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Novel *iboga* alkaloid congeners block nicotinic receptors and reduce drug self-administration

Christopher J. Pace[a], Stanley D. Glick[a], Isabelle M. Maisonneuve[a], Li-Wen He[b], Patrick A. Jokiel[b], Martin E. Kuehne[b], Mark W. Fleck[a,*]

[a] *Center for Neuropharmacology and Neuroscience, The Albany Medical College, MC-136, 47 New Scotland Avenue, Albany, NY 12208, USA*
[b] *University of Vermont, Chemistry Department, Burlington, VT, USA*

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Abstract

18-Methoxycoronaridine, a novel *iboga* alkaloid congener, reduces drug self-administration in animal models of addiction. Previously, we proposed that these effects are mediated by the ability of 18-methoxycoronaridine to inhibit nicotinic α3β4 acetylcholine receptors. In an attempt to identify more potent 18-methoxycoronaridine analogs, we have tested a series of 18-methoxycoronaridine congeners by whole-cell patch clamp recording of HEK 293 cells expressing recombinant nicotinic α3β4 receptors or glutamate NR1/NR2B *N*-methyl-D-aspartate (NMDA) receptors. The congeners exhibited a range of inhibitory potencies at α3β4 receptors. Five congeners had IC_{50} values similar to 18-methoxycoronaridine, and all of these were ineffective at NMDA receptors. The congeners also retained their ability to reduce morphine and methamphetamine self-administration. These data are consistent with the importance of nicotinic α3β4 receptors as a therapeutic target to modulate drug seeking. These compounds may constitute a new class of synthetic agents that act via the nicotinic α3β4 mechanism to combat addiction.

Keywords: 18-Methoxycoronaridine; Ibogaine; Nicotinic receptor; *N*-methyl-D-aspartate receptor; Addiction

1. Introduction

18-Methoxycoronaridine is a novel congener of the *iboga* alkaloid ibogaine that is being studied as a potential treatment for multiple forms of drug abuse. In rats, both 18-methoxycoronaridine and ibogaine decrease the self-administration of several addictive agents including morphine (Glick et al., 1996; Maisonneuve and Glick, 1999), cocaine (Glick et al., 1996), methamphetamine (Glick et al., 2000a), nicotine (Glick et al., 2000a), and ethanol (Rezvani et al., 1995, 1997). However, they differ in their effects on responding for a non-drug reinforcer (water); ibogaine decreases responding whereas 18-methoxycoronaridine does not (Glick et al., 1996). Although ibogaine has been reported to effectively reduce drug cravings and withdrawal symptoms in addicts (Sheppard, 1994), its tremorigenic, hallucinogenic, neurotoxic, and cardiovascular side effects (see Alper, 2001) have prevented its approval as a treatment for addiction. On the other hand, 18-methoxycoronaridine, although not yet tested in humans, has no apparent side effects in rats, presumably because it is more selective pharmacologically than ibogaine.

Despite its promise for the treatment of addiction, the predominant mechanism of action of 18-methoxycoronaridine has remained elusive. Although 18-methoxycoronaridine binds with low affinity to several types of receptors (Glick and Maisonneuve, 2000; Glick et al., 2000b), we have proposed that its effects on drug-seeking behavior result from antagonism of the α3β4 subtype of nicotinic acetylcholine receptor (Glick et al., 2002a). Evidence for this hypothesis comes from previous in vitro patch-clamp studies showing that 18-methoxycoronaridine is a somewhat selective antagonist of nicotinic α3β4 receptors (Glick et al., 2002a). 18-Methoxycoronaridine, unlike ibogaine, has little or no effect at nicotinic α4β2 receptors or at NMDA-type glutamate receptors (Glick et al., 2002a), and like ibogaine it does not act on α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid or kainate-selective glu-

* Corresponding author. Tel.: +1-518-262-6536; fax: +1-518-262-6534.
E-mail address: fleckm@mail.amc.edu (M.W. Fleck).

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tamate receptor channels, γ-aminobutyric acid-A, or glycine receptor channels (Chen et al., 1996; Glick et al., 2002a; unpublished observations). Furthermore, combinations of 18-methoxycoronaridine with other agents known to block nicotinic α3β4 receptors (mecamylamine, dextromethorphan, bupropion) selectively reduce drug-seeking behavior in rats at doses that are ineffective when administered alone (Glick et al., 2002a,b). These results suggested that the effects of these agents on drug self-administration are mediated by their common actions at nicotinic α3β4 receptors. Finally, nicotinic α3β4 receptors are localized in brain regions, particularly within the habenulointerpeduncular pathway (Klink et al., 2001; Quick et al., 1999) that are well suited to modulate, either directly (e.g., Klink et al., 2001) or indirectly (e.g., Nishikawa et al., 1986; Quick et al., 1999), the mesolimbic dopamine system involved in drug reward (see Maisonneuve and Glick, 2003). Together, these findings suggest that antagonism of acetylcholine's actions at nicotinic α3β4 receptors may be an important mechanism for reducing the rewarding effects of a variety of drugs.

Part of the rationale for our current investigation was to create a compound(s) with pharmacological and therapeutic profiles superior to 18-methoxycoronaridine. We also sought to further test the hypothesis that nicotinic α3β4 receptors are part of the drug reward mechanism. A series of 18-methoxycoronaridine congeners were tested at nicotinic α3β4 receptors and promising candidates were cross-tested to confirm a lack of interaction with NMDA receptors. We show that these compounds inhibit nicotinic α3β4 receptors, but are ineffective at NMDA receptors, and retain the ability to reduce morphine and methamphetamine self-administration in rats.

