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Chain-Lengthened and Imidazoline Analogues of Nicotine

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Abstract—Analogues of nicotine (**1**) and azanicotine (**3**) were prepared with an additional methylene group inserted between the two rings (i.e., homonicotine and homoazanicotine; **6** and **5**, respectively). Although **6** ($K_i = 3110$ nM) and **3** ($K_i = 206$ nM) bind at nACh receptors with ≥100-fold lower affinity than nicotine ($K_i = 2.1$ nM), **5** displays high affinity ($K_i = 7.8$ nM). Like nicotine ($ED_{50} = 12$ μg/mouse), both **3** and **5** ($ED_{50} = 21$ and 19 μg/mouse, respectively) produced antinociceptive activity in the tail-flick assay following intrathecal administration. The antinociceptive actions of **3** and **5**, unlike those of nicotine, were not antagonized by mecamylamine. Compounds **3** and **5** might represent novel analgesic agents that act via a non-nicotinic mechanism, or via a nicotinic mechanism that is distinct from that mediating the antinociceptive actions of nicotine.

(−)-Nicotine (**1**) produces some of its effects via interaction with nicotinic acetylcholinergic (nACh) receptors in the brain.[1,2] Of late, nicotine research has gained popularity because several different subpopulations of nACh receptors have been identified, and because there has been a realization that nicotinic agents lacking the deleterious side effects of nicotine (**1**) might be useful in the management or treatment of anxiety, pain, appetite, schizophrenia, and certain neurological disorders.[1] Neuronal nACh receptors are primarily of two types, $\alpha_4\beta_2$ receptors and α_7 receptors. Nicotine binds with low affinity at the latter but binds with high affinity (i.e., $K_i = 1$–5 nM) at the $\alpha_4\beta_2$-type receptors.[1,2]

There have been several attempts to identify a 'nicotinic pharmacophore' and the most widely accepted is that proposed by Sheridan and co-workers (reviewed[3]). The exact bioactive conformation of nicotine is unknown. However, a feature of the pharmacophore is that nicotinic ligands should possess both an onium group (e.g., as represented by the pyrrolidine nitrogen atom in nicotine) and a hydrogen bond acceptor (e.g., as represented by the pyridine nitrogen atom) situated about 4.8 Å from one another. Of course it is possible that different pharmacophores eventually will be identified for various subpopulations of nACh receptors,[3] and although there has been some controversy regarding optimal pharmacophoric distances (e.g., see ref 2 for discussion), the Sheridan pharmacophore seems to apply to the $\alpha_4\beta_2$ receptors with respect to structures that are similar to that of nicotine.

The structure of nicotine has been modified in an attempt to identify the minimal structural requirements for binding. For example, **1** can be abbreviated to the aminomethylpyridine derivative **2** ($K_i = 28$ nM) with only about a 10-fold reduction in affinity.[4] In contrast, introduction of substituents at the 3′-, 4′-, or 5′-position of nicotine typically results in a significant reduction in affinity,[5] and limits the extent of structural modification that can be explored in the pyrrolidine ring. However, it might be possible to replace the pyrrolidine portion of nicotine with an imidazoline moiety (e.g., **3**). The inter-nitrogen (i.e., pyridine nitrogen to imidazoline *N*-methyl nitrogen) distance should approximate that found in nicotine. Because the substituted nitrogen of **3** is now in conjugation with the aromatic ring, the basicity of the ring amino group would likely be reduced relative to that found in nicotine. However, it has yet to be demonstrated that base strength is related to affinity.[2] Furthermore, introduction of a second nitrogen atom, in the form of an imidazoline, would allow delocalization of the charge over a greater span of atoms, and might offer additional possibilities for binding. Hence, azanicotine (**3**) was prepared for evaluation.

