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Development of Radioiodinated Receptor Ligands for Cerebral Single Photon Emission Tomography

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ABSTRACT

In the last decade the use of radiolabeled ligands for the imaging of cerebral receptors by emission computed tomography (ECT) has seen rapid growth [1,2]. The opportunity to routinely perform cerebral single photon emission tomography (SPET) with iodine-123-labeled ligands depends on the availability of receptor ligands into which iodine can be introduced without decreasing the required high target receptor specificity. The use of iodine-123-labeled receptor-specific ligands also depends on the availability of high purity iodine-123 at reasonable costs and the necessary imaging instrumentation. In this paper, the development and current stage of evaluation of various iodine-123-labeled ligands for SPET imaging of dopaminergic, serotonergic and muscarinic acetylcholinergic receptor classes are discussed.

Molecular Design, Radiochemistry and Brain Imaging

The pioneering studies of Paul Ehrlich early in this century formed the basis for contemporary studies with neurotransmitters and the use of radiolabeled ligands for studying neuroreceptor binding[3]. Since binding of neurotransmitters to cerebral receptors is required for neurotransmission, the external imaging of binding of radiolabeled receptor antagonists has presented a challenge to the medicinal chemist and biologist to synthesize and evaluate analogues or new molecular structures with altered affinities which can be used to evaluate receptor binding. There are currently a large number of radiolabeled ligands for the dopaminergic, serotonergic, and acetylcholinergic receptors which have been developed and classified based on their binding to specific receptor systems (Table 1). This rapidly growing field offers increasing opportunities for the specialty of nuclear medicine to provide important information in improving health care. As an example, although three classes of dopaminergic receptors have been described for several years,

recently two additional receptor classes have been reported[4,5,6].

A large number of receptor-specific ligands have been radiolabeled and evaluated *in vitro* and *in vivo* as potential agents for SPET imaging of cerebral receptors. There are only a limited number of single-photon emitting radioisotopes, however, which can be introduced into such ligands without compromising the strict molecular requirements necessary for receptor binding. For this reason, many of the metallic radioisotopes widely used in nuclear medicine for a number of other imaging applications, such as technetium-99m and indium-111, would not be expected to be useful for radiolabeling receptor-specific molecules because of the relatively large chemical groups containing the multiple binding sites ("multi dentate") required to stabilize the metallic isotope.

The importance of molecular size and shape on receptor binding is even more vividly illustrated by the significantly reduced specificity encountered with many radioiodinated ligand analogues (*vide infra*). The iodine atom is about the size of a methyl ($-CH_3$) group and introduction of iodine into regions of a molecule whose size is critically important for receptor binding can thus interfere with binding to the receptor. Such strict structural requirements are especially true for ligands which bind to cerebral receptors, where the additional structural requirement for passage through the blood-brain barrier imposes further limitations on molecular weight and lipophilic character of the ligand. This paper is not intended to be a comprehensive overview of all the radioiodinated ligands which have been synthesized and evaluated, but to point out examples of ligands for the key receptors and to discuss recent work we have conducted in the Nuclear Medicine Group at the Oak Ridge National Laboratory.

Chemical Methods for Introduction of Radioiodine

An issue of central importance for the use of radioiodine is the availability of appropriate methods

for the introduction and stabilization of iodine on receptor ligand analogues. The relatively low bond energy (56.3 kcals/mole) of iodine attached to an sp^3 hybridized carbon (e.g. saturated carbon) often results in the facile loss of radioiodine from such molecules *in vivo*. Thus, iodine must usually be "stabilized" by attachment to the ligands in the appropriate manner[7,8]. The main approach involves the attachment of radioiodide to sp^2 hybridized carbon atoms (e.g. alkene and aromatic carbon atoms), and many approaches have thus been developed for attaching radioiodine to an aromatic ring or to a vinyl group (Figure 1). Most of these methods involve various iododemetallation reactions by the reaction of an iodonium ion (I^+) with the prefabricated substrate.

