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(−)6-*n*-Propylnicotine Antagonizes the Antinociceptive Effects of (−)Nicotine

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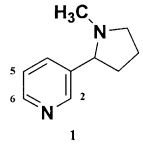
Abstract—Several 6-alkyl analogues of nicotine were examined in radioligand binding and in vivo functional assays. Although (−)6-ethylnicotine (**3**) binds with high affinity at nACh receptors ($K_i = 5.6$ nM) and produces nicotine-like actions, its *n*-propyl homologue (−)**4** ($K_i = 22$ nM) failed to produce such effects. In fact, (−)**4** antagonized the antinociceptive effects of (−)nicotine in the tail-flick assay in mice, but not the spontaneous activity or discriminative stimulus effects of (−)nicotine. Compound (−)**4** appears to selectively antagonize only one of the three effects examined and is an interesting cholinergic agent for subsequent investigation.

Nicotine (**1**) is a naturally occurring substance that is believed to produce certain of its effects through nicotinic acetylcholine (nACh) receptors. There is evidence that neuronal nicotinic receptors might be involved in appetite, memory, analgesia, and various other physiological processes as well as in anxiety and certain neurological disorders.[1–3] Although nicotine is also associated with a variety of toxic side effects,[1] there is no reason to believe that these side effects are inextricably linked to the beneficial effects of nicotine. This has led to a search for nACh receptor agents with reduced toxicity and to investigations of the structure–activity relationships of nicotine.[3–5] With respect to binding at nACh receptors, nicotine can tolerate certain substituents at the pyridine ring 6-position. For example, 6-chloro- and 6-bromonicotine bind with several times the affinity of nicotine.[6,7] These agents are also more potent than nicotine in in vivo assays indicative of nicotinic activity (e.g., tail-flick assay and spontaneous activity in mice).[8] Interestingly, 6-methylnicotine is three to five times more potent than nicotine in these assays even though its affinity is no greater than that of nicotine.[8] The 6-halogenated analogues of nicotine are also more potent than expected on the basis of their affinities. One explanation for this effect is that the presence of the 6-methyl or 6-halo groups enhances the lipophilicity of nicotine and allows greater in vivo distribution. Another explanation is that 6-position substituents influence the intrinsic activity of nicotine. Consequently, it was of interest to examine several additional 6-substituted derivatives of nicotine and, in particular, to compare their affinities with their potencies in several in vivo assays. Specifically, we examined 6-alkyl-substituted analogues of nicotine where the 6-position substituents ranged from methyl to *n*-pentyl.



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Compounds **2**–**4** were available from a previous study.[7] The free base of the (−)6-*n*-butyl analogue (−)**5**, although known,[9] was prepared by free radical alkylation of *S*(−)-nicotine using the Seeman et al.[10] approach as reported for **3** and **4**.[7] An extended analogue of **5**, (±)6-*n*-pentylnicotine, (±)**6**, was formed by

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the reaction of (±)**2** with *n*BuLi (1:3) in dry THF under an N_2 atmosphere, for 30 min at −70 °C, followed by standard workup. The free base of (±)**6** was purified by column chromatography (acetone/*n*-hexane; 1:7), then converted to its di-HCl salt and recrystallized from MeOH/Et_2O; mp 174–176 °C. Both analogues, (−)**5** and (±)**6**, analyzed correctly for CHN and their structures were confirmed by ^1H NMR.

Radioligand binding data are reported in Table 1.[11] The compounds were also examined in three functional assays in which nicotine shows activity: an antinociceptive assay (i.e., tail-flick assay) in mice, a spontaneous activity assay in mice, and substitution in a drug discrimination assay in rats trained to discriminate (−)nicotine from saline vehicle. Table 1 shows that as the size of the 6-position substituent increases, affinity decreases. Within the alkyl series, 6-methylnicotine (**2**; $K_i = 1.8$ nM) binds with the highest affinity whereas 6-*n*-pentylnicotine (**6**; $K_i = 72$ nM) binds with the lowest affinity. These results are consistent with a prior finding that affinity decreases as the molecular volume of 6-position alkyl substituent increases.[7]

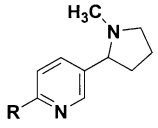
As with 6-methylnicotine (**2**), (−)6-ethylnicotine, (−)**3**, ($K_i = 5.6$ nM) binds with slightly lower affinity than nicotine but is more potent than nicotine in both the tail-flick assay and spontaneous activity assay in mice (Table 1) following subcutaneous injection. 6-Ethylnicotine also substituted for (−)nicotine in tests of stimulus generalization. Interestingly, however, the 6-*n*-propyl derivative (±)**4** ($K_i = 25$ nM) binds only with about 4-fold lower affinity than its ethyl counterpart but failed to produce antinociceptive effects in the tail-flick assay at more than 30 times the ED_{50} dose of (−)**3** (i.e., at doses of up to 70 μmol/kg). Because the latter was initially examined as the racemate whereas the 6-ethyl compound was investigated as its (−)isomer, we examined (−)6-*n*-propylnicotine (−)**4**. Compound (−)**4** ($K_i = 22$ nM) behaved similarly to its racemate. That is, it was inactive in producing antinociceptive effects in mice up to doses of 70 μmol/kg. In addition, it failed to have an effect on spontaneous activity at doses of up to 35 μmol/kg. Also, unlike the 6-methyl and 6-ethyl analogues, (−)**4** failed to produce nicotine-like stimulus effects in rats at doses of up to 50 μmol/kg. Clearly, the actions of (−)**4** are quite different than those of (−)**3**.