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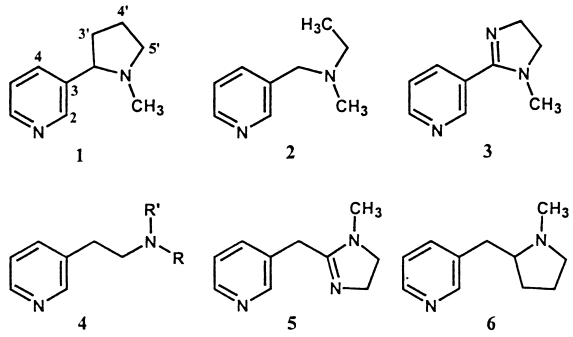

In a departure from what might have been expected, we recently found that chain-lengthening in the aminomethylpyridine series results in retention of affinity.[6] For example, compound **4** binds with an affinity ($K_i = 18$ nM where R = -CH_3 and R′ = -C_2H_5) comparable to that of the corresponding aminomethylpyridine **2**. A chain-lengthened homologue of **3** (i.e., **5**) would reduce the possibility of conjugation associated with **3**. Consequently, compounds **3** and **5** were prepared for evaluation. Compound **6**, homonicotine, a chain-lengthened analogue of nicotine, was also prepared for purposes of comparison with **5** and nicotine.

Chemistry

Homonicotine (**6**) was prepared by the method of Secor and Seeman[7] and characterized as its fumarate salt. Azanicotine (**3**) was obtained in 38% yield by reaction of *N*-(methyl)ethylenediamine with trimethylaluminum followed by the addition of methyl nicotinate. Homoazanicotine (**5**) was prepared in 42% yield in the same manner using ethyl 3-pyridylacetate in place of methyl nicotinate.

Results

Table 1 shows the nACh receptor affinities of the compounds investigated in the present study. Azanicotine (**3**; $K_i = 206$ nM) binds with 100-fold lower affinity than (−)-nicotine ($K_i = 2.1$ nM). Unlike what was seen upon chain-lengthening of **2**, the chain-extended analogue of nicotine, homonicotine (**6**; $K_i = 3110$ nM), binds with significantly (1500-fold) decreased affinity compared to (−)-nicotine. Interestingly, the chain-extended analogue of azanicotine (i.e., homoazanicotine, **5**; $K_i = 7.8$ nM) also does not behave like **2**; indeed, **5** binds with about 25-fold higher affinity than azanicotine (**3**), and with an affinity in the range of (−)-nicotine.

Table 1. nACh receptor affinities and antinociceptive potencies of compounds examined

Compound	nACh receptor affinity K_i, nM (±SEM)	Antinociceptive potency (ED_{50}, μg/mouse; 95% CL)
(−)-Nicotine (**1**)	2.1 (±0.8)	12 (8–16)
Azanicotine (**3**)	206 (±5)	21 (19–22)
(±)-Homonicotine (**6**)	3110 (±140)	>100[a]
Homoazanicotine (**5**)	7.8 (±1.0)	19 (18–21)

[a]Produced only 4 (±2)% of the maximal possible effect at this dose.

Nicotine produces an antinociceptive effect in mice. The antinociceptive actions of compounds **3**, **5**, and **6** were examined and compared with that of (−)-nicotine (**1**). Like nicotine (Table 1), compounds **3** and **5** were active (ED_{50} values = 21 and 19 μg/mouse) when administered via the intrathecal route. Compound **6** was inactive in this assay and produced only 4% of the maximal possible nicotine effect at a dose of 100 μg/mouse. Compound **6** was also examined for its ability to antagonize the activity of nicotine but failed to produce an antagonist effect at doses of up to 100 μg/mouse. Although both **3** and **5** produced antinociceptive effects, unlike what was observed with nicotine these effects were not antagonized by pretreatment of the animals with 1 mg/kg of mecamylamine.