Availability of Iodine-123

Based on availability and nuclear properties, the number of radioisotopes available for radiolabeling receptor agents for single photon tomography (SPET) is extremely limited. Therefore iodine-123 is expected to continue to be the most widely used radioisotope for radiolabeling receptor ligands for SPET. The accelerator production pathways involving proton irradiation of tellurium or iodine targets (Figure 2) also produce unwanted radionuclide impurities which either significantly increase the patient absorbed radiation dose (iodine-124 and iodine-125) or emit high energy photons (iodine-124) which can result in significant "image degradation" from collimator septa penetration. The availability of carrier-free, high purity iodine-123 was thus a problem for many years until the routine and reliable production of iodine-123 via proton irradiation of enriched xenon-124 gas targets became available[9]. High purity iodine-123 is now regularly available in North America, Europe and Japan. The availability of this important radioisotope is now not an impediment for the development and testing of iodine-123-labeled receptor imaging agents. Before the routine clinical use of these agents can be realized, however, a reduction of the high cost of iodine-123 will probably be required to make these procedures

competitive and cost effective. The maximum theoretical specific activity of iodine-123 is 2.4 X 10^5 Ci/mmole, which is well below the nanomolar concentration of the various cerebral receptors.

SPET Instrumentation

Parallel to the development and testing of new iodine-123-labeled receptor ligands there continues to be improvements in imaging instrumentation hardware and computer software. While only single head rotating gamma cameras using traditional back-projection algorithms were used for cerebral imaging just a few years ago, a variety of three-head cameras which are capable of the higher spacial resolution required for receptor imaging are now available. Examples include the General Electric "Neurocam" Star™, Picker "PRSIM™", Trinoix "Triad" and Toshiba "GCA-9300A" gamma camera systems. In addition, dedicated ring systems such as the Adac "Aspect" camera are also now available. Use of these systems increases both the sensitivity and resolution. For single tomographic acquisitions the time required for the imaging studies can be significantly decreased, thereby both increasing patient comfort and patient throughput. More importantly, the opportunity to preform dynamic SPET with multiple, sequential acquistions will undoubtedly provide new opportunities for evaluation of regional changes in tracer concentration at the regions of interest.

Radioiodinated Ligands for the Dopaminergic Receptor

The development of radiolabeled ligands for SPET and PET have in many cases been complementary. A good example is the number of early ligands for dopaminergic receptor binding based on the spiroperidol nucleus (Figure 3). Because of the importance of apparent changes in dopamine-mediated neurotransmission in many diseases such as Alzheimer's dementia (decreased) and schizophrenia (increased), various investigators have focussed on the preparation of

radioiodinated ligands specific for the D2 receptor. The successful imaging of dopamine D2 receptors in humans with both fluorine-18 and carbon-11 labeled ligands[10,11] stimulated research into the possibility of introduction of iodine into similar molecules while maintaining their receptor binding properties. Several approaches have been used to introduce iodine-123 into spiroperidol analogues, including attachment to the 2'-position (Figure 3) of spiroperidol[12,13,14]. Another example, based on the success of attaching ω-fluoroalkyl groups to the lactam nitrogen[15,16], involves attachment of the iodophenyl[17] or the iodoethyl group[18] to the lactam nitrogen. The latter approach is apparently one of the few examples of stability of iodine-saturated carbon bond *in vivo* .

Because of the expected greater stability of the iodine attached to an olefin, a group at the Johns Hopkin Medical Institution[19,20] and our group at the Oak Ridge National Laboratory have prepared the N-3-(E-iodoallyl) substituted spiroperidol analogue[21,22]. Although this analogue shows high specific binding to dopamine rich areas of the brain, unfortunately these spiroperidol analogues show only modest global uptake into brains of mice and rats and therefore have not been further evaluated. Presumably, the increased lipophilicity of this analog and/or alterations in the overall molecular structure affect cerebral localization. N-Alkylated analogues of spiroperidol, in addition to binding to dopamine D2 receptor subtypes, also have affinity for serotonin 5-HT_2 receptor subtype.

To overcome this problem of specificity for more than one receptor subtype, other ligands are being developed to increase the specificity for a single receptor. More recently, the potent antagonist action of substituted benzamides for the dopamine D2 receptor has been described[23,24,25]. Radioiodinated ligands based on the benzamide nucleus (Figure 4) are thus being studied in detail and may show greater promise for receptor *in vivo* imaging with SPET than

the radioiodinated analogues based on the spiroperidol nucleus. Iodine-123-labeled benzamide analogues currently show great promise for SPET imaging of cerebral dopamine D2 receptor[26,27]. The IBZM benzamide analogue, ((S)-(-)-3-iodo-2-hydroxy-6-methoxy-N-[1-ethyl-2-pyrrolidinyl)methyl]-benzamide)[28,29,30], was one of the first analogues in this series of compounds which was evaluated both *in vitro* and *in vivo* in animals and humans. More recently, epidepride (Figure 4) has been studied by several groups and shows very high specificity for the dopamine D2 receptor [31,32,33]. In Europe, various benzamide analogues are the first commercially available iodine-123-labeled dopamine D2 ligands, which include IBZM (Cygne, Inc., Eindoven, The Netherlands) and Iomazinil (Paul Scherrer Institute, Wuerinlingen, Switzerland).