A portion of this research has appeared in abstract form.

2. Materials and methods

2.1. Receptor functional analyses

Receptor functional analyses were performed as described previously (Glick et al., 2002a). Briefly, human embryonic kidney 293 fibroblasts (ATCC CRL1573) were cultured in minimum essential medium supplemented with 10% fetal bovine serum and 2 mM glutamine (Life Technologies). The culture media for cells transfected with the NMDA receptor was further supplemented with 10–100 μM DL-2-amino-5-phosphono-valeric acid to block excitotoxic levels of receptor activation. Cells were plated at $2-3 \times 10^4$ cells per milliliter on poly-D-lysine-coated 35-mm Nunc dishes. We used the following cDNAs (GenBank accession no.): nicotinic acetylcholine receptor-α3 (L31621), -β4 (U42976), NMDA-type glutamate receptors (NR1; X63255), and NR2B (M91562). Cells were transfected with the cDNAs for the nicotinic α3/β4 receptor at 1:1 and with the glutamate NR1/NR2B receptor at 1:2 with a total DNA concentration of 0.5–1.0 μg/ml. Cotransfection of enhanced green fluorescent protein (10% of total cDNA) provided a visual marker for transfected cells. Cells were transfected using either the Lipofectamine PLUS or the Lipofectamine 2000 reagents (Life Technologies). We performed functional analyses between 12 and 36 h post-transfection. Transfected cells were selected for enhanced green fluorescent protein expression and examined by voltage-clamp recording at −70 mV in the whole-cell configuration using an Axopatch 200A patch-clamp amplifier (Axon Instruments). Thin-walled borosilicate glass microelectrodes (TW150F, World Precision Instruments) had resistances of 3–5 MΩ when filled with an internal solution containing (in mM): 135 CsCl, 10 CsF, 10 HEPES, 5 EGTA, 1 $MgCl_2$, 0.5 $CaCl_2$ at pH 7.2. When recording from nicotinic acetylcholine receptors, we added 10 mM phosphocreatine to the internal solution to reduce rundown of acetylcholine-evoked currents. Current responses were filtered at 5 kHz with an 8-pole Bessel filter (Cygnus Technologies), digitized at 1 kHz, and stored on a Macintosh PowerPC-G3 computer using an ITC-16 interface (Instrutech) under control of the data acquisition and analysis program Synapse (Synergy Research). Cells were continuously superfused with extracellular solution containing (in mM): 150 NaCl, 3 KCl, 5 HEPES, 1 $MgCl_2$, 1.8 $CaCl_2$, 10 glucose, and 0.1 mg/ml phenol red, pH 7.3 (Mg^{2+} was eliminated from all solutions used to study NMDA receptors). Agonist stocks were made up in extracellular solution. All compounds were racemic mixtures unless otherwise indicated. 18-Methoxycoronaridine, 18-methoxycoronaridine congener, and 18-methoxycoronaridine enantiomer stocks were made up at 10–20 mM in dimethyl sulfoxide or 50% extracellular solution/50% dimethyl sulfoxide. All stocks were kept at −20 and 4 °C for long-term and short-term storage, respectively. 18-Methoxycoronaridine enantiomers (King et al., 2000) and 18-methoxycoronaridine congeners (Kuehne et al., 2003; Fig. 1; Table 1) were synthesized as described previously. Agonist and compound solutions were diluted in extracellular solution immediately before use; final concentration of dimethyl sulfoxide was 0.25% or lower. Control, agonist, and compound solutions were applied to individual cells by rapid perfusion with a 30-s intertrial interval. Solutions were driven by a syringe pump through a flowpipe having 4 or 6 inputs that converge at a single common output of

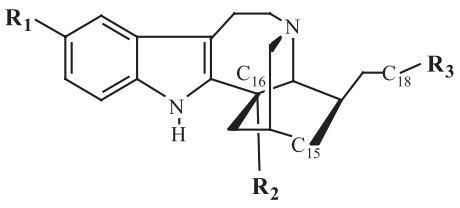

Fig. 1. Chemical structures of ibogaine, 18-methoxycoronaridine, and 18-methoxycoronaridine congeners. The chemical structure is numbered using the LeMen and Taylor system (see Table 1).

Table 1
Chemical modifications of congeners

Compound	R1	R2	R3
Ibogaine	OCH_3	H	H
18-Methoxycoronaridine (18-MC)	H	CO_2CH_3	OCH_3
#4 18-Hydroxycoronaridine (18-HC)	H	CO_2CH_3	OH
#5 18-Heptoxycoronaridine (18-HOC)	H	CO_2CH_3	$OCH_2C_6H_5$
#7 18-MethoxyIbogamine-16-sodium carboxylate (18-MISC)	H	CO_2Na	OCH_3
#8 *N,N*-Dimethylaminoethyl 18-methoxycoronaridinate (DAE-18-MC)	H	$CO_2C_2H_4NH_2$	OCH_3
#9 2-Hydroxyethyl 18-methoxycoronaridinate (HE-18-MC)	H	$CO_2C_2H_4NOH$	OCH_3
#10 2-Acetylaminoethyl 18-methoxycoronaridinate (AAE-18-MC)	H	$CO_2C_2H_4NAc$	OCH_3
#11 2-Methoxyethyl 18-methoxycoronaridinate (ME-18-MC)	H	$CO_2C_2H_4OCH_3$	OCH_3
#12 18-Methoxycoronaridine *N,N*-Dimethyl-ethylenediamine amide (18-MC DEAA)	H	$CONHC_2H_4NH_2$	OCH_3
#13 18-Methoxycoronaridine 2-hydroxyethylamide (18-MC-HEA)	H	$CONHC_2H_4OH$	OCH_3
#14 18-Methoxycoronaridine 2-methoxyethylamide (18-MC-MEA)	H	$CONHC_2H_4OCH_3$	OCH_3
#18 18-Methylaminocoronaridine (18-MAC)	H	CO_2CH_3	$NHCH_3$
#19 18-Dimethylaminocoronaridine (18-DAC)	H	CO_2CH_3	$N(CH_3)_2$
#36 15-*nor*-18-methoxycoronaridine (15-*nor*-18-MC)	H	CO_2CH_3	OCH_3

