Synthesis and Pharmacological Characterization of [^{125}I]Iodomethyllycaconitine ([^{125}I]Iodo-MLA). A New Ligand for the α_7 Nicotinic Acetylcholine Receptor

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Since the nicotinic acetylcholine receptors (nAChRs) have been targeted for the development of drugs for cognitive function, Parkinson's disease, analgesia, inflammatory bowel disorder, schizophrenia, anxiety, depression, Tourette's syndrome, and smoking cessation, considerable effort has been directed toward the identification and characterization of radioligands for this receptor system.[1,2] Two major classes of nicotinic receptors have been identified in rat and human brain based on whether they demonstrate high affinity binding for either [^{3}H]nicotine or [^{125}I]α-bungarotoxin ([^{125}I]α-BGT).[3] Heteromeric receptors composed of α and β subunits bind [^{3}H]nicotine with high affinity. The $\alpha_4\beta_2$ receptor is the most common subtype comprising almost 90% of rat brain nAChRs.[4] Receptors with high affinity for [^{125}I]α-BGT contain only the α_7 subunit[5−7] and display a regional distribution distinct from the $\alpha\beta$ heteromeric receptors.[3,5,8] Several new tritium and iodine-125 ligands have been developed for studying the pharmacological properties of $\alpha_4\beta_2$ nAChRs.[9−15] In addition, several carbon-11, fluorine-18, and iodine-123 positron emission tomography (PET) and single-photon emission computed tomography (SPECT) tracers have been developed for in vivo imaging of $\alpha_4\beta_2$ nAChRs.[9,12−14,16−29] At present, [^{125}I]α-BGT is the only iodine labeled radioligand specific for the α_7 nAChR. α-BGT is a 7800−8000 kDa 74 amino acid polypeptide isolated from the venom of the snake *Bugarus multicinctus*.[30] The radioligand has the disadvantage of high nonspecific binding in filtration-based assays. Moreover, α-BGT does not cross the blood-brain barrier, limiting its use for in vivo binding and imaging studies for the α_7 nAChR. Methyllycaconitine (**1**, MLA), which is isolated from the seeds of *Delphinium brownii*, is a ligand that is highly selective for the α_7 nAChR.[1,2] In contrast to α-BGT, MLA is a relatively small reversible-binding compound that has been shown to cross the blood-brain barrier after peripheral administration.[31] In this study we present the synthesis and characterization of [^{125}I]iodomethyllycaconitine ([^{125}I]iodo-MLA, [^{125}I]-**8**) and report that it is a useful radioligand for studying the α_7 nAChR.

The [^{125}I]-**8** was synthesized by the route outlined in Scheme 1. MLA (**1**) was isolated from *Delphinium elatum* (Pacific giant) seeds according to the procedure developed by Pelletier and co-workers.[32] Alkaline hydrolysis of MLA using 5% potassium hydroxide in ethanol gave lycoctonine (**2**).[33] Treatment of 5-iodoanthranilic acid (**3**) with (*S*)-methylsuccinic anhydride (**4**) provided a mixture of the iodomethyllycoctonic acids (**5** and **6**). The ^{1}H NMR spectrum of the acids showed two equal doublets at 1.29 and 1.26 ppm (J = 7.1 Hz) for the methyl groups which suggests that the acids are a 1:1 mixture of **5** and **6**. The mixture of acids was refluxed under a Dean Stark tube in toluene containing triethylamine for 24 h to yield (*S*)-2-(methylsuccinimido)-5-iodobenzoic acid (**7**). The structure and single-isomer nature of **7** was established by the ^{1}H NMR spectrum which showed only one doublet at 1.37 ppm (J = 6.8 Hz) for the methyl group. The acid **7** was coupled to the primary hydroxyl group of lycoctonine in the presence of *p*-toluenesulfonyl chloride and pyridine to give iodo-MLA. Refluxing iodo-MLA with hexamethyldistannane in toluene in the presence of palladium-tetrakis-triphenylphosphine provided trimethylstannyl-MLA (**9**) which was the precursor needed to prepare [^{125}I]-**8**.

A sample of **9** used for radioiodination was purified by HPLC to eliminate any trace of **8** from the precursor, as the presence of unlabeled **8** would reduce the specific activity of the final radiolabeled product. HPLC analysis showed that the contamination of **8** in the trimethylstannyl-MLA precursor **9** was less than 0.027%. Since the ratio of **9**/[^{125}I]sodium iodide (1875 Ci/mmol) used in the synthesis of [^{125}I]-**8** was 17, the effect on specific activity of the labeled product was less than 0.46% (0.027% × 17), which was within the normal experimental error. The radioiododestannylation of the precursor **9** was completed within 1 min at room temperature using chloramine-T as oxidant. The total radiochemical yield of [^{125}I]-**8** after HPLC purification was 74%.