Along these same lines, benzazepines analogues have been reported to be highly selective ligands for dopamine D1 receptor subtype. This has led to the investigation of a series of radioiodinated benzazepine derivatives which include iodine-123 labeled-SCH 23982[34], IBZP[35], FISCH[36], and TISCH[37] (Figure 5). Preliminary *in vivo* studies in animals have indicated that these ligands may be useful for the imaging of dopamine D1 receptors in human subjects.

Radioiodinated Ligands for the Serotonin $5HT_2$ Receptor

Because of the apparent important involvement of serotonin-mediated neurotransmission in various mood disorders, there is also interest in the potential use of iodine-123-labeled serotonin receptor-specific ligands for the evaluation of the activity or population of cerebral serotonin receptors. The initial description of the potent antagonistic activity of ketanserine (Figure 6) for the serotonin 5-HT_2 receptor[38], was perfectly tailored for the development of a PET tracer since the parent molecule already contained the para-fluoro substituent in the phenyl ring. Demonstration of the high *in vitro* binding of ketanserine to 5-HT_2 receptors with subsequent imaging of receptors *in*

vivo with carbon-11-labeled ketanserine[39] provided the impetus for the synthesis and screening of iodinated analogues for potential radiolabeling with iodine-123. These encouraging results using the ketanserine nucleus suggested that the radioiodinated analogues should also be prepared. Analogues to the work preformed on the spiroperidol nucleus, introduction of iodine at the 2-prime position of the fluorophenyl ring did not greatly affect receptor specificity[40,41], and patient studies with this new analogues have been reported[42].

A good example of the rapid transition from chemical synthesis and pharmacological testing to radiolabeling and *in vivo* evaluation was the reported significantly increased specificity of the altanserine, an analogue of ketanserine, for the serotonin 5-HT_2 receptor subtype. In altanserine (Figure 6), one carbonyl oxygen in the heterocyclic ring system has been replaced with sulfur. This structural modification greatly increases the serotonin 5-HT_2 specificity while decreasing uptake to the D2 and α_1 receptor sites[43]. The fluorine-18 labeled analogue has recently been studied in rats[44] and humans[45]and shows good promise for the PET evaluation of the neurological involvement of these receptors in various disease states.

The description of the increased specificity of altanserine for the serotonin 5-HT2 receptor *in vivo* has prompted attempts to prepare radioiodinated analogues. The presence of the sulfur in the heterocyclic ring system, however, interfered with the chemical methods used previously for the preparation of [I-123]-2'-iodoketanserine, which involves the cuprous ion-assisted iodine displacement of bromide from the 2'-bromoketanserine substrate[40]. Because such sulfur compounds have traditionally been used as ligands to bind cupric ions, the heterocyclic ring system was found to interfere with the iodine-bromine exchange reaction[46] and evidently 2'-iodoaltanserine (Figure 6) can not be prepared by this approach.

For these reasons, we are currently approaching the synthesis of the 2'-iodoaltanserine analogue by the addition of the iodine before the sulfur moiety is introduced into the molecule. Our attempts have thus far been unsuccessful, but we are optimistic that the iodine-123-labeled 2'-iodoaltanserine can be synthesized and will hopefully represent a potential new ligand for the evaluation of its specificity for the serotonin 5-HT_2 cerebral receptor.

Radioiodinated Ligands for the Cholinergic-Muscarinic Receptor

The most widely studied radioiodinated analogue for the muscarinic acetylcholine receptor (mAChR) is 1-azabicyclo[2.2.2]oct-3-yl α-hydroxy-α-(4-iodophenyl)-α-phenylacetate, (4IQNB), an analog of QNB which is a potent muscarinic antagonist (Figure 7). 4IQNB was prepared several years ago[47] and has been used for the study of mAChR in humans[48,49,50]. 4IQNB exists in two diastereomeric forms, and the more rapid clearance of the (R,S)-isomer from the receptor-rich regions of tissues in comparison to the (R,R)-isomer has also been recently studied[51]. More recently, the carbon-11 labeled QNB has been synthesized and evaluated as a PET tracer for the cerebral m-AChR[52]. The radioiodination of 4IQNB involves use of a "triazine" substrate (Figure 8) in which the nitrogen substituent is displaced by iodide. Since this approach apparently provides the radioiodinated target molecule in only low yields (~18%)[53], we have pursued the synthesis and screening of other analogues which would retain their receptor binding *in vivo* but could be more readily be radiolabeled in higher yields.