approximately 100-μm diameter. Rapid switching between inputs was achieved using a set of upstream solenoid valves (Lee) under computer control; the solution exchange rate was determined to be ≤ 5 ms from liquid junction currents.

As an initial test of the potency of the various 18-methoxycoronaridine congeners, we first tested them at 20 μM and then 1 μM for inhibition of nicotinic α3β4 receptors. We characterized their overall potency by measuring the percent inhibition at the end of the drug co-application, the rate of inhibition during drug co-application and the amount of persistent inhibition 1 s after drug removal. Percent inhibition and persistent inhibition were calculated using the equation $(I_{control} - I_{drug}/I_{control}) \times 100$ after normalizing to the peak of the control current. Using these measures as a guide, we selected the most promising candidates for further in vitro and in vivo analyses.

For concentration–response analyses, data are presented as the mean ± S.E.M. and curve fits are given for the logistic equation: $I = I_{max}/(1+[drug]/IC_{50})$. The calculated Hill slope (n_H) for each concentration–response curve was near unity in all cases, and therefore was fixed at 1 and the data were refitted as presented.

2.2. Animals

Naïve female Long-Evans derived rats (250 g; Charles River, NY) were maintained on a normal 12-h light cycle (lights on at 7:00 a.m., lights off at 7:00 p.m.). For all experiments, the "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1997) were followed.

2.3. Self-administration procedure

The i.v. 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 i.v. 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. 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 μl infusion of drug solution (0.025 mg of either morphine sulfate or methamphetamine sulfate) in about 1 s. Because all rats generally weighed 250 ± 20 g, each response delivered approximately 0.1 mg/kg of morphine or 0.1 mg/kg of methamphetamine. Experiments to assess the effects of experimental treatments were begun when baseline self-administration rates stabilized (< 10% 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 1 week apart. Due to the limited quantities available, the congeners were all initially tested at only a single dosage (20 mg/kg, i.p., 15 min before testing) in both self-administration models ($N = 3–6$/congener/model); at this dosage, 18-methoxycoronaridine decreases morphine and methamphetamine self-administration by approximately 50% (Glick et al., 1996, 2000a). To the extent available, a few congeners were tested at lower doses to determine if any of them was more potent than 18-methoxycoronaridine. Lastly, in other groups of rats, using exactly the same schedule and protocol used for drug self-administration, several congeners were tested in a control (non-drug) model for drug self-administration: responding for an oral solution of sucrose (15% w/v, 0.01 ml/response) that maintains response rates comparable to those maintained by drugs.

3. Results

3.1. 18-Methoxycoronaridine inhibits nicotinic α3β4 acetylcholine receptors

Human embryonic kidney 293 cells expressing α3 and β4 subunits of nicotinic acetylcholine receptors were voltage-clamped at −70 mV and examined by whole-cell patch clamp recording with fast perfusion of acetylcholine and drug solutions. Application of 1 mM acetylcholine produced a large, inward, desensitizing current (Fig. 2) similar to that described previously. Consistent with our previous studies showing that 18-methoxycoronaridine potently inhibits nicotinic α3β4 receptors (Glick et al., 2002a), co-application of 20 μM 18-methoxycoronaridine produced a rapid and nearly complete (98%, on average) inhibition of the inward currents produced by acetylcholine ($N=9$, Figs. 2A and 3). This inhibition was slow to be relieved after removal of 18-methoxycoronaridine, persisting at 80% after 1 s. The inhibition produced by 18-methoxycoronaridine at nicotinic α3β4 receptors was concentration-dependent with an IC_{50} of 0.90 μM (Fig. 4).

3.2. Effects of (+)- and (−)-18-methoxycoronaridine enantiomers on nicotinic α3β4 acetylcholine receptors

It was previously shown that (+)-18-methoxycoronaridine has 15–25 times higher affinity than (−)-18-methox-

