i.p. with 4 mg/kg clorgyline plus 4 mg/kg deprenyl. Five rats received saline alone. Rats were observed for withdrawal signs at 22 h following nicotine withdrawal.

Expt. 3 procedure: The effects of MAO A inhibition in nicotine-dependent rats

Fourteen rats were infused with nicotine as above, and the nicotine pumps were removed on the seventh day of infusion. At 19 h following pump removal, seven rats were injected i.p. with 4 mg/kg clorgyline in saline, while seven received saline alone. Blinded observers recorded withdrawal signs over 20 min beginning 3 h later at 22 h post-pump removal.

Expt. 4 procedure: The effects of MAO A inhibition in nicotine-dependent rats

Fifteen rats were infused with nicotine as above, and the nicotine pumps were removed on the seventh day of infusion. At 19 h following pump removal, seven rats were injected i.p. with 4 mg/kg deprenyl in saline, while eight received saline alone. Blinded observers recorded withdrawal signs over 20 min beginning 3 h later at 22 h post-pump removal.

Expt. 5 procedure: The Effects of drugs on MAO A and B enzymatic activity

MAO A and B enzymatic activity was determined in individual whole brain homogenates in phosphate buffer from four of the saline-treated rats, three of the clorgyline-treated rats, four of the deprenyl-treated rats and three of the rats treated with both drugs. The Amplex® Red Monoamine Oxidase Assay Kit (Life Technologies, New York) with *p*-tyramine as the substrate was used according to the manufacturer's instructions with the following minor exceptions. For MAO B activity determination, 50% additional clorgyline was added to more thoroughly block MAO A activity. For MAO A activity determination, 50% additional pargyline was added to more thoroughly block MAO B activity.

Results

Expt. 1: The effects of MAO A and B inhibition in nicotine-dependent rats

As shown in Fig. 1 (panel B), nicotine-infused rats injected with saline had 27.7 ± 3.0 (M ± SEM) overall withdrawal signs cumulated across all categories. Nicotine-infused rats injected with clorgyline plus deprenyl had 64.7 ± 11.3 overall withdrawal signs. This difference was significant, $t(12) = 3.17$, $p = 0.004$. As shown in Fig. 2, the predominant category of withdrawal sign was gasps and writhes. There was a significant difference between treatment groups in gasps/writhes, $t(12) = 3.71$, $p = 0.0015$, and in teeth chattering/chews, $t(12) = 1.91$, $p = 0.04$. There were no significant differences in the other categories.

Expt. 2: The effects of MAO A and B inhibition in saline-infused rats

As shown in Fig. 1 (panel A) saline-infused, nicotine-naïve rats averaged 14.2 ± 6.2 overall signs. Clorgyline and deprenyl-injected rats averaged 19.5 ± 4.3 overall signs. This difference was not significant, $t(9) = 0.71$, NS. There were no significant differences in any category of withdrawal sign.

Expt. 3: The effects of MAO A inhibition in nicotine-dependent rats

As shown in Fig. 2, clorgyline injection significantly increased overall nicotine withdrawal signs, $t(12) = 3.06$, $p = 0.005$. It also significantly increased the predominant withdrawal sign, gasps and writhes, $t(12) = 3.26$, $p = 0.004$. There were no significant differences in any other category of sign.

Expt. 4: The effects of MAO B inhibition in nicotine-dependent rats

As shown in Fig. 3, deprenyl injection significantly *decreased* overall nicotine withdrawal signs, $t(13) = 2.47$, $p = 0.014$. It also significantly decreased the predominant withdrawal sign, gasps and writhes, $t(13) = 2.15$, $p = 0.026$. In addition, it also significantly reduced shakes and tremors, $t(13) = 1.91$, $p = 0.044$, as well as instances of ptosis, $T(13) = 3.07$, $p = 0.015$. There were no significant differences in chewing/teeth chattering or miscellaneous less frequent signs.

Expt. 5: Effects of drugs on MAO A and B enzymatic activity

One-way Analysis of variance (ANOVA) of MAO A activity in nicotine-dependent rats by the four treatments revealed a significant overall treatment effect, $F(3,10) = 30.18$, $p < 0.001$. Relative to saline injection, clorgyline caused a highly significant 99.5% reduction in MAO A enzyme activity, $p < 0.001$ (Fisher's LSD). The combined clorgyline plus deprenyl treatment caused a highly significant, $p < 0.001$, 90.5% reduction. The injection of deprenyl alone caused a much more modest 37% reduction, which was nevertheless significant, $p = 0.007$. However, compared with the deprenyl-treated brains, MAO A activity in brains from clorgyline-treated rats or from rats receiving the combination treatment, was significantly lower, $p < 0.001$ and $p = 0.001$, respectively (Fig. 4 left side).

One-way ANOVA of MAO B activity revealed a significant effect of the four treatments, $F(3,10) = 19.71$, $p < 0.001$. Relative to saline treatment, deprenyl resulted in a highly significant, $p < 0.001$, 100%