We focussed on the preparation of analogues (Figure 7) based on the "three point of attachment" model[54] for m-AChR. These new analogues were synthesized by a variety of radioiodination techniques which were used as substrates for electrophilic introduction of iodine in the last step of

the reaction sequence[55]. While "IQNX" [1-azabicyclo[2.2.2]oct-3-yl (7-iodo)xanthene-9-carboxylate] and "IQNA" [1-azabicyclo[2.2.2]oct-3-yl α-hydroxy-α-(4-iodophenyl)-α-methylacetate] analogues do not show receptor-specific cerebral distribution in studies in rats, the "IQNP" analogue [1-azabicyclo[2.2.2]oct-3-yl α-hydroxy-α-(1-iodo-1-propen-3-yl)-α-phenylacetate] exhibits high specific uptake in those regions of the rat brain rich in acetylcholinergic receptors[56]. As an example, tissue distribution studies with iodine-125-labeled IQNP in female Fisher VAF rats over a six hour period demonstrated high uptake of radioactivity in those areas of brain containing high concentrations of the m-AChR while cerebellar uptake is low (Table 2). Striatal/cerebellar ratios of about 7 to 1 (e.g. striatum) were observed after six hours.

The synthesis of IQNP has been more recently studied further and an improved synthesis developed which involves the use of a peroxide solution rather than chloramine-T for the requisite oxidation of iodine-123-iodide to the iodonium ion required for replacement of the tributylstannyl group (Figure 8). A number of pre-blocking and displacement studies utilizing QNB , a potent muscarinic antagonist, demonstrate that IQNP is reversibly bound to the cerebral muscarinic receptor. The effects of one hour pre-injection of QNB (5 mg/kgm) were evaluated on the regional distribution of radioactivity in rat brains before administration of [I-125]-IQNP (Figure 9). A similar experiment was conducted to evaluate the displacement of radioactivity from rat brains by two hour post-injection of QNB following intravenous administration of the [I-125]-IQNP. These results clearly indicated that the striatal concentration of the new IQNP analogue is blocked by pre-injection of the m-AChR-antagonist QNB and that striatal uptake of radioactivity after injection of [I-125]-IQNP is displaced by QNB.

Additional blocking studies have also been conducted with ligands which are specific for various cerebral receptors. These studies included pre-blocking prior to [I-125]-IQNP injection with

spiperone (D2 dopamine antagonist), (+)-butaclamol (D2/D1 dopamine antagonist), (-)-butaclamol (inactive enantiomer), dexetimide (muscarinic antagonist) and ketanserine (5-HT_2 antagonist). The results of these studies (Figure 10) demonstrate that pre-treatment with QNB and with dexetimide, which are muscarinic antagonists, block the localization of radioiodinated IQNP and provide evidence that IQNP is specific and selective for cerebral m-AChR and is a good candidate for further study.

Other investigators have focussed on the development and evaluation of various radiolabeled dexetimide analogues (Figure 11). The fluorine-18-labeled analogue[56] and the carbon-11-labeled analogue[57] have been prepared and are being studied as potential PET ligands. In addition, the radioiodinated dexetimide and levetimide have been prepared[58,59] and *in vivo* studies in the quantification of m-AChR in the human brain are being pursued[60].

Although the nonspecific binding of iododexetimide to cardiac m-AChR is relatively high[61], by using the "upregulation" of muscarinic receptors in rats, 4-[I-125]-dexetimide binding has been shown to reflect changes in atropine-induced muscarinic receptors[62]. Heart to blood ratios of ~30 and heart to lung ratios of ~4 have been reported for the methiodide salt of QNB[63] in the study of cardiac m-AChR. In addition, carbon-11-label methiodide QNB has been used to study cardiac m-AChR[63]. Therefore, with its ease of preparation, the methiodide salt of IQNP could be an ideal candidate for the study of these receptor populations. Thus, it remains to be established which iodine-123-labeled analogue will be the best for myocardial or cerebral m-AChR receptor imaging.