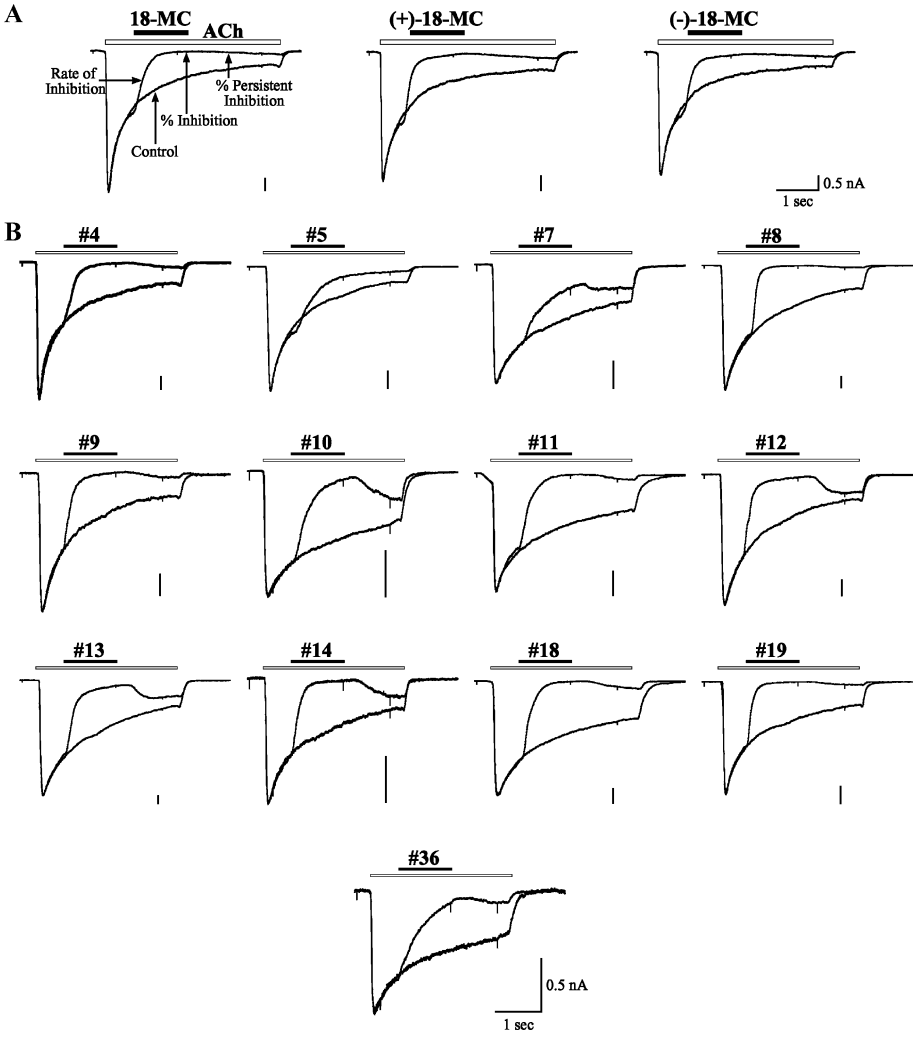

Fig. 2. Inhibition of nicotinic α3β4 receptors by 18-methoxycoronaridine, 18-methoxycoronaridine enantiomers, and 18-methoxycoronaridine congeners. Recombinant receptors were expressed in human embryonic kidney 293 cells and examined by whole-cell patch clamp recordings with rapid application of acetylcholine and drug solutions. (A) Whole-cell current evoked by application of 1 mM acetylcholine in transfected cells was nearly eliminated by co-application of 20 μM 18-methoxycoronaridine (left). Current traces for the control and drug co-application are superimposed. Open bar depicts the timing of acetylcholine application. Solid bar depicts the timing of co-application of 18-methoxycoronaridine. Arrows point to measures presented in Fig. 3. Co-application of 20 μM (+)-18-methoxycoronaridine (middle) or (−)-18-methoxycoronaridine (right) had similar effects on acetylcholine-evoked currents. Horizontal scale bar applies to all traces in A. (B) Inhibition of 1 mM acetylcholine-evoked currents by various 18-methoxycoronaridine congeners (18–20 μM). Conventions are as in A. Horizontal scale bar applies to all traces in B; vertical bars are 0.5 nA for all traces.

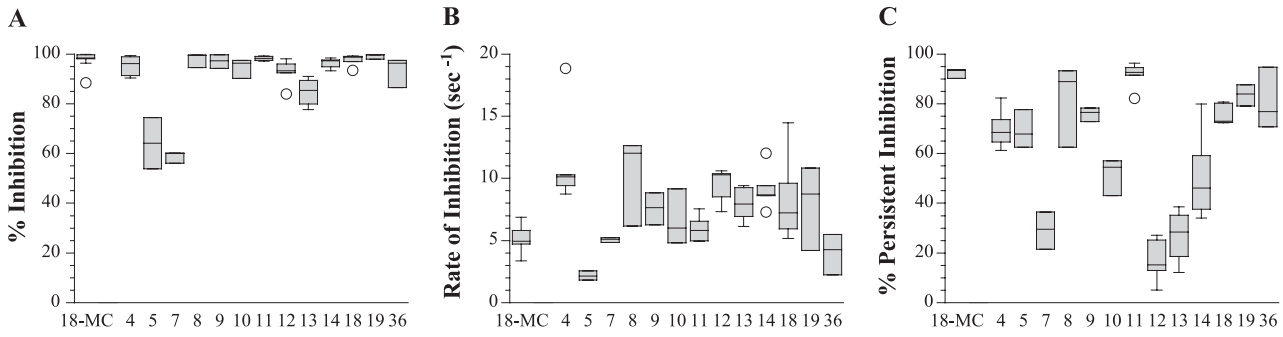

Fig. 3. Effects of application of 18-methoxycoronaridine and 18-methoxycoronaridine congeners on nicotinic α3β4 receptors. For each compound, the box represents the second and third quartile of the data (middle 50%), the horizontal line inside the box represents the median, the horizontal lines above and below the box represent the range excluding outliers, which are represented by open circles. (A) Percent inhibition at the end of the drug co-application; (B) rate of inhibition; (C) percent inhibition at 1 s after the removal of the drug. See Fig. 2A for example of measures, $N=3-8$ observations per compound.

