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Brain regions mediating $\alpha 3\beta 4$ nicotinic antagonist effects of 18-MC on nicotine self-administration

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Abstract

18-methoxycoronaridine (18-MC), a putative anti-addictive agent, has been shown to decrease the self-administration of several drugs of abuse in rats. 18-MC is a potent antagonist at $\alpha 3\beta 4$ nicotinic receptors. Consistent with high densities of $\alpha 3\beta 4$ nicotinic receptors being located in the medial habenula and the interpeduncular nucleus, 18-MC has been shown to act in these regions to decrease both morphine and methamphetamine self-administration. The present study was conducted to determine if 18-MC's effect on nicotine self-administration is mediated by acting in these same brain regions. Because moderate densities of $\alpha 3\beta 4$ receptors occur in the dorsolateral tegmentum, ventral tegmental area, and basolateral amygdala, these brain areas were also examined as potential sites of action of 18-MC. Local administration of 18-MC into either the medial habenula, the basolateral amygdala or the dorsolateral tegmentum decreased nicotine self-administration. Surprisingly, local administration of 18-MC into the interpeduncular nucleus increased nicotine self-administration while local administration of 18-MC into the ventral tegmental area had no effect on nicotine self-administration. Similar effects were produced by local administration of either mecamylamine or conotoxin AuIB. These data are consistent with the hypothesis that 18-MC decreases nicotine self-administration by indirectly modulating the dopaminergic mesolimbic pathway via blockade of $\alpha 3\beta 4$ nicotinic receptors in the medial habenula, basolateral amygdala, and dorsolateral tegmentum. The data also suggest that an action of 18-MC in the interpeduncular nucleus may attenuate aversive and/or depressive effects of nicotine.

Keywords

18-methoxycoronaridine; α -conotoxin AuIB; mecamylamine; nicotine; medial habenula; interpeduncular nucleus; basolateral amygdala; ventral tegmental area; dorsolateral tegmentum; $\alpha 3\beta 4$ nicotinic receptors; drug self-administration; drug addiction

1. Introduction

18-Methoxycoronaridine (18-MC), an *iboga* alkaloid congener, has been shown to decrease the self-administration of several drugs of abuse (Glick et al. 1996, 2000a; Rezvani et al., 1997) and to attenuate signs of opioid withdrawal (Rho and Glick, 1998) in rats. 18-MC's

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anti-addictive properties appear to be attributable to selective antagonism of $\alpha 3\beta 4$ nicotinic receptors (Glick et al., 2002a, 2002b; Pace et al., 2004), acting as a negative allosteric modulator that stabilizes the ligand bound, desensitized state of the receptor (Pace et al., 2003; Yuan et al., 2007).

Alpha3beta4 nicotinic receptors in the brain are localized in the medial habenula and interpeduncular nucleus and, to a lesser extent, in other brain regions such as the ventral tegmental area (e.g., Klink et al., 2001; Quick et al., 1999), the dorsolateral tegmentum and the basolateral amygdala; Perry et al., 2002; Zhu et al., 2005). For nearly three decades it has been known that the habenulo-interpeduncular pathway is involved in reward mechanisms, either directly (e.g., Sutherland and Nakajima, 1981; Rompre and Miliareisis, 1985; Blander and Wise, 1989; Vachon and Miliareisis, 1992) or indirectly by modulating the dopaminergic mesolimbic system (Sutherland and Nakajima, 1981; Nishikawa et al., 1986). In view of 18-MC's $\alpha 3\beta 4$ nicotinic antagonist action, we hypothesized that the habenulo-interpeduncular pathway might mediate 18-MC's putative anti-addictive properties. Consistent with this hypothesis, we subsequently found that local administration of 18-MC into either the medial habenula or interpeduncular nucleus decreased both morphine (Glick et al., 2006) and methamphetamine (Glick et al., 2008) self-administration in rats. In the present study, we have sought to determine if locally administered 18-MC also affects the self-administration of nicotine. The ventral tegmental area, the basolateral amygdala, and the dorsolateral tegmentum were also included as potential sites of action since each of these areas is involved in reward mechanisms and each has at least low or moderate densities of $\alpha 3\beta 4$ nicotinic receptors. Lastly, as a means of confirming 18-MC's nicotinic antagonist effects, two other agents (mecamylamine and α -conotoxin AuIB) that block $\alpha 3\beta 4$ nicotinic receptors were tested as well.