SUMMARY

An issue of central importance is how imaging of receptors by SPET can be related to important clinical problems. As the efforts for development of both more selective radioiodinated receptor specific ligands and imaging systems with higher sensitivity and spacial resolution continue, this will allow SPET imaging of cerebral receptors to become a useful tool for neurologists and psychiatrists. When this point is finally reached in the future, these important molecules will change from merely being chemical curiosities to useful radiopharmaceuticals.

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FIGURE LEGENDS

Figure 1. Examples of Stable Attachment of Iodine to Organic Molecules

Figure 2. Reaction Pathways for Accelerator Production of Iodine-123

Figure 3. Examples of Radioiodinated Spiroperidol Analogues

Figure 4. Radiolabeled Benzamide-Substituted Ligands

Figure 5. Radiolabeled Benzazephine-Substituted Ligands.

Figure 6. Radiolabeled Analogues Specific for the Serotonin $5HT_2$ Receptor

Figure 7. Radioiodinated Analogues for the Muscarinic Cholinergic Receptor (m-AChR)

Figure 8. Synthesis of "4IQNB" and "IQNP"

Figure 9. Blocking studies with [I-125]-IQNP

QNB (5mg/kg) post-injected 2 hour after IQNP
QNB (5mg/kg) pre-injected 1 hour before IQNP
Animals sacrificed 3 hours post-injection IQNP

Figure10. Blocking Studies with [I-125]-IQNP Uptake by Various Receptor-Specific Ligands

Ligands (5mg/kg) injected 1 hour pre-injection of IQNP
Animals sacrificed 3 hours post-injection of IQNP

Figure 11. Dexetimide Analogues for Muscarinic Receptor Uptake Studies

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Table 1. Examples of Categorization of the Principal Cerebral Receptor Sub-Groups.

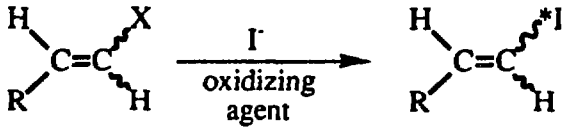
Receptor	Antagonist	Radioiodinated Ligand	Reference
Dopamine			
D1	SCH 23390	^{123}I-SCH 23982	34
		^{123}I-FISCH	36
		^{123}I-TISCH	37
D2	Sulpiride	^{123}I-IBZM	29
		^{123}I-Epidepride	33
		^{123}I-2'-Spiperone	66
		^{123}I-ILIS	67
Serotonin			
5-HT1B	Iodocyanopindolol	^{125}I-ICYP	68
5-HT1C	MES	^{125}I-LSD	69
5-HT2	Ritanserine	^{123}I-Altanserine	70
5-HT3/1A	OH-DPAT	^{125}I-Iodoethyl-spirotraxine	71
Acetylcholinergic			
M1	Pirenzepine	^{123}I-IQNB	48
M2	AFDX-116	^{123}I-Dexetimide	60
M3	HHSiD	^{125}I-IQNP	56

Table 2. Distribution of Radioactivity in Blood and Cerebral Tissues of Female Fisher VAF Rats Following Intravenous Administration of [I-125]-IQNP.

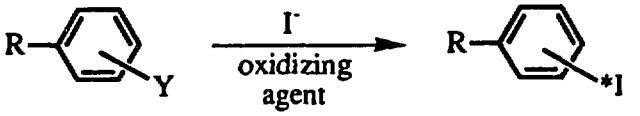
	Mean Percent Injected Dose/Gm ± SD[1]			
	Minutes After Injection			
Tissue	15	60	120	360
Blood	0.85±0.15	0.38±0.05	0.38±0.03	0.21±0.02
Cortex	0.99±0.09	0.86±0.05	0.64±0.02	0.47±0.06
Striatum	0.82±0.10	0.86±0.11	0.93±0.12	0.41±0.07
Cerebellum	0.54±0.05	0.30±0.02	0.24±0.01	0.06±0.01
Remainder of Brain	0.72 ±0.06	0.58±0.07	0.41±0.03	0.27±0.03
Striatal/Cerebellar Ratio[2]	1.51	2.86	3.87	6.83
Striatal/Blood Ratio[2]	0.96	2.26	2.45	1.95

[1]Mean values for five rats.

[2]Ratios of the mean values.



