

PII: S0960-894X(97)10139-1

A NOVEL PHOTOAFFINITY LABEL FOR THE DOPAMINE TRANSPORTER BASED ON N-SUBSTITUTED 3α-[BIS(4'-FLUOROPHENYL)METHOXY]TROPANE

Gregory E. Agoston,^a Roxanne Vaughan,^b John R. Lever,^c Sari Izenwasser,^a Phyllis D. Terry,^a and Amy Hauck Newman^{*a}

^aPsychobiology Section and ^bMolecular Neurobiology Branch, National Institute on Drug Abuse, Intramural Research Program, National Institutes of Health, 5500 Nathan Shock Drive, Baltimore, MD 21224, U. S. A. ^cDepartment of Environmental Health Sciences, The Johns Hopkins University School of Public Health, Baltimore, MD 21205, U. S. A.

Abstract: A novel photoaffinity label for the dopamine transporter (DAT) based on N-substituted 3α -[bis(4'-fluorophenyl)methoxy]tropane has been synthesized in five steps and has been characterized. Preliminary binding studies indicated this ligand bound irreversibly to the dopamine transporter. Preparation of the ¹²⁵I analog and its photoactivation in the presence of membrane bound DAT demonstrated it covalently binds to the DAT. Published by Elsevier Science Ltd

Cocaine abuse and addiction in the United States and abroad is a serious health and social concern. The euphoric and reinforcing effects of cocaine are believed to be primarily due to its binding to the dopamine transporter (DAT) and inhibition of the uptake of dopamine. While there are many structurally diverse ligands that are known to bind to the DAT and inhibit the uptake of dopamine, the exact nature of these compound's interactions within the DAT primary sequence has not been elucidated.¹ The synthesis of compounds that probe the interaction of substrates with the DAT may furnish a functional characterization and ultimately lead to the development of a mechanistically based pharmacotherapeutic for cocaine abuse.

Recently, it was reported that photoaffinity labels based on GBR 12909 (DEEP, **1**, Figure 1) and RTI 55 (RTI 82, **2**) had different sites of incorporation in the DAT primary sequence.^{2,3} A class of dopamine uptake inhibitors based on 3α -(diphenylmethoxy)tropane have recently been described, which are hybrids of the RTI and GBR structures in that they contain the tropane ring of RTI 55 and the diphenyl ether moiety of GBR 12909.^{4,6} Meltzer has reported structure–activity relationships for a series of N-substituted-2-carbomethoxy- 3α -[bis(4'-fluorophenyl)methoxy]tropanes that appear to be correlated with the GBR analog series.⁷ Our group has recently reported on a series of N-methyl- 3α -(diphenylmethoxy)tropane analogs and has tested them in behavioral models of cocaine abuse.^{4,6} Unlike most dopamine uptake inhibitors, N-methyl- 3α -(diphenylmethoxy)tropane analogs typically do not generalize to a cocaine cue in animal models of drug abuse.^{4,5} Given this unique behavioral profile (GBR and RTI analogs are cocaine-like in the same behavioral model), the structural similarities between both GBR 12909 and RTI 55 and the structure–activity relationships for N-methyl- 3α -(diphenylmethoxy)tropanes, it would be useful to design a probe to explore the interaction of this ligand at the dopamine transporter. To this end, the

design, synthesis, and preliminary DAT binding studies of an N-substituted 3α -[bis(4'-fluorophenyl)methoxy]tropane-based photoaffinity label are reported herein.



We recently reported a series of N-substituted 3α -[bis(4'-fluorophenyl)methoxy]tropanes that bound with high affinity and selectivity to the dopamine transporter.⁸ The most potent and selective in this series was N-*n*butylphenyl- 3α -[bis(4'-fluorophenyl)methoxy]tropane (**3**). This compound could serve as a template for a photoaffinity label (**4**) since addition of substitutents (e.g., 4'-NO₂, 4'-NH₂) on the N-substituted aromatic ring did not substantially decrease binding affinity to the DAT (Table 1). Additionally, **4** could be readily obtained from commercially available materials using standard synthetic operations.