2. Materials and Methods

2.1 Treatment Drugs

Treatment drugs included 18-methoxycoronaridine hydrochloride (1–20 μ g; Albany Molecular Research, Inc., Albany, NY and Obiter Research, LLC, Champaign, IL), mecamylamine hydrochloride (10 μ g; Sigma/RBI, St. Louis, MO), and α -conotoxin AuIB (25 pmol; generously provided by Dr. J. Michael McIntosh, University of Utah). All treatments were injected intracerebrally immediately before behavioral testing; dosages of all treatments were in the same range as those used in previous studies of morphine and methamphetamine self-administration (Glick et al., 2006, 2008).

2.2 Animals

Naïve female Long-Evans derived rats (250 g; Charles River, NY), housed individually, were maintained on a normal 12 h light cycle (lights on at 7:00 a.m., lights off at 7:00 p.m.). Female rats were used in part because female rats grow at a much lower rate than males and are less likely to outgrow their intravenous cannulas; also, while females are usually more sensitive than males to the rewarding effect of nicotine, there appears to be no effect of the estrous cycle on fixed ratio self-administration of nicotine (e.g., Donny et al., 2000). For all experiments, the "Guide for the Care and Use of Laboratory Animals" (National Academy of Sciences, 1996) was followed.

2.3 Self-administration procedure

The intravenous self-administration procedure has been described previously (e.g., Glick et al., 1996, 2000a). Briefly, responses on either of two levers (mounted 15 cm apart on the front wall of each operant test cage) were recorded on an IBM compatible computer with a Med Associates, Inc. interface. The intravenous self-administration system consisted of

polyethylene-silicone cannulas constructed according to the design of Weeks (1972), Instech harnesses and swivels, and Harvard Apparatus infusion pumps (#55–2222). Shaping of the bar-press response was initially accomplished by training rats to bar-press for water after being water deprived for 23 hours; water (as well as food) was provided *ad libitum* thereafter. Cannulas were then implanted in the external jugular vein according to procedures described by Weeks (1972). Self-administration testing began with a 16-h nocturnal session followed by daily 1-h sessions, 5 days (Monday–Friday) a week. A lever-press response produced a 50 μ l infusion of drug solution (0.02 mg of nicotine hydrogen bitartrate) in about 1 s. Since all rats generally weighed 250 ± 20 g, each response delivered approximately 0.08 mg/kg of nicotine (0.028 mg/kg free base). Surgery to implant cannulae for intracerebral drug administration was performed when baseline self-administration rates stabilized ($\pm 20\%$ variation from one day to the next across 5 days), usually after 2 weeks of testing. Each rat typically received two or three different treatments spaced at least one week apart.

2.4 Intracerebral Drug Administration

Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and secured in a stereotaxic instrument. A midline incision was made, the bone was exposed, and bilateral holes for the microinjection guide cannulae were drilled. Microinjection guide cannulae (22-gauge; Plastics One, Roanoke, VA, USA) were lowered into place such that, when inserted, the tips of injectors would be located in the medial habenula or interpeduncular nucleus. The coordinates for the medial habenula and interpeduncular nucleus injections were, respectively, as follows: AP -4.2 mm, ML ± 2.9 mm, DV -5.0 mm, using a 24° angle; AP -6.3 , ML ± 2.6 mm, DV -8.7 mm, using a 15° angle (Paxinos and Watson, 1986). Some injections were also made into the ventral tegmental area, basolateral amygdala and dorsolateral tegmentum; coordinates were, respectively, as follows: AP -6.0 mm, ML ± 2.6 mm, DV -8.0 mm, using a 14° angle; AP -2.2 mm, ML ± 4.8 mm, DV -6.0 mm, using a 0° angle; AP -7.8 mm, ML ± 3.5 mm, DV -6.7 mm, using a 14° angle. The microinjection guide cannulae were permanently secured with stainless steel screws and cranioplastic cement. Rats were allowed to recover for approximately 24 hours before resuming self-administration testing. Intracerebral injections were made with the use of microsyringe (Hamilton; Reno, Nevada). Treatment drugs (or vehicle) were administered in a volume of 1 μ l over 1 minute to prevent backflow through the microinjection guide; the injection cannula (26 gauge) was kept in place for an additional minute after drug infusion. All intracerebral injections were made bilaterally, immediately before starting a self-administration session.