X = $B(OH)_2$, $Sn(Bu)_3$



Y = $B(OH)_2$, $Sn(Me)_3$, $Sn(Bu)_3$, HgOAc, $Th(TFA)_2$

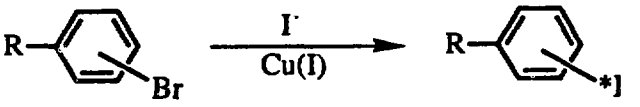


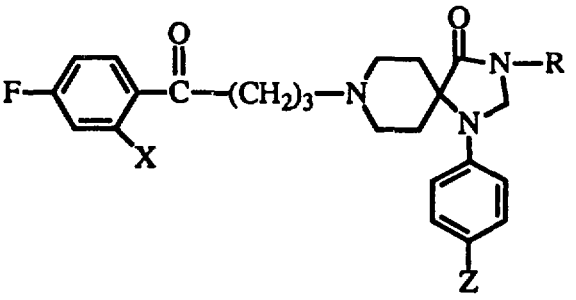
Figure 1

^{123}Te (p, 2n) ^{123}I

^{127}I (p, 5n) ^{123}Xe $\longrightarrow$ ^{123}I

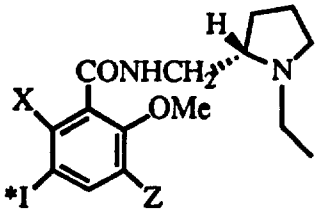
^{124}Xe (p, 2n) ^{123}Cs $\longrightarrow$ ^{123}Xe $\longrightarrow$ ^{123}I

Figure 2



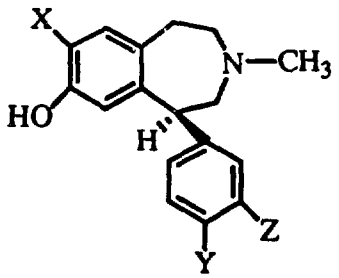
2'-Iodospiroperidol	R = Z = H	X = *I
4-Iodospiroperidol	R = X = H	Z = *I
Iodoethylspiroperidol	X = Z = H	R = Iodoethyl
Iodoallylspiroperidol	X = Z = H	R = Iodoallyl
p-Iodophenylspiroperidol	X = Z = H	R = p-Iodophenyl

Figure 3



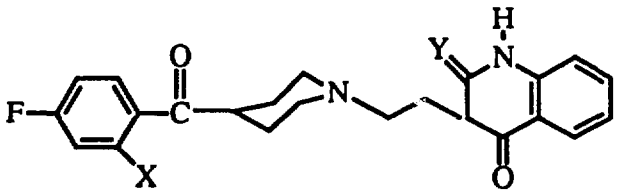
Iodopride	X = H	Z = H
IBZM	X = OH	Z = H
Epidepride	X = OH	Z = OMe

Figure 4



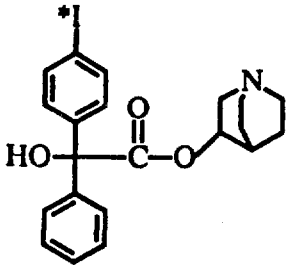
IBZP	X = I	Y = H	Z = H
(±) FISCH	X = Cl	Y = I	Z = H
R-TISCH	X = Cl	Y = H	Z = I

Figure 5

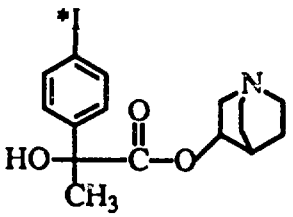


Ketanserine	X = H	Y = O
2'-Iodoketanserine	X = I	Y = O
Altanserine	X = H	Y = S
2'-Iodoaltanserine	X = I	Y = S

Figure 6



"4IQNB"



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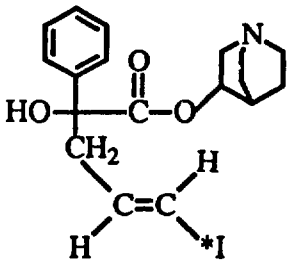
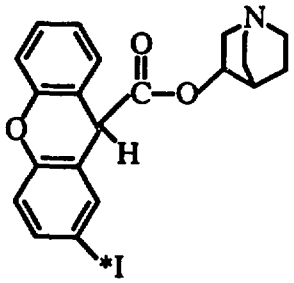
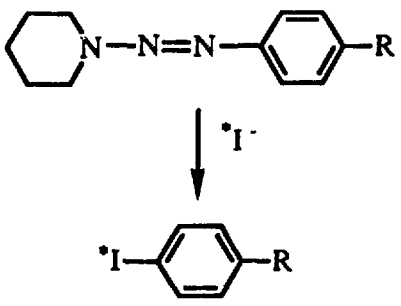