Table 1:

Compound #	N-Substitutent	K _i DAT (nM), (%error) ^a
3	4-phenyl-n-butyl	8.51 (14) ^b
5	4-(4'-nitrophenyl)-n-butyl	20.2 (11) ^b
6	4-(4'-aminophenyl)-n-butyl	29.66 (12)
4	4-(4'-azido-3'-iodophenyl)-n-butyl	159 (13)

^aEach K_i value represents data from at least three independent experiments, each performed in triplicate. ^bFrom reference 8.

Compound **4** was prepared in five steps as depicted in Scheme 1 by coupling 4-(4-nitrophenyl)butyric acid and nor- 3α [bis(4'-fluorophenyl)methoxy]tropane with DCC and HOBt to give amide **8**, which was reduced with allane to give amine **5** (67% from nor-amine and acid). The nitro group of **5** was converted to an amine by catalytic reduction with Pd/C (10%) in the presence of H₂ to give **6** (72%). The amino-iodo intermediate **9** was prepared by iodination of **6** using ICl in acetic acid (57%). Conversion to the azido-iodo analog **4** was accomplished using nitrous acid with sodium azide.⁹ The crude product was purified by crystallization of the oxalate salt in acetone and ether (75%).

Scheme 1



Compound 4 bound to the DAT with a $K_i = 159$ nM in rat caudate putamen. In binding experiments where 4 was equilibrated with rat caudate putamen and either photolyzed with UV light or not photolyzed, wash-resistant binding was observed. Consequently, it was not clear if 4 bound covalently to the DAT following photoactivation. One potential reason that 4 is wash resistant is that it is highly lipophilic and may be interacting with the DAT "pseudo-irreversibly."^{10,11} The [¹²⁵I]-4 analog was prepared which permitted the analysis of the radiolabelled protein by SDS-polyacrylamide gel electrophoresis and the direct demonstration of the interaction of 4 to the DAT.

As shown in Scheme 2, a one-flask radiosynthesis of $[^{125}I]$ -labeled 4 was performed using methodology previously described in detail for the preparation of $[^{125}I]$ -DEEP (1)¹² and $[^{125}I]$ -RTI 82 (2).¹³ In brief, electrophilic radioiodination of **6** with-NaI (2.05 mCi) under no-carrier-added conditions using chloramine-T as the oxidant was followed by diazotization and treatment with sodium azide. Isolation by reverse-phase HPLC afforded $[^{125}I]$ -4 in

G. E. AGOSTON et al.

good yield (76%) and purity (>99%). The radioligand displayed high specific activity (2075 mCi/ μ mol), and exhibited a chromatographic profile identical to that of non-radioactive 4.



DATs were photoaffinity labeled with [125 I]-4, using the procedures and analyses as described previously for [125 I]-DEEP (1) and [125 I]-RTI 82 (2).^{2,3} Figure 2 shows the results of labeling rat striatial membranes with either [125 I]-4 or [125 I] RTI-82. Both compounds labeled a similar spectrum of proteins, including a protein of about 80 kDa (see arrow, Figure 2), the molecular mass of DAT. Positive identification of this protein as DAT was performed by immunoprecipitation with a DAT-specific antibody (lanes 3 and 6). The immunoprecipitated [125 I]-4 labeled protein migrated with an identical molecular mass and appearance as authentic DAT labeled with [125 I] RTI 82 (2), and preimmune serum (lanes 2 and 4) did not recognize either the [125 I]-4 or [125 I] RTI 82 (2) labeled proteins. These results are strong evidence that the protein labeled with [125 I]-4 is DAT.