Upon completion of an experiment, rats were sacrificed, and their brains were removed, frozen, and sliced (40 μ m sections) in a cryostat. All sections containing a cannula track were traced and the locus of each track was mapped. The anterior-posterior extent was determined by making a composite of the drawings in which the track was present and referencing its full extent to the position of neuroanatomical landmarks. Intracerebral injection placements were verified without knowledge of the behavioral data.

2.5 Statistical Analysis

Statistical analyses of 18-MC effects initially employed one way analyses of variance across doses, separately for each brain region (e.g., effect of interpeduncular 18-MC on nicotine self-administration; subsequently, paired t-tests were employed to compare effects at individual doses with each animal's baseline performance. Only the latter analyses were conducted for assessing the effects of mecamylamine and α -conotoxin AuIB as only single dosages of these agents were tested. The outcomes of the analyses of variance are reported in the text of the results section while the outcomes of the paired t-tests are included in the figure and table legends.

3. Results

Figure 1 shows the effects of local administration of 18-MC into the medial habenula and interpeduncular nucleus on nicotine self-administration. Infusion of 18-MC into the medial habenula decreased nicotine self-administration [$F(4,36) = 3.06, P < 0.05$], while infusion of 18-MC into the interpeduncular nucleus increased nicotine self-administration [$F(4,28) = 2.78, P < 0.05$]. The dose-response function was non-monotonic in both cases, with the highest dosage (20 μg) having no effect in either site.

Figure 2 shows the effects of local administration of 18-MC into the ventral tegmental area, basolateral amygdala, and dorsolateral tegmentum. Infusion of 18-MC into the ventral tegmental area had no effect on nicotine self-administration, while infusion of 18-MC into either the basolateral amygdala or dorsolateral tegmentum decreased nicotine self-administration [basolateral amygdala: $F(4,27) = 3.27, P < 0.05$; dorsolateral tegmentum: $F(4,29) = 2.72, P < 0.05$]. With regard to decreasing nicotine self-administration, 18-MC appeared to be most potent in the medial habenula (Figure 1) and least potent in the dorsolateral tegmentum: 1 μg had a significant effect in the medial habenula while 5 μg was required to produce a significant effect in the basolateral amygdala and 10 μg was required to produce a significant effect in the dorsolateral tegmentum. However, unlike the medial habenula and interpeduncular nucleus, the highest dosage (20 μg) of 18-MC still had significant effects in both the basolateral amygdala and dorsolateral tegmentum.

Table 1 shows the effects of locally administered mecamylamine (10 μg) and α -conotoxin AuIB (25 pmol). Both agents significantly increased nicotine self-administration when infused into the interpeduncular nucleus and decreased nicotine self-administration when infused into the medial habenula, basolateral amygdala or dorsolateral tegmentum. Neither agent had any effect when infused into the ventral tegmental area.

4. Discussion

Previous work has indicated that 18-MC appears to act in three circuits that potentially modulate the dopaminergic mesolimbic pathway: medial habenula-interpeduncular nucleus, basolateral amygdala-nucleus accumbens, and dorsolateral tegmentum-ventral tegmental area (Glick et al., 2006, 2008; Maisonneuve and Glick, 2003). The importance of each pathway for the actions of 18-MC appears to vary with the particular reward, i.e., whether it be morphine, methamphetamine, nicotine or sucrose. Thus the basolateral amygdala is apparently much less important for opioid reward (Olmstead and Franklin, 1997; Alderson et al., 2000) than for stimulant reward (Hiroi and White, 1991; Whitelaw et al., 1996) or sucrose reward (Everitt et al., 1991), consistent with data from this laboratory indicating that infusion of 18-MC into the basolateral amygdala has no effect on morphine self-administration but decreases methamphetamine (Glick et al., 2008), nicotine (present study), and sucrose self-administration (Glick et al., 2008). In the present study, infusion of 18-MC into the dorsolateral tegmentum (which includes the pedunculopontine nucleus) decreased nicotine self-administration; previously, such infusions were found to have no effect on morphine or methamphetamine self-administration while decreasing responding for sucrose (Glick et al., 2008). Other studies have also strongly implicated the dorsolateral tegmentum in the mediation of nicotine's rewarding effect (e.g., Lanca et al., 2000; Corrigan et al., 2001; Laviolette et al., 2002; Ishibashi et al., 2009) as well as in sucrose reward (Ainge et al., 2006).

