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Quaternary Ammonium 3-(Aminoethoxy)pyridines as Antinociceptive Agents

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Abstract—Quaternization via *N*-methylation of the terminal amines of a series of 3-(dialkylaminoethoxy)pyridines resulted in analogues that displayed up to 50–60-fold enhanced affinity for nicotinic acetylcholinergic (nACh) receptors. Several of these compounds displayed antinociceptive properties in mice using the tail-flick assay and serve as possible leads for the development of novel analgesic agents.

There are several therapeutic approaches for the treatment of pain,[1] and chief among them are agents that bind at opioid receptors and inhibitors of cyclooxygenase.[2] Both general classes of agents have undesirable side effects associated with their use,[2] and this has prompted a search for mechanistically different analgesic agents.[1] Nicotinic acetylcholinergic (nACh) mechanisms, although not yet well investigated, also seem to play a role in analgesia.[2–6] Nicotine itself possesses analgesic activity (e.g., refs 3 and 4) and epibatidine, a naturally occurring nicotinic agonist, is about 200 times more potent than morphine as an antinociceptive agent in animals.[7] But nACh receptor ligands are not without their own unique shortcomings; that is, certain nicotinic agents have been shown to produce, for example, hypothermia, ataxia, and seizures, and nicotine itself is associated with dependence.[2,8] nACh receptors are composed of several types or subpopulations and evidence suggests that different subpopulations of nACh receptors might be related to different pharmacological actions;[5] however, this issue is far from being resolved. Nevertheless, it is possible that agents that bind selectively at the different subpopulations might display somewhat different pharmacology, and attempts are now directed toward identifying more selective nicotinic ligands. In any event, one population of receptors that seems involved with antinociception are the α4β2 nACh receptors.[5]

Many of the actions of nicotine are centrally mediated, and this is likely very true of its dependence producing properties. The possibility exists, then, that the central actions and abuse liability of nicotinic agents might be lessened if an agent is less brain penetrant. Quaternization (via *N*-methylation) of the pyrrolidine nitrogen atom of nicotine doubles its affinity for α4β2 nACh receptors, whereas quaternization of the pyridine nitrogen dramatically decreases or abolishes affinity.[9,10] In theory, then, quaternization of the more basic amine of a nicotinic ligand could result in an agent that retains analgesic properties but in one that possesses reduced ability to penetrate the blood–brain barrier, and might afford a peripherally-acting analgesic with reduced side effects. This approach has not been previously explored.

In the course of our investigations we identified compound **1** as a novel nACh receptor agonist.[11] Compound **1** ($K_i = 21$ nM)[11] binds at α4β2 nACh receptors with an affinity several-fold lower than that of nicotine. It was expected that quaternization of **1** might result in enhanced affinity and in a compound that would not readily penetrate the blood–brain barrier. Consequently, we prepared compound **2**. The nature of amine substitution and the introduction of halogen at the pyridine ring of nicotine has an impact on nACh receptor affinity and agonist action; that is, an *N*-methyl group

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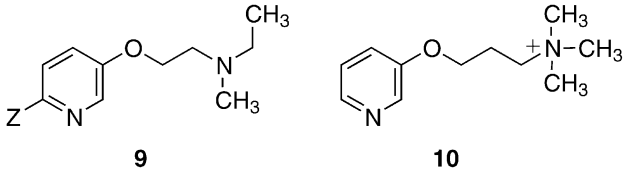
seems optimal and introduction of a 6-chloro or bromo group increases affinity by up to several-fold and can increase agonist potency by up to 15-fold.[12,13] However, for analogues of **1**, an *N*-ethyl substituent is optimal.[14] Consequently, we prepared and examined compounds **3**–**5** where the *N*-substituent was varied, and **6** and **7** where halogen was introduced. Compound **8**, which bears an electron donating group, was examined for comparison. Compound **10** represents a chain-extended analogue of **2** where an additional methylene group was inserted into the alkyl chain.

The compounds prepared for this investigation are shown in Table 1. nACh receptor binding data were obtained for all targets, and selected compounds were examined for their antinociceptive properties using the mouse tail-flick assay (Table 1).

Radioligand Binding

The affinity of **1** was re-determined and found to be consistent with the previously reported value (Table 1). *N*-Monomethylation of **1** (i.e., **2**, *N*,*N*-dimethyl AXP-Q; $K_i = 0.5$ nM) resulted in nearly a 50-fold increase in affinity. This was unexpected given that *N*-monomethylation of nicotine-related analogues typically results in, at best, only a several-fold increase in affinity. *N*-Monomethylation of the *N*-ethyl homologue of **1** (**9**, Z = H; $K_i = 22$ nM)[11] resulted in a 10-fold increase in affinity (i.e., **3**; $K_i = 2.2$ nM), whereas methylation of its 1-*n*-propyl homologue ($K_i = 138$ nM)[11] increased affinity only by about 5-fold (i.e., **5**; $K_i = 29.2$ nM). The size of the R substituent (see Table 1) influences affinity in that as size is increased, affinity decreases. This effect might be more specifically related to substituent length because the isopropyl derivative **4** ($K_i = 13.8$ nM) binds with twice the affinity of **5**.