"IQNP"

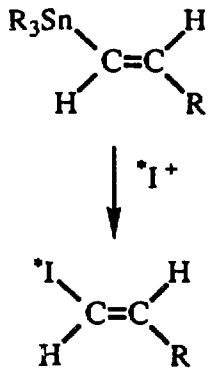


"IQNX"

Figure 7



Triazene Approach
for "4IQNB"



Trialkylstannyl Approach
for "IQNP"

Figure 8

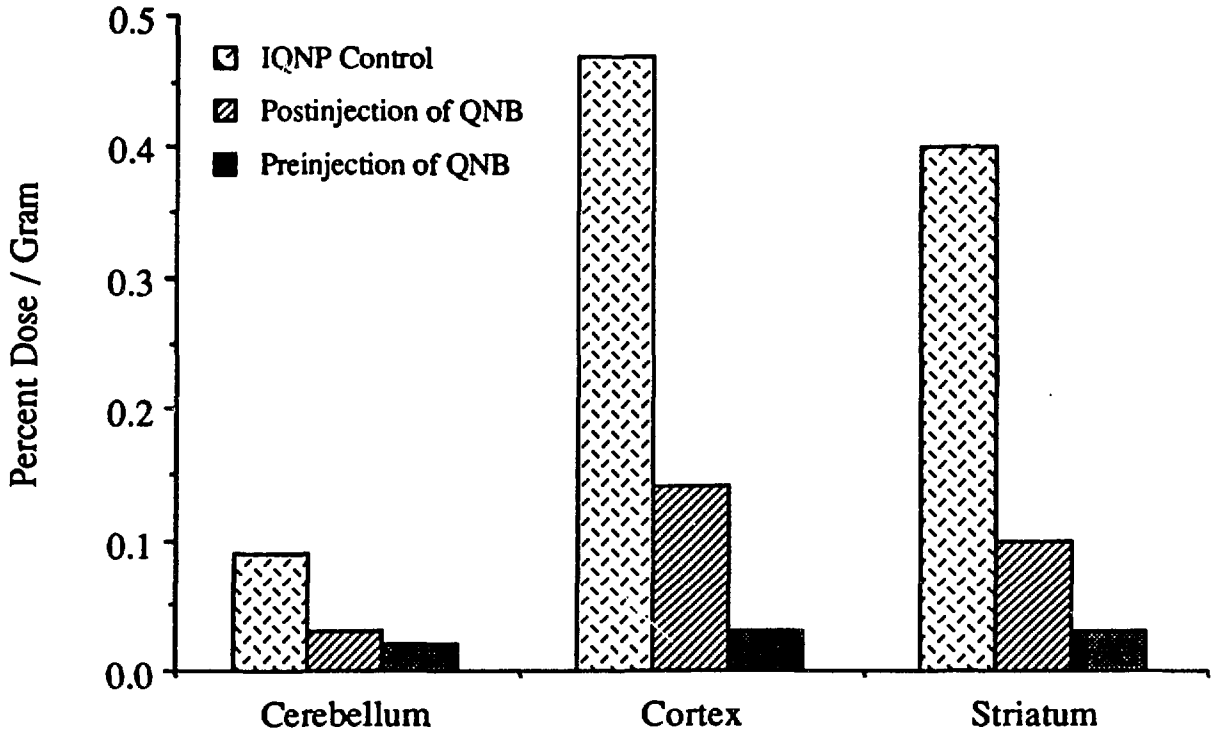

0.5
0.4
0.3
0.2
0.1
0.0
Percent Dose / Gram
IQNP Control
Postinjection of QNB
Preinjection of QNB
Cerebellum
Cortex
Striatum