Discussion

It was expected that homonicotine (**6**) and homoazanicotine (**5**) might bind with affinities comparable to that of (−)-nicotine (**1**) and azanicotine (**3**). This was not found to be the case; homonicotine binds with 1500-fold lower affinity than nicotine, whereas homoazanicotine binds with 25-fold higher affinity than azanicotine. On the basis that parallel structural modification of **1** and **3** does not result in a parallel shift in affinity, it seems that these compounds are not binding in the same manner at nACh receptors. Nicotine produces an antinociceptive effect in mice following intrathecal administration. Likewise, compounds **3** and **5** produced this effect (Table 1) and all three compounds were similar in potency despite their 100-fold spread in nACh receptor affinity. Interestingly, however, the nicotinic antagonist mecamylamine antagonized the antinociceptive actions of nicotine, but not those of **3** or **5**, indicating possible differences in mechanism of action.

The present findings indicate that homonicotine (**6**) lacks the high nACh receptor affinity and antinociceptive potency of nicotine. Azanicotine (**3**) binds with about 100-fold lower affinity than nicotine but, like nicotine, retains antinociceptive actions. Homoazanicotine (**5**) retains both the nACh receptor affinity and antinociceptive potency of nicotine. But, whereas the antinociceptive actions of nicotine are antagonized by mecamylamine, those of **3** and **5** are not. It not only seems that these compounds are binding at nACh receptors in a different manner, but the similar antinociceptive potencies of **1**, **3**, and **5**, coupled with the mecamylamine-insensitive actions of the latter two compounds, suggest that the compounds are producing their antinociceptive effects via different mechanisms. It remains to be determined if **3** and **5** act via a non-nicotinic mechanism or via a nicotinic mechanism that is different than that mediating the antinociceptive effects of nicotine. Such studies will be undertaken in the future. In any event, compounds **3** and **5** represent novel antinociceptive agents that are worthy of further investigation.

Experimental

Chemistry

Melting points were taken in glass capillary tubes on a Buchi SMP-20 or Thomas Hoover melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded with a Varian EM-390 spectrometer, and peak positions are given in parts per million (δ) downfield from tetramethylsilane as the internal standard. The microanalyses were performed in the Microanalytical Laboratory of the University of Camerino, or by Atlantic Microlab and the results are within 0.4% of theory. Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.040–0.063 mm, Merck) by flash chromatography. Reactions and product mixtures were routinely monitored by thin-layer chromatography (TLC) on precoated F_{254} Merck silica gel plates.

3-(1-Methyl-4,5-dihydro-1*H*-2-imidazolyl)pyridine or azanicotine oxalate (3)

A solution of *N*-(methyl)ethylenediamine (0.98 mL, 11.03 mmol) in dry toluene (6.5 mL) was added in a dropwise manner to a vigorously stirred solution of 2 M trimethylaluminum (11.04 mL, 22.07 mmol) in dry toluene (18.26 mL) at 0 °C under an inert (N_2) atmosphere. The reaction mixture was stirred at room temperature for 1 h, cooled to 0 °C, and a solution of methyl nicotinate (1.51 g, 11.03 mmol) in dry toluene (9.1 mL) was added in a dropwise manner. The reaction mixture was heated at reflux for 1.5 h, cooled to 0 °C, and cautiously quenched by the dropwise addition of MeOH (4.06 mL), followed by water (0.8 mL). After addition of $CHCl_3$ (32 mL), the mixture was heated at reflux for 1 h at 70 °C to ensure the precipitation of the aluminum salts. The mixture was dried (Na_2SO_4) and the organic portion was evaporated in vacuo to give a light-yellow oil which was purified on a silica gel column by eluting with EtOAc:EtOH (abs):NH_4OH 28% (7:3:0.1). The free base was converted to the oxalate salt and recrystallized from 2-PrOH/absolute EtOH to yield 1.18 g (38%) of **3**; mp 83–85 °C. ^{1}H NMR (DMSO-d_6) δ 3.10 (s, 3H, N–CH_3), 3.90–4.20 (m, 4H, NCH_2CH_2N), 7.65–8.95 (m, 4H, ArH), 10.30 (broad s, 1H, NH). Anal. ($C_9H_{11}N_3 \cdot 1.5C_2H_2O_4$) C, H, N.