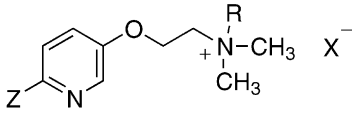


The 6-choro derivative of **1**-homologue **9** (where Z = Cl) has not been reported. Nevertheless, 6-chloro compound **6** ($K_i = 1.3$ nM) binds only with half the affinity of **2**. Bromo derivative **7** ($K_i = 5.0$ nM) binds with half the affinity of quaternary parent amine **3**. In the case of **7**, the effect of quaternization could be determined because the corresponding tertiary amine is known; tertiary amine **9**, where Z = -Br, binds only with modest affinity ($K_i = 290$ nM).[14] Hence, quaternization to **7** represents about a 60-fold increase in affinity. This is the second instance, then, where quaternization by *N*-methylation resulted in unusually enhanced affinity. The 6-methoxy analogue **8** ($K_i = 37$ nM) displayed reduced affinity relative to **2**.

To determine whether affinity is related to chain length, or perhaps simply to the presence of a quaternary amine function, the chain-extended analogue of **2** (i.e., **10**) was examined. The >20,000-fold decrease in affinity shown by **10** ($K_i > 10{,}000$ nM) versus **2** indicated that binding is highly dependent on the length of the aminoethoxy chain and is not due solely to the presence of a charged amine species.

Although it is not yet known exactly how nicotine binds at nACh receptors, it has been proposed that the basic

Table 1. Physicochemical, nACh receptor binding characteristics, and antinociceptive properties of compounds studied[15]



	R	Z	X	Mp (°C)	% Yield	Empirical formula[a]	K_i (nM) (±SEM)[16]		ED_{50}, mg/kg or % MPE;[g] sc		ED_{50} µg/mouse or % MPE;[h] it	
1	H	H	Ox[b]	—	—	—	24.0	(2.4)	15	(12–18)	—	—
2	Me	H	Cl	167–170	51	$C_{10}H_{17}N_2O$[c]	0.5	(0.1)	1.4	(0.9–2.2)	19	(16–23)
3	Et	H	Cl	80–86	44	$C_{11}H_{19}ClN_2O$[d]	2.2	(0.2)	5%	[20]	14	(10-19)
4	*i*Pr	H	Cl	126–127	10	$C_{12}H_{21}ClN_2O$[e]	13.8	(2.1)	3%	[15]	2%	[20]
5	*n*Pr	H	Cl	118–122	22	$C_{12}H_{21}ClN_2O$[e]	29.2	(2.7)	1%	[15]	3%	[20]
6	Me	Cl	I	245–248	60	$C_{10}H_{16}ClN_2O$	1.3	(0.1)	5%	[10]	4%	[15]
7	Et	Br	I	145–146	93	$C_{10}H_{18}BrIN_2O$	5.0	(1.8)	0%	[10]	0%	[25]
8	Me	OMe	Cl	165–167	23	$C_{11}H_{19}ClN_2O_2$[f]	37.0	(2.2)	—	—	—	—
	(−)Nicotine						2.4[i]		1.5	(0.9–1.7)	12	(10–18)

[a]Compounds were washed with dry acetone (i.e., **2**) or anhydrous Et_2O prior to microanalysis, and analyzed within 0.4% of theory for C, H, and N.
[b]Compound **1** was evaluated as its oxalate salt and its synthesis was previously reported.[11]
[c]Crystallized with 0.25 mol H_2O.
[d]Crystallized with 2.75 mol H_2O.
[e]Crystallized with 1.75 mol H_2O.
[f]Crystallized with 1 mol H_2O.
[g]ED_{50}, mg/kg (95% CL) or % MPE [highest dose tested] following subcutaneous (sc) administration.[17]
[h]ED_{50} µg/mouse (95% CL) or % MPE [highest dose tested] following intrathecal (it) administration.[17]
[i]K_i value, determined under similar assay conditions, from Dukat et al.[18]

amine participates in a cation–π interaction with aromatic amino acid residues of α and/or β subunits.[19–21] As such, it might be expected that quaternized nicotinic ligands, exemplified by acetylcholine itself, should bind. Indeed, *N*-methylnicotine binds at α4β2 receptors with an affinity at least comparable to that of nicotine[9] and functions as an agonist with potency similar to nicotine in electrophysiological studies.[20] While the manner of binding of the present compounds relative to nicotine at α4β2 receptors has yet to be defined, compounds such as **2** ($K_i = 0.5$ nM) bind with an affinity similar to that of (−)nicotine ($K_i \approx 2$ nM) and it is not unlikely, being quaternary amines, that they too might participate in such cation–π interactions. Of particular interest is that Beene et al.,[20] have found that cation–π interactions at nACh receptors are quite sensitive to variations in the structure at the cationic center. Hence, the progressive decrease in affinity seen with the present quaternary amines bearing increasingly bulky or longer *N*-alkyl substituents (i.e., comparing **2**–**5**) might simply reflect the lack of steric tolerance by the receptor (e.g., in forming such cation-π interactions) for ligands possessing larger amine substituents.