Figure 9

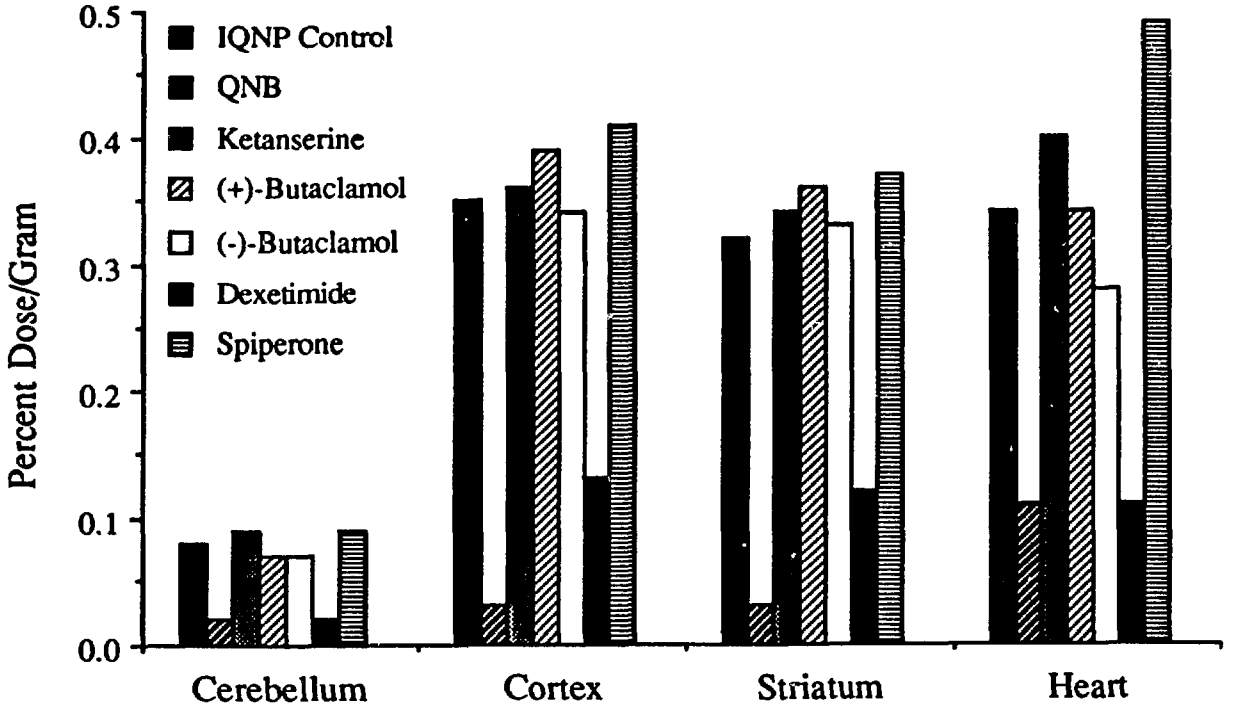
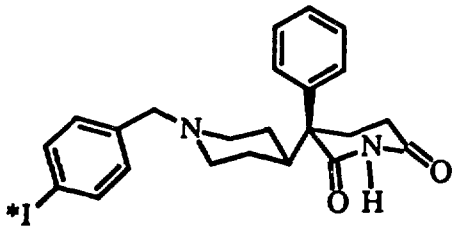
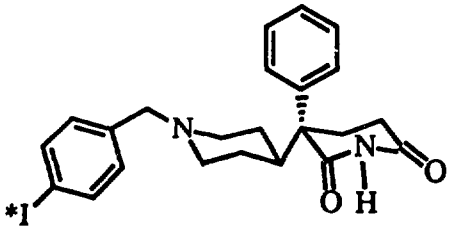

0.5
0.4
0.3
0.2
0.1
0.0
Percent Dose/Gram
IQNP Control
QNB
Ketanserine
(+)-Butaclamol
(-)-Butaclamol
Dexetimide
Spiperone
Cerebellum
Cortex
Striatum
Heart

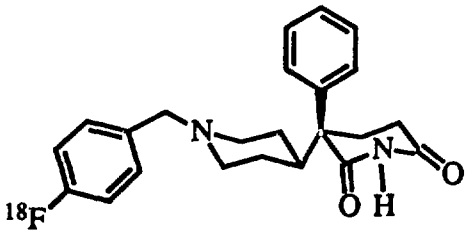
Figure 10



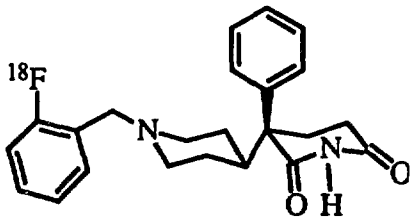
Iododexetimde



Iodolevetimide



4-[^{18}F]-Fluorodexetimide



2-[^{18}F]-Fluorodexetimide

Figure 11