The specificity of **8** for the α_7 nAChR relative to $\alpha_4\beta_2$ was assessed in two to three preliminary competition binding experiments using [^{125}I]α-BGT, [^{3}H]MLA, or [^{3}H]epibatidine (Table 1). The iodo-MLA analogue **8** showed affinity for the α_7 nAChR almost identical to that of MLA. Both MLA and **8** exhibited poor affinity at the $\alpha_4\beta_2$ nAChRs labeled by [^{3}H]epibatidine. The results show that the insertion of an iodine at the meta position of the benzoyl fragment in MLA does not decrease its potency or selectivity at the α_7 nAChR and suggest that [^{125}I]-**8** might be a useful radioligand for the studies of α_7 nAChR.

[^{125}I]-**8** binding was characterized in rat brain cerebral cortex homogenates. Specific binding of [^{125}I]-**8** was typically 70−80% of total binding at 100 pM, and it was linear with protein concentration (up to 300 μg protein/assay tube; not shown). The data from the saturation binding experiments (N = 6) revealed that the binding was saturable and that the specific binding was best fit by a one-site model that gave an affinity constant (K_d) of 1.8 ± 0.4 nM and a B_{max} of 68 ± 3 fmol/mg protein. Both values are in general agreement with corresponding values determined for [^{3}H]MLA[34] and with the B_{max} measured in rat brain using [^{125}I]α-BGT[35] (Table 2). The specific binding data from the association binding

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Scheme 1

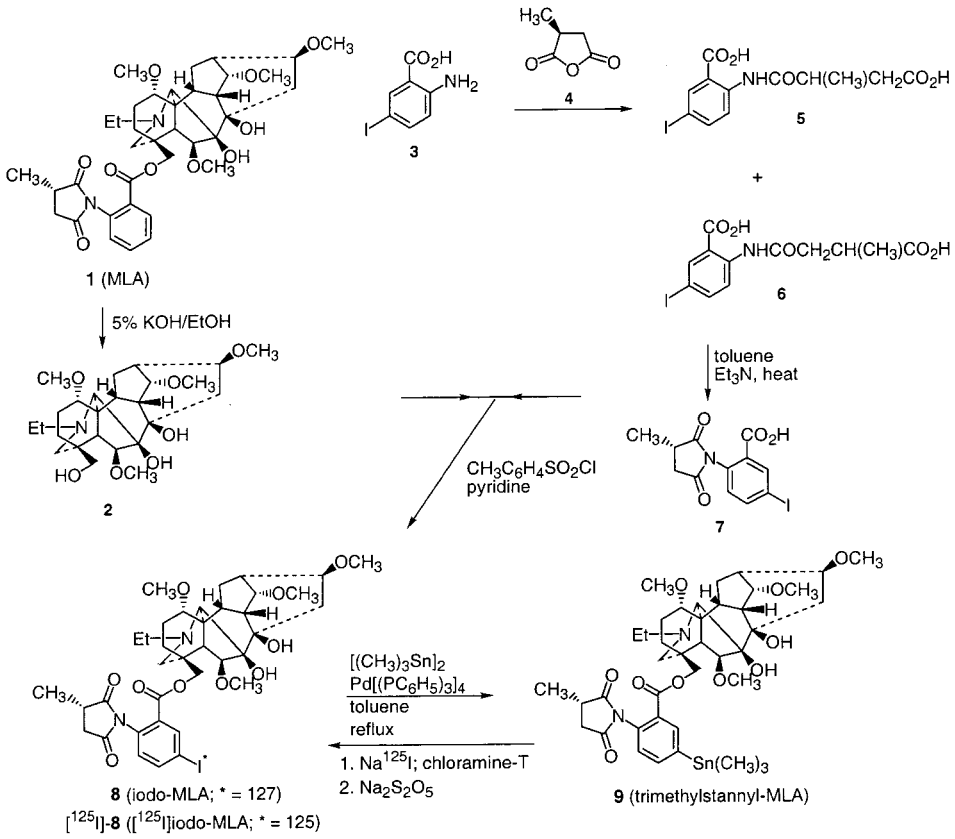

Table 1. K_i Values of MLA and **8** (Iodo-MLA)

compound	binding affinity (K_i in nM)		
	[^{125}I]α-BGT	[^{3}H]MLA	[^{3}H]epibatidine
MLA	0.8 ± 0.1	0.6 ± 0.02	>1 μM
8 (iodo-MLA)	1.3[a]	1.6 ± 0.4	>1 μM

[a] Represents a single determination.