A selectivity profile was obtained for **2** at 75 populations of receptors and transporters (Cerep; Celle l'Evesca It, France); **2** displayed <20% inhibition at a concentration of 10,000 nM at all receptors tested (including m_1–m_5 muscarinic cholinergic, 5-HT_3 serotonin, and all opioid receptors). As such, **2** is uniquely selective for nACh receptors.

Antinociceptive Activity

Administered subcutaneously, compound **1** ($ED_{50} = 15$ mg/kg; Table 1) produced an antinociceptive effect in mice as measured using the tail-flick assay, and is one-tenth as potent as (−)nicotine ($ED_{50} = 1.5$ mg/kg). The *N*-methyl quaternary amine counterpart of **1** (i.e., **2**; $ED_{50} = 1.4$ mg/kg) was essentially equipotent with (−)nicotine. The antinociceptive effects of **2** and (−)nicotine were antagonized by the nACh antagonist mecamylamine (data not shown). The remaining quaternary amines, **3**–**7**, failed to elicit >5% of the maximal possible effect (MPE) at doses up to 10–20 mg/kg.

Due to the decreased ability of quaternary amines to penetrate the blood–brain barrier, compounds **2**–**7** were also examined following intrathecal administration. Compounds **2** and **3** were active via this route ($ED_{50} = 19$ and 14 μg/mouse, respectively) and were similar in potency to (−)nicotine ($ED_{50} = 12$ μg/mouse; Table 1). Quaternary amines **4**–**7** failed to produce >5% MPE. Because the latter compounds bind at nACh receptors but lacked antinociceptive properties, they were examined as possible antagonists of (−)nicotine-induced antinociception. Although compounds **4**, **6**, and **7** did not block the antinociceptive actions of (−)nicotine at doses of up to 15–25 mg/kg, the *n*-propyl derivative **5** ($ED_{50} = 5.5$ mg/kg; 95% CL = 1–30 mg/kg) antagonized the effect of (−)nicotine in a dose-dependent fashion (data not shown).

Summary

On the basis of these results, optimal affinity with the quaternized aminoethoxypyridines is apparently associated with the structurally simplest member of the series (i.e., **2**), and as the size of the R group (Table 1) increases, affinity progressively decreases. It is speculated that this effect might reflect greater steric hindrance in the ability of the quaternary amines to participate in a cation–π interaction due to the increased size of the amine substituents. Furthermore, when compared with their corresponding tertiary amines, *N*-methylation results in a progressively diminishing affinity-enhancing effect as the size of R is increased in length. Compounds **2** and **7** both displayed greater nACh receptor affinity than might have been expected from what is known about the quaternization of nicotine. Some of the compounds displayed antinociceptive actions. Compound **2**, in particular, was similar in potency to (−)nicotine in producing antinociceptive effects in mice. It remains to be determined whether this effect is peripherally mediated, or whether **2** can penetrate the blood–brain barrier. For example, **3** displayed antinociceptive character when administered via the intrathecal route but not following subcutaneous administration. Also, compound **5** lacked antinociceptive activity but, when administered subcutaneously, antagonized the actions of (−)nicotine and might represent a novel type of nACh antagonist. The binding of these quaternary amines at other nACh receptor populations also remains to be explored. Nevertheless, the present investigation indicates that quaternary amine analogues of nicotinic ligands can retain the antinociceptive properties of their tertiary amine counterparts and suggests that this approach might prove effective for the development of novel analgesics.

Acknowledgements

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15. The synthesis of **1** was reported.[11] Compounds **2**–**5** and **8** were prepared in a similar manner using the appropriate pyridoxyalkyl chloride and the requisite tertiary amine. Iodides **6**, **7** and **10** were prepared by methylation of their corresponding tertiary amines; for example, **6** was prepared by reaction of 2-chloro-5-hydroxypyridine, *N,N*-dimethyl-2-chloroethylamine and K_2CO_3 in CH_3CN, followed by *N*-methylation and purification by column chromatography.
16. The binding assay was conducted as previously reported[18] using rat brain (minus cerebellum) homogenates and [^{3}H](−)nicotine. IC_{50} values were determined from a plot of the log concentration versus percent displacement and converted to K_i values (at least in triplicate).
17. Tail-flick assay: Male ICR mice (20–25 g; Harlan Laboratories, Indianapolis, IN, USA), were housed in an AALAC approved facility in groups of six and had free access to food and water. The study was approved by the Institutional Animal Care and Use Committee of VCU. Antinociception was assessed by the tail-flick method as previously described.[18] Antinociceptive response was calculated as percent maximum possible effect (% MPE) where % MPE = [(-test−control)/(10−control)] ×100. Groups of 6–12 mice were used for each dose, and mice were tested 5 min after either subcutaneous or intrathecal injections. Antagonism studies were carried out by pretreating mice either with saline or drug at different times before (−)nicotine. Intrathecal injections were performed free-hand between the L5 and L6 lumbar space in unanesthetized mice according to the method of Hylden and Wilcox[22] using a 30-gauge needle attached to a glass microsyringe. The injection volume in all cases was 5 μL.
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