Increasing the length of the alkyl substituent beyond *n*-propyl had little additional effect on affinity. The affinity of the *n*-butyl analogue (−)**5** ($K_i = 21$ nM) was similar to that of (−)**4**, whereas the *n*-pentyl analogue **6** displayed about 3-fold reduced affinity relative to **4**. Compounds (−)**5** and **6** were also inactive in the tail-flick and spontaneous activity assays (Table 1). Both (−)**4** and (−)**5** were also examined for agonist activity in the tail-flick assay via intrathecal administration (data not shown); both failed to exhibit an effect at doses of up to 50 μg/mouse.

In a separate set of experiments, the actions of (±)**1**, (−)**1**, **2**, and **3** were challenged with 1 mg/kg of mecamylamine to determine if this noncompetitive nicotinic antagonist would attenuate the observed effects. In each case, mecamylamine successfully antagonized nicotine-like actions (data not shown).

Because compound (−)**4** binds at nACh receptors yet failed to display any nicotine-like actions, it was examined as an antagonist. This compound was demonstrated to antagonize the antinociceptive actions of 2.5 mg/kg of (−)nicotine as measured in the tail-flick assay, $AD_{50} = 4.9$ (95%CL = 1.4–14.8) μmol/kg. However, (−)**4** did not antagonize either the spontaneous activity or stimulus effects of nicotine at doses of up to 35 and 50 μmol/kg, respectively. Evidently, (−)**4** is a rather unique nACh receptor antagonist in that it blocks only one of the three in vivo measures of nicotine-like activity. Compound (−)**5** also exhibited antagonist activity in the tail-flick assay, $AD_{50} = 9.2$ (95% CL = 1.0–68) μmol/kg, but was not examined in the other two assays.

Table 1. Binding and functional data for nicotine analogues **1**–**6**



Compd	R	K_i, nM (SEM)	Tail-flick assay ED_{50} (μmol/kg)[a,b]	Spontaneous activity ED_{50} (μmol/kg)[a,b]	Drug discrimination assay ED_{50} (μmol/kg)[b]
(±)**1**	–H	1.3 (±0.1)	9.9 (6.1–16.0)	4.9 (3.1–8.0)	0.6 (0.1–3.0)
(−)**1**	–H	1.2 (±0.1)	7.4 (5.4–9.6)	3.1 (2.6–6.6)	0.6 (0.2–2.6)
(±)**2**	–Me	1.8[c]	3.5 (2.3–4.7)	1.0 (0.2–5.0)	0.4 (0.2–1.5)
(−)**3**	–Et	5.6[c]	2.1 (0.7–5.2)	1.4 (0.7–2.8)	1.1 (0.4–3.3)
(±)**4**	–*n*Pr	25[c]	>70	—	>20
(−)**4**	–*n*Pr	22(±2)[d]	>70	>35	>50
(−)**5**[e]	–*n*Bu	21 (±1)	>50	—	—
(±)**6**	–*n*Pe	72 (±10)	>50	>50	—

[a]Values are means of at least three separate experiments; where an ED_{50} dose is reported, the effect was antagonized by 1 mg/kg of mecamylamine.
[b]The '>' symbol indicates that the compound failed to produce the effect at doses up to that shown.
[c]K_i value previously reported.[7]
[d]K_i value was re-determined; previously reported $K_i = 17.3$ nM.[7]
[e]Preceding pharmacological evaluation, (−)**5** was converted to its HCl salt; mp 61–62 °C.

A number of nicotinic antagonists have been reported, but few bind at nACh receptors (i.e., they are noncompetitive antagonists).[12] Dihydro-β-erythroidine (DHβE) is one of the very few competitive antagonists to have seen relatively broad application. Interestingly, as with (−)**4**, DHβE blocks the antinociceptive effects of nicotine and nicotine-like agents;[13,14] but, unlike (−)**4**, DHβE also blocks the stimulus effects of nicotine.[15] apparently, the antagonist profiles of (−)**4** and DHβE are not identical. 5-Methoxynicotine is a newer example of a competitive nACh receptor antagonist that blocks (−)nicotine's antinociceptive effects in the tail-flick assay, but not its locomotor actions.[16] Given the possibility that multiple subtypes of nACh receptors might be involved in the different actions of nicotinic agonists, indeed—even in producing the antinociceptive actions of nicotinic agonists[13,14]—compounds such as DHβE, (−)**4**, and 5-methoxynicotine should prove useful in unraveling the functional consequences of nACh receptor-mediated events.

Acknowledgements

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