3-[(1-Methyl-4,5-dihydro-1*H*-2-imidazolyl)methylene]-pyridine or homoazanicotine oxalate (5)

The same procedure used for the preparation of **3** was followed except that the methyl nicotinate was replaced by ethyl 3-pyridylacetate (1.82 g, 11.03 mmol). The crude oily product was purified by silica gel column chromatography by eluting with EtOAc:EtOH:NH_4OH 28% (6:4:0.3). The product was converted to its oxalate salt and recrystallized from absolute EtOH to yield 1.56 g (42%) of **5**; mp 154–156 °C. ^{1}H NMR (DMSO-d_6) δ 3.10 (s, 3H, N–CH_3), 3.70–4.00 (m, 4H, NCH_2CH_2N), 4.10 (s, 2H, CH_2–C=N), 7.40–8.65 (m, 4H, ArH), 10.30 (broad s, 1H, NH). Anal. ($C_{10}H_{13}N_3 \cdot 2C_2H_2O_4$) C, H, N.

(±)-1-Methyl-2-(3-pyridylmethyl)pyrrolidine or homonicotine fumarate (6)

Homonicotine was prepared according to the method of Secor and Seeman.[7] Because it was originally characterized as a picrate complex, for the purpose of the present investigation the free base was converted to its water-soluble fumarate salt; mp 108–110 °C after recrystallization from a mixture of absolute EtOH/anhydrous Et_2O. Anal. ($C_{11}H_{16}N_2 \cdot 1.5C_4H_4O_4$) C, H, N.

Radioligand binding assay

The binding assay was conducted as previously described.[6] In brief, rat brain without cerebellum was homogenized in 10 volumes of ice-cold 0.05 M Na–K phosphate buffer (pH 7.4) and centrifuged at 17,000 *g* (4 °C) for 30 min. The pellet was resuspended in 20 volumes of ice-cold glass-distilled H_2O and allowed to incubate on ice for 60 min before centrifugation as described above. The resulting pellet was resuspended to a final tissue concentration of 40 mg/mL of buffer. [^{3}H]Nicotine was incubated with 0.5 mL of tissue homogenate in a final incubation volume of 1 mL for 2 h at 0 °C. The samples were rapidly filtered through Whatman GF/C filters. Specific binding was defined as the difference in the amount of binding in the presence and absence of 100 μM (−)-nicotine tartrate. Following three consecutive washes with ice-cold buffer the filters were allowed to air-dry and were placed in scintillation vials for quantitation of radioactivity. Following transformation of the data by the Scatchard method, the K_D and B_{max} values were determined using the program LIGAND. Displacement of tritiated nicotine binding at 1 nM was determined in the presence of increasing concentrations of various ligands and converted to percent displacement of specific binding. The IC_{50} values were determined from a plot of log concentration versus percent displacement and converted to K_i values by the method of Cheng and Prusoff. All K_i values represent a minimum of triplicate determinations.

Analgesic activity

The procedure used was the tail-flick method of D'Amour and Smith[8] as modified by Dewey et al.[9] A control response (2–4 s) was determined for each animal before treatment and a test of latency was determined after drug administration. A maximum latency of 10 s was used to avoid tissue damage. The antinociceptive response was calculated as percent maximal possible effect (%MPE) where %MPE = [(test−control)/(10−control) × 100. Groups of 8–12 male ICR mice (20–25 g; Harlan Laboratories, Indianapolis, IN) were used to examine each dose of compound. Agents were administered via intrathecal injections performed free-hand between the L5 and L6 lumbar space in unanesthetized animals according to the method of Hylden and Wilcox.[10] The injection was performed using a 30-gauge needle attached to a glass microsyringe. An injection volume of 5 μL was used and animals were tested 5 min post injection. We have previously described these techniques in further detail.[11]

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