ycoronaridine for μ and δ opioid receptors (King et al., 2000). To determine whether this difference extends to other transmitter systems, we first investigated the 18-methoxycoronaridine enantiomers for inhibition of acetylcholine-evoked currents at nicotinic α3β4 receptors. Like (±)-18-methoxycoronaridine, 20 μM of the (+)- and (−)-enantiomers potently and rapidly inhibited acetylcholine-evoked currents (95% and 93%, respectively, $N=3$ for each; Fig. 2A). This inhibition was somewhat reduced (to 73% and 77%, respectively) at 1 s after removal of the drug. Furthermore, at 1 μM, (+)- and (−)-18-methoxycoronaridine effectively inhibited acetylcholine evoked currents, although to a lesser extent (60% and 55%, respectively, $N=3$ for each) and more slowly. This suggests that their IC_{50} values are slightly below 1 μM and both are similar to that of the racemate. Furthermore, the (+)- and (−)-enantiomers produced similar amounts of persistent inhibition at 1 s after drug removal (45% and 54%, respectively).

3.3. Effects of 18-methoxycoronaridine congeners on nicotinic α3β4 acetylcholine receptors

We next tested a series of 18-methoxycoronaridine congeners having substitutions at C_{16} and C_{18} for inhibition at nicotinic α3β4 receptors (Fig. 1, Table 1; Kuehne et al., 2003). At 20 μM, all 13 compounds tested inhibited the inward currents produced by application of acetylcholine (Fig. 2); however, they exhibited a range of potencies (Fig. 3). Compound numbers 4, 8, 9, 11, 18, 19, and 36 ($N=3-6$ for each) were particularly potent. On average, each of these compounds inhibited acetylcholine-evoked currents by greater than 94% with persistent inhibition of 70% or greater at 1 s after the drug was removed. With the exception of compound #36, which had considerably slower onset, all had mean inhibition rates faster than 18-methoxycoronaridine (Fig. 3). Thus, these compounds interact rapidly with and have high affinity for their active site on the receptors. Compound numbers 10, 12, 13, and 14 ($N=3-6$ for each)

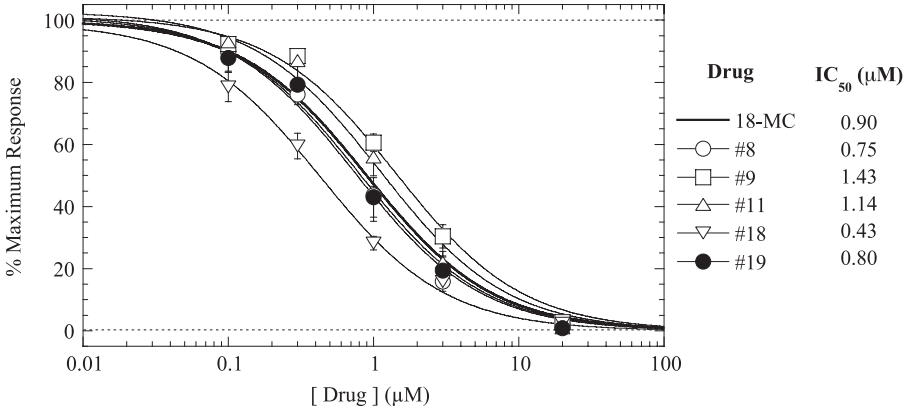

Fig. 4. Inhibition of 1 mM acetylcholine evoked currents by various concentrations of 18-methoxycoronaridine and 18-methoxycoronaridine congeners. Inhibition was measured at the end of the drug pulse (see Fig. 2A). For the lower concentrations (i.e. ≤0.3 μM), the length of the drug co-application was increased from 1.5 to 2.5 s to reach equilibrium. Data are the mean ± S.E.M. for three to nine observations per point. Curve fits are given for the logistic equation: $I=I_{max}/(1+[drug]/IC_{50})$. Best-fitting IC_{50} values are given in the figure for 18-methoxycoronaridine and 0.75, 1.43, 1.14, 0.43, and 0.80 μM for compound numbers 8, 9, 11, 18, and 19, respectively. Hill slopes of the concentration–effect curves were near unity in all cases and were assumed to be 1 for IC_{50} determinations.

inhibited the acetylcholine-evoked currents by 85% or more; however, the persistent inhibition was less than 52% at 1 s after the drug was removed. These compounds had mean inhibition rates that were faster than 18-methoxycoronaridine. Their fast inhibition rates suggest that they bind rapidly to the receptors, but their limited persistent inhibition suggests they also unbind rapidly, and so have relatively lower affinity. Finally, compound numbers 5 and 7 ($N=3$ and 4, respectively) were the least potent; both inhibited the acetylcholine-evoked currents by less than 65%, suggesting they also have relatively lower affinity.

To extend these findings, we explored the concentration–response relationships of the 18-methoxycoronaridine congeners (excluding compound numbers 5, 7, and 12). The inhibition produced by each of the congeners tested was concentration-dependent, showing less inhibition at 1 μM (data not shown). These findings were largely consistent with the affinity profiles described above; compound numbers 10, 12, 13, 14, and 36 were of lower affinity, whereas compound numbers 8, 9, 11, 18, and 19 were of higher affinity. For this latter group of more potent compounds, we carried out further concentration–response experiments. The estimated IC_{50} values for these compounds ranged from 0.43 to 1.43 μM (Fig. 4).