In summary, we have synthesized and characterized a novel DAT photoaffinity label based on N-substituted 3α [bis(4'-fluorophenyl)methoxy]tropane. This ligand bound with moderate affinity to the DAT and washout experiments demonstrated that this ligand bound "pseudo-irreversibly" with or without photoactivation. The ¹²⁵I analog of **4** was prepared and was shown to bind covalently to the DAT in membrane preparations following photoactivation as demonstrated by use of an immunoprecipitation assay. Further studies are currently underway to elucidate the binding domains of **4** on the DAT and determine whether this ligand accesses a binding domain that is similar to or distinct from those reported for DEEP (**1**) and RTI 82 (**2**). Further characterization of the DAT and correlations to the behavioral and pharmacological effects of these compounds may lead to the development of a treatment for cocaine abuse.

Scheme 2



Figure 2

Legend: Photoaffinity labeling of dopamine transporters with $[^{125}I]$ -4 and $[^{125}I]$ RTI 82 (2). Rat striatal membranes were photoaffinity labeled with $[^{125}I]$ -4 (lanes 1-3) or $[^{125}I]$ RTI 82 (2) (lanes 4–6), and subjected to immunoprecipitation with DAT antiserum. Lanes 1 and 4, total membranes; lanes 2 and 5, samples precipitated with preimmune serum; lanes 3 and 6; samples precipitated with immune serum.

Acknowledgment: We thank Cheryl Mayo for expert technical assistance. Animals used in this study were maintained in facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC) and all experimentation was conducted according to the guidelines of the Institutional Care and Use Committee of the Division of Intramural Research, National Institute on Drug Abuse, NIH and the Guide for Care and Use of Laboratory Animals of the Institute of Laboratory Animal Research Council, Department of Health, Education and Welfare, Publication (NIH) 85-23, revised 1985.

References:

1. Carroll, F. I.; Lewin, A. H.; Kuhar, M. J. Neurotransmitter Transporters: Structure, Function and Regulation; Reith, M. E. A., Ed.; Humana: Totowa, 1996, Chapter 9, pp 263–295.

2. Vaughan, R. A. Mol. Pharm. 1995, 47, 956.

- 3. Vaughan, R. A.; Kuhar, M. J. J. Biol. Chem. 1996, 271, 21672.
- 4. Newman, A. H.; Allen, A. C.; Izenwasser, S.; Katz, J. L. J. Med. Chem 1994, 37, 2258.
- 5. Newman, A. H.; Kline, R. H.; Allen, A. C.; Izenwasser, S.; George, C.; Katz, J. L. J. Med. Chem. 1995, 38, 3933.
- 6. Kline, R. H.; Izenwasser, S.; Katz, J. L.; Joseph, J. B.; Bowen, W. D.; Newman, A. H. J. Med. Chem. 1997, 40, 851.
- 7. Meltzer, P. C.; Liang, A. Y.; Madras, B. K. J. Med. Chem. 1996, 39, 371.
- 8. Agoston, G. E.; Wu, J. H.; Izenwasser, S.; George, C.; Katz, J. L.; Kline, R. H.; Newman, A. H. J. Med. Chem. 1997, in press.
- 9. Carroll, F. I.; Gao, Y.; Abraham, P.; Lewin, A. H.; Lew, R.; Patel, A.; Boja, J. W.; Kuhar, M. J. J. Med. Chem. 1992, 35, 1813.
- 10. Xu, H. K., C.-H.; Zhu, Y. C.; Weber, R. J.; Rice, K. C.; Rothman, R. B.; Neuropharm. 1991, 30, 455.
- 11. Ni, Q.; Xu, H.; Partilla, J. S.; Stark, P. A.; Carroll, F. I.; Brine, G. A.; Rothman, R. B. Synapse 1993, 15, 296.
- 12. Wilson, A. A.; Grioriadis, D. E.; Dannals, R. F.; Ravert, H. T.; Wagner, H. N., Jr. J. Labelled Compd. Radiopharm. 1989, 27, 1299.
- 13. Lever, J. R.; Carroll, F. I.; Patel, A.; Abraham, P.; Boja, J.; Lewin, A.; Lew, R. J. Labelled Compd. Radiopharm. 1993, 33, 1131.

(Received in USA 22 September 1997; accepted 24 October 1997)