Table 2. Comparison of K_d and B_{max} Values for [^{125}I]Iodo-MLA, [^{3}H]MLA, and [^{125}I]α-BGT in Rat Brain

compound	K_d (nM)	B_{max} (fmol/mg protein)
[^{125}I]iodo-MLA	1.8 ± 0.4	68 ± 3
[^{3}H]MLA	1.9 ± 0.3[a]	65 ± 5[a]
[^{125}I]α-BGT	1.5 ± 0.7[b]	63 ± 17[b]

[a] Taken from ref 34. [b] Taken from ref 35.

experiments (N = 3) were best fit by a one-phase exponential association equation with a K_{obs} = 0.08 ± 0.02 min^{-1} and a $t_{1/2}$ = 10.5 ± 3.1 min. The kinetic dissociation data determined from three experiments were best fit by a one-phase exponential decay equation which gave a K_{off} of 0.07 ± 0.01 min^{-1} and a $t_{1/2}$ = 10.3 ± 1.6 min. On the basis of these two rate constants, the K_{on} was calculated to be 0.033 M^{-1} min^{-1}, and the derived K_d was equal to 2.1 nM.

The specificity of [^{125}I]-**8** binding was characterized through competition binding experiments using known α_7 ligands MLA, α-bungarotoxin, and 3-cinnamylidine-anabasine, the $\alpha_4\beta_2$ agonist and antagonist nicotine and dehydro-β-erythrodine, respectively, and the noncompetitive nAChR antagonist mecamylamine (Table 3). MLA, α-bungarotoxin, and 3-cinnamylidine-anabasine competed effectively with [^{125}I]-**8** binding with K_i values of 3.2, 1.9, and 13.2 nM, respectively. Nicotine, dihydro-β-erythrodine, and mecamylamine were weak or ineffective at competing with [^{125}I]-**8** binding. This pharmacological profile is similar to that reported for MLA,[36,37] [^{3}H]MLA,[34] and [^{125}I]α-BGT,[3,38−40] and thus consistent with [^{125}I]-**8** being specific for the α_7 nAChR subtype.

The specificity of binding was further examined by measuring the binding of [^{125}I]-**8** in rat brain regions containing high (hippocampus and thalamus/hypothalamus) and low (cerebellum and striatum) α_7 nAChR numbers based on [^{125}I]α-bungarotoxin binding.[8] For each brain region, the ratio of specific binding (fmol/mg protein) to specific cerebellar binding (control region) was calculated. The ratios were highest in the hippocampus (8.2 ± 0.9; N = 2; mean ± SD) and thalamus/

Table 3. Comparison of Inhibition of [^{125}I]Iodo-MLA Binding to That Reported for [^{125}I]α-BGT and [^{3}H]MLA

compound	N	K_i (nM) [^{125}I]iodo−MLA	K_i (nM) [^{125}I]α-BGT	K_i (nM)[b] [^{3}H]MLA
methyllycaconitine	5	3.2 ± 0.7	1.4[a]	0.98
α-bungarotoxin	2	1.9 ± 0.1	0.7[b]	1.78
3-cinnamylidene-anabasine	4	13.2 ± 2.1	39[c]	
(−)-nicotine	5	667 ± 49	820[d]	6070
dihydro-β-erythroidine	3	>10000		
mecamylamine	3	>10000	>10000[e]	>20000

[a] Taken from ref 39. [b] Taken from ref 34. [c] Taken from ref 40. [d] Taken from ref 8. [e] Taken from ref 3.

hypothalamus (4.2 ± 1.4), whereas the striatal [^{125}I]-**8** binding ratio was close to unity (1.1 ± 0.8). The regionally selective increases in the [^{125}I]-**8** binding pattern are consistent with the ligand being selective for α_7 nAChRs.

In summary, we found that [^{125}I]-**8** binds with high specificity to α_7 nAChRs, making it a viable alternative to [^{125}I]α-BGT in the study of this nAChR subtype. Moreover, its high specific activity makes it suitable for use in high throughput screening assays aimed at identifying nAChR subtype-specific ligands using either brain tissue, which has low concentrations of α_7 receptors, or cloned α_7 receptors. Since the addition of iodine-125 to MLA does not alter its specificity for the α_7 nAChR, it is likely that an iodine-123 labeled MLA could be a useful ligand for imaging this nAChR subtype in vivo. Finding nAChR subtype-specific ligands that may be useful as imaging agents is particularly relevant since reduced numbers of nAChRs have been observed in Parkinson's and Alzheimer's diseases and in schizophrenia.[41]

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Supporting Information Available: Experimental details and data analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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