3.4. Effects of 18-methoxycoronaridine congeners on N-methyl-D-aspartate receptors

Another known action of ibogaine is its ability to inhibit NMDA-type glutamate receptor currents (Popik et al., 1995; Chen et al., 1996, Glick et al., 2002a). Unlike 18-methoxycoronaridine, which has no effect on these receptors, 10 μM ibogaine produces >80% inhibition of NMDA receptor currents (Table 2; Glick et al., 2002a). Therefore, we next tested 18-methoxycoronaridine and several congeners for inhibition of NMDA receptors. Human embryonic kidney 293 cells expressing NR1 and NR2B subunits of glutamate receptors were voltage-clamped at −70 mV and stimulated with 100 μM glutamate and 10 μM glycine (glutamate/glycine) in the absence of extracellular Mg^{2+}. Application of glutamate/glycine produced an inward current that only moderately desensitized in the continuous presence of agonist (Fig. 5). Consistent with our previous findings, 18-methoxycoronaridine failed to inhibit the glutamate/glycine-evoked currents at NR1/NR2B receptors at 10 μM ($N=9$; Fig 5; Table 2) or 50 μM ($N=4$). Like 18-methoxycoronaridine, the seven congeners tested (compound numbers 4, 8, 9, 11, 13, 18, and 19) all failed to inhibit NR1/NR2B receptors at 10 μM ($N=3–7$; Fig. 5; Table 2) or at 50 μM ($N=4–6$). The small amount of inhibition produced by some of the compounds is within the range of experimental variability and attributable to artifact.

Table 2
Effects of 18-methoxycoronaridine and 18-methoxycoronaridine congeners on glutamate/glycine-evoked currents at glutamate NR1/NR2B receptors

	Percent inhibition (mean ± S.E.M.)	
	[10 μM]	[50 μM]
Ibogaine[a]	82 ± 3[a]	n.d.
18-MC	2 ± 2	7 ± 5
#4	0 ± 0	0 ± 0
#8	3 ± 2	19 ± 6
#9	2 ± 2	11 ± 3
#11	12 ± 9	7 ± 5
#13	7 ± 4	7 ± 5
#18	0 ± 0	5 ± 4
#19	0 ± 0	16 ± 3

The small amount of inhibition produced by some of the compounds is likely artifact. $N=3–9$. n.d. = not determined.

[a] Values for ibogaine have been added for comparison. Ibogaine data are taken from Glick et al. (2002a) who also showed that 20 μM ibogaine inhibits glutamate NR1/NR2B receptor currents by >90%.

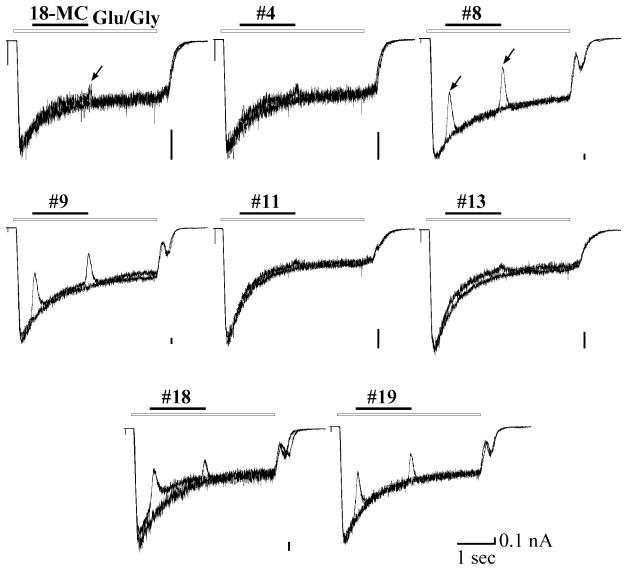

Fig. 5. Lack of inhibition of glutamate NR1/NR2B receptors by 18-methoxycoronaridine and 18-methoxycoronaridine congeners. Recombinant receptors were expressed in human embryonic kidney 293 cells and examined by whole-cell patch clamp recordings with rapid application of 100 μM glutamate/10 μM glycine and 10 μM drug solutions. Like 18-methoxycoronaridine, all of the congeners tested failed to inhibit glutamate NR1/NR2B receptors. Similar results were obtained using 50 μM drug (Table 2). Current traces for the control and drug co-application are superimposed. Open bar depicts the timing of glutamate/glycine application. Solid bar depicts the timing of co-application of drug. Small arrows indicate artifact caused by solution switching. Horizontal scale bar applies to all traces; vertical scale bars are 0.1 nA for all traces.