Certainly the most surprising result of this study was that infusion of 18-MC into the interpeduncular nucleus increased nicotine self-administration while infusion of 18-MC into the medial habenula decreased nicotine self-administration. There are several potential

reasons why such different results were observed in these two regions. The habenulo-interpeduncular system has been long considered an alternate reward pathway in the brain, due to its direct and indirect connections to the mesolimbic dopamine pathway (Nishikawa et al., 1986). A neighboring structure, the lateral habenula, has emerged as a region processing negative motivational information; it projects to the ventral tegmental area via the rostromedial mesopontine tegmental nucleus (Jhou et al., 2009; Balcita-Pedicino et al., 2011) and inhibits dopamine neurons (Christoph et al., 1986; Ji and Shepard, 2007; Matsumoto and Hikosaka, 2007). In the present study, nicotine might have acted in the medial habenula to inhibit the lateral habenula, consistent with the asymmetrical connection between the medial and lateral habenular nuclei (Kim and Chang, 2005). Thus nicotine reward may in part be mediated by inhibition of an inhibitory input from the lateral habenula to the ventral tegmental area, and infusion of 18-MC into the medial habenula would, as observed, decrease nicotine self-administration.

As noted above, the increase in nicotine self-administration that occurred when 18-MC was administered into the interpeduncular nucleus was unexpected. However, these results are consistent with a recent study demonstrating that either genetic deletion of the $\alpha 5$ subunit, or inactivation of this region using lidocaine increases nicotine self-administration (Fowler et al., 2011). Fowler and colleagues have speculated that inactivation of the interpeduncular nucleus interrupts nicotine-stimulated glutamatergic transmission, and disrupts inhibitory motivational signals in this region, thereby increasing nicotine self-administration (Fowler et al., 2011). It is possible that, in the interpeduncular nucleus, $\alpha 3\beta 4$ blockade may have a similar effect. From previous reports it is clear that nicotine does not have rewarding effects when administered directly into the interpeduncular nucleus; rather it induces sedation (Hentall et al., 1992), hypoactivity (Hentall et al., 1995), and aversion (Shoaib and Stolerman, 1995) rather than reward (Ikemoto et al., 2006). These effects of nicotine, as well as the present effect of 18-MC, could be mediated by projections from the interpeduncular nucleus to the serotonin-containing medial and dorsal raphe nuclei (Groenewegen et al., 1986). Consistent with serotonergic mediation, ketanserin, a 5-HT₂ serotonin receptor antagonist, has been reported to decrease nicotine self-administration in rats (Levin et al., 2008).

The effects of mecamylamine and α -conotoxin AuIB were comparable to those of 18-MC in all brain regions examined. These results are therefore consistent with the premise that 18-MC's primary mode of action is to block $\alpha 3\beta 4$ nicotinic receptors (Glick et al., 2002a; Pace et al., 2004). While mecamylamine blocks all nicotinic receptor subtypes and has some selectivity for the $\alpha 3\beta 4$ subtype (Papke et al., 2001), α -conotoxin AuIB is a specific antagonist of $\alpha 3\beta 4$ nicotinic receptors (Luo et al., 1998).

The dose-effect relationship for 18-MC was non-monotonic in the medial habenula and interpeduncular nucleus; similar results were observed previously on morphine, methamphetamine and sucrose self-administration (Glick et al., 2006, 2008). The lack of effect of higher doses suggests that 18-MC has an opposing but less potent action at another receptor, i.e., possibly at the 5-HT₃ serotonin or M1 muscarinic receptor (Glick et al., 2000b; Glick et al., 2002a). Curiously, in the dorsolateral tegmentum and basolateral amygdala, 18-MC was still effective at the highest dosage (20 μ g), suggesting that 18-MC's action at a secondary site was less important in these brain areas. When infused into the ventral tegmental area, 18-MC had no effect on nicotine self-administration. Aside from showing that 18-MC does not directly influence the ascending mesolimbic pathway, this lack of effect is important in that it rules out the possibility that, when injected into the interpeduncular nucleus, 18-MC might have diffused to the ventral tegmental area to produce its effect.