3.5. 18-Methoxycoronaridine congeners reduce drug self-administration

Table 3 shows the effects of 18-methoxycoronaridine and the congeners tested on morphine and methamphetamine self-administration. With the exception of congener #4 and #13, all the congeners tested retained the ability to reduce both morphine and methamphetamine self-administration. In general, similar to 18-methoxycoronaridine (Glick et al.,

Table 3
Effects of 18-methoxycoronaridine and 18-methoxycoronaridine congeners on morphine, methamphetamine, and sucrose self-administration

	Percent of baseline ([a]mean ± S.E.M.)		
	Morphine	Methamphetamine	Sucrose
Vehicle	102.2 ± 10.6	100.5 ± 12.7	98.2 ± 8.8
18-MC	40.4 ± 6.6[b]	55.4 ± 6.4[b]	88.1 ± 10.8
#4	63.2 ± 8.1[b]	89.8 ± 9.1	111.2 ± 15.6
#8	31.0 ± 10.3[b]	82.0 ± 5.6[b]	91.8 ± 17.1
#10	31.9 ± 9.5[b]	76.9 ± 10.6[b]	89.8 ± 11.9
#11	45.3 ± 6.7[b]	75.1 ± 10.8[b]	96.6 ± 13.5
#13	80.2 ± 8.4[b]	101.7 ± 18.2	108.9 ± 7.1
#18	18.0 ± 9.1[b]	71.8 ± 9.5[b]	115.4 ± 19.5
#19	41.0 ± 13.4[b]	72.6 ± 16.1[b]	n.d.
#36	84.8 ± 10.6	82.2 ± 3.8[b]	118.2 ± 20.5

All compounds were tested at 20 mg/kg, administered i.p. 15 min before a 1-h drug or sucrose self-administration test session.

[a] Baseline mean infusions per hour or sucrose responses per hour = 28.3 ± 1.7, 21.7 ± 1.5, and 43.5 ± 4.2 for morphine, methamphetamine, and sucrose, respectively.

[b] Significantly different from baseline, $P < 0.05–0.01$, paired t test. n.d. = not determined.

1996, 2000a), most of the congeners (#36 being the one exception) were more effective on morphine self-administration than on methamphetamine self-administration. Several congeners (#8, #10, #11, #18, and #19) reduced morphine self-administration to levels that were similar to or better than 18-methoxycoronaridine, whereas none of the congeners were as effective as 18-methoxycoronaridine at reducing methamphetamine self-administration. Interestingly, for the congeners tested, there was a trend relating the inhibitory potency at nicotinic α3β4 receptors and the effects on drug self-administration. Those compounds that were more effective at inhibiting nicotinic α3β4 receptors tended also to be more effective on either morphine or methamphetamine drug self-administration.

The minimally effective dose (i.e., significant effect) of 18-methoxycoronaridine on both morphine and methamphetamine self-administration is 10 mg/kg (Glick et al., 1996, 2000a). In view of the limited quantities available, a selection (#8, #11, #18, and #19) of the congeners were tested at one other dosage, 5 mg/kg, to determine if any of them was clearly more potent than 18-methoxycoronaridine. Only one compound, #11, was significantly effective: It reduced morphine self-administration to 49.2 ± 6.6% of baseline ($N = 4$; $P < 0.01$, t test) and methamphetamine self-administration to 80.7 ± 8.4% of baseline ($N = 3$; $P < 0.05$, t test).

Lastly, 18-methoxycoronaridine and compound numbers 4, 8, 10, 11, 13, 18, and 36 were tested at 20 mg/kg for their effects on responding for sucrose. These compounds are representative of the range of inhibitory potency at nicotinic α3β4 receptors and drug self-administration. None of these compounds had significant effects in this model, indicating that the congeners selectively decrease drug vs. non-drug self-administration (Table 3).

4. Discussion

We show that 18-methoxycoronaridine and 18-methoxycoronaridine congeners effectively inhibit nicotinic α3β4 acetylcholine receptors, yet the congeners tested were ineffective at NMDA-type glutamate receptors. This extends our previous findings that 18-methoxycoronaridine and, to a lesser extent, ibogaine are selective for nicotinic α3β4 receptors (Glick et al., 2002a). Previously, we showed that ibogaine and 18-methoxycoronaridine inhibit nicotinic α3β4 receptors in the nanomolar range ($IC_{50} \sim 0.2$ and 0.8 μM, respectively) and block serotonin 5-HT_3 receptors but with considerably lower potency ($IC_{50} \sim 20$ μM). Ibogaine also inhibits nicotinic α4β2 receptors ($IC_{50} = 1–5$ μM) and NMDA receptors ($IC_{50} = 1–10$ μM), whereas 18-methoxycoronaridine has little effect on either of these receptor types but retains anti-addiction properties. These data lead us to propose that inhibition of nicotinic α3β4 receptors might be the primary mechanism by which 18-methoxycoronaridine and ibogaine decrease drug-seeking behavior in rats (Glick et al., 2002a).

Also consistent with this idea, we found that (±)-18-methoxycoronaridine and the (+)- and (−)-18-methoxycoronaridine enantiomers are equipotent at inhibiting nicotinic α3β4 receptors. These data are consistent with our hypothesis because the ability of 18-methoxycoronaridine to inhibit drug self-administration is, likewise, not stereoselective (King et al., 2000). In contrast, (+)-18-methoxycoronaridine binds with nearly 20-fold higher affinity than (−)-18-methoxycoronaridine to both μ and δ opioid receptors (King et al., 2000), suggesting these receptors are not involved in the anti-addiction actions of 18-methoxycoronaridine. On the other hand, (±)-, (+)- and (−)-18-methoxycoronaridine have similar affinities at κ opioid (King et al., 2000), and inhibition of κ opioid receptors has been proposed to be involved in the action of ibogaine (Pearl et al., 1995; Glick et al., 1997). However, the ability of 18-methoxycoronaridine and 18-methoxycoronaridine congeners to inhibit binding at κ opioid receptors, as reported by Kuehne et al. (2003), shows no clear relationship to the effects of these compounds on the self-administration of morphine or methamphetamine reported here. This suggests that κ opioid receptors can account for, at most, only part of the action of 18-methoxycoronaridine and 18-methoxycoronaridine congeners.

In this series of compounds, we found five congeners that were about as potent as 18-methoxycoronaridine at blocking nicotinic α3β4 receptors (IC_{50} values were 0.4–1.4 μM), whereas these congeners had no effect at NMDA receptors at up to 50 μM. In other experiments, we found that the ability of most of the 18-methoxycoronaridine congeners to inhibit binding to μ, κ, and δ opioid receptors was similar to 18-methoxycoronaridine, having affinities in the low micromolar range (Glick et al., 1996; Kuehne et al., 2003). We acknowledge that there are other receptor types, even of the nicotinic class, that may participate in the actions of these compounds but have not been tested.

Experiments are in progress to test some of these candidate receptor types.

We also report here that the congeners maintained their ability to reduce morphine and methamphetamine self-administration in rats. Interestingly, there was a trend to correlation between the inhibitory effects of the congeners at nicotinic α3β4 receptors and the effects on drug self-administration. The most potent compounds in vitro were also more effective at reducing drug self-administration, but with some differences between morphine and methamphetamine. These findings are particularly noteworthy given that only the most potent compounds were tested in vivo and that we know little regarding their pharmacokinetics. We acknowledge that our in vivo assessment of the potency of the 18-methoxycoronaridine congeners is limited because, due to limiting quantities of the compounds, we were unable to generate complete dose–effect curves. However, our current findings support the hypothesis of nicotinic α3β4 receptor involvement in drug reward and are consistent with earlier reports that combinations of agents with only one common known mechanism of action, that being α3β4 antagonism (mecamylamine, Papke et al., 2001; dextromethorphan, Hernandez et al., 2000; bupropion, Fryer and Lukas, 1999), decrease the self-administration of several addictive drugs. Thus, at doses that are normally ineffective when administered alone, 18-methoxycoronaridine combined with mecamylamine or with dextromethorphan reduce the self-administration of morphine and methamphetamine (Glick et al., 2002a). Likewise, 18-methoxycoronaridine combined with mecamylamine, dextromethorphan, or bupropion reduce the self-administration of nicotine (Glick et al., 2002b). We proposed that the reduced drug self-administration in these studies was due to the combined action of the various nicotinic α3β4 receptor antagonists. Thus, combining low doses of 18-methoxycoronaridine, 18-methoxycoronaridine congeners, and/or other nicotinic α3β4 receptor antagonists may be an alternative treatment for drug addiction. Although it is difficult to extend the findings of the present study to addiction in humans, we should note that ibogaine and 18-methoxycoronaridine are roughly as potent at inhibiting recombinant human nicotinic α3β4 receptors as they are at the rat receptors (unpublished observations).

Clearly the common and likely critical target of ibogaine, 18-methoxycoronaridine, and 18-methoxycoronaridine congeners are nicotinic α3β4 receptors. Yet, little is known regarding their mechanism of action at these receptors. We propose that these compounds act as non-competitive, allosteric modulators to inhibit the bound receptor by promoting the desensitized state. Preliminary data indicate that inhibition of nicotinic α3β4 receptors by ibogaine, 18-methoxycoronaridine, and several of the congeners is voltage-independent and does not depend on the concentration of acetylcholine, suggesting they are neither open-channel blockers nor competitive antagonists. This is consistent with previous reports of the noncompetitive action of ibogaine at nicotinic receptors (Badio et al., 1997; Fryer and Lukas, 1999). Experiments are ongoing to discern the mechanism of action of ibogaine, 18-methoxycoronaridine, and 18-methoxycoronaridine congeners at nicotinic α3β4 receptors.

The ability of the 18-methoxycoronaridine congeners to inhibit nicotinic α3β4 receptors and reduce drug self-administration support the hypothesis that antagonism of nicotinic α3β4 receptors is a potential mechanism to modulate drug-seeking behavior. However, this may not directly involve the dopaminergic mesolimbic pathway implicated in drug dependence. Although low densities of nicotinic α3β4 receptors reside in the dopaminergic nuclei of the ventral tegmental area (Klink et al., 2001), central nicotinic α3β4 receptors are mainly located in the medial habenula and the interpeduncular nucleus (e.g., Klink et al., 2001; Quick et al., 1999). The interpeduncular nucleus receives its main input from the medial habenula, and there are multiple avenues for interaction between this habenulointerpeduncular pathway and the mesolimbic system (see Maisonneuve and Glick, 2003). Thus, for example, the medial habenula receives input from the nucleus accumbens and sends efferents to the ventral tegmental area. The interpeduncular nucleus also sends efferent connections to the brain stem raphe nuclei and the medial dorsal thalamic nucleus, both of which, directly or indirectly, signal to the ventral tegmental area. Functional interactions between the habenulointerpeduncular and mesolimbic pathways have in fact been demonstrated (Nishikawa et al., 1986). Still, the action of these drugs has yet to be localized.

In summary, several *iboga* alkaloid congeners were found to both block recombinant nicotinic α3β4 acetylcholine receptors in human embryonic kidney 293 cells and decrease drug self-administration in rats. The consistent effects in vitro and in vivo support our hypothesis that nicotinic α3β4 receptors may be a novel and viable target for treating multiple forms of drug addiction.

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