As discussed previously (Glick et al., 2008), how 18-MC alters the interactions between each of the circuits noted above and the mesolimbic pathway may depend on the activity of these pathways and on how drugs of abuse exert their effects; that is, an action of 18-MC in each of these circuits may modulate rather than simply inhibit the activity of the mesolimbic pathway. As also noted previously, one modulatory mechanism common to all of these circuits might involve acetylcholine-induced activation of GABAergic neurotransmission (e.g., Zhu and Chiapinelli, 1999; Dani and Bertrand, 2007). Acetylcholine appears to preferentially activate presynaptic $\alpha 3\beta 4$ receptors on GABA neurons, releasing GABA (Lena et al., 1993; Zhu et al., 2005). 18-MC should promote desensitization of these receptors (Pace et al., 2004; Yuan et al., 2007) and reduce an inhibitory GABAergic influence, thereby altering the output of these circuits and their influence on mesolimbic responses involved in reward. There are several routes, both direct and indirect, by which mesolimbic activity could be altered by each of the circuits (now potentially four of them) affected by 18-MC. The specific connections involved in these routes have been reviewed previously (Maisonneuve and Glick, 2003; Glick et al., 2008).

In summary, along with other data from this laboratory (Glick et al., 2006, 2008; Taraschenko et al., 2007), the present data indicate that 18-MC acts in one or more of four circuits to decrease drug (morphine, methamphetamine, nicotine) and sucrose self-administration. Exactly which circuits mediate the effects of 18-MC appears to be specific to each reward. In each instance, however, 18-MC appears to indirectly dampen dopaminergic mesolimbic activity by antagonizing $\alpha 3\beta 4$ nicotinic receptors in a related brain area.

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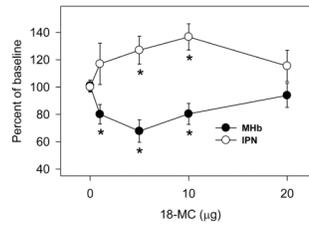


Figure 1. Effects of local infusion of 18-MC into the medial habenula (MHb) and interpeduncular nucleus (IPN) on nicotine self-administration. Baseline nicotine infusions averaged (\pm S.E.M.) 22.1 ± 1.1 and 23.6 ± 1.3 for rats in the MHb and IPN groups, respectively. Each data point represents the mean (\pm S.E.M.) percent of baseline of 6–9 rats. *Significant difference between drug and baseline (paired t-test, $P < 0.05$ – 0.02).

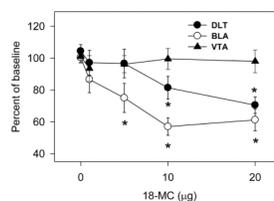


Figure 2. Effects of local infusion of 18-MC into the basolateral amygdala (BLA), dorsolateral tegmentum (DLT) and ventral tegmental area (VTA) on nicotine self-administration. Baseline nicotine infusions averaged (\pm S.E.M.) 21.5 ± 1.1 , 22.5 ± 1.4 and 22.3 ± 1.2 for rats in the BLA, DLT and VTA groups, respectively. Each data point represents the mean (\pm S.E.M.) percent of baseline of 6–7 rats. *Significant difference between drug and baseline (paired t-test, $P < 0.05$ – 0.02).

Table 1

Effects of locally administered mecamlamine (10 μ g) and α -conotoxin AuIB (25 pmol) on rates (mean responses per hour \pm S.E.M.; Ns= 5–7) of nicotine self-administration

<u>Site^a</u>	<u>Treatment</u>		
	vehicle	mecamlamine	α -conotoxin uIB
MHb	22.1 \pm 2.2	17.1 \pm 2.7 ^b	13.4 \pm 2.3 ^b
IPN	22.3 \pm 1.7	29.5 \pm 2.0 ^b	27.4 \pm 1.8 ^b
BLA	21.9 \pm 1.6	12.4 \pm 1.5 ^b	15.5 \pm 1.9 ^b
DLT	22.6 \pm 2.0	16.8 \pm 1.7 ^b	14.6 \pm 2.5 ^b
VTA	22.4 \pm 1.8	21.0 \pm 2.6	21.2 \pm 2.0

^aMHB= medial habenula; IPN= interpeduncular nucleus; BLA= basolateral amygdala; DLT= dorsolateral tegmentum; VTA= ventral tegmental area

^bsignificantly different from baseline, $p < 0.05$ – 0.02 , paired t-test