Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **42**, 641-652 (1999)

(-)-6',7'-[^{11}C]Dihydroroten-12α-ol ((-)-[^{11}C]DHROL) for *In Vivo* Measurement of Mitochondrial Complex I

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SUMMARY

Deficits in Complex I (NADH-ubiquinone oxidoreductase) of the electron transport chain may play an important role in the inception and progression of neurodegenerative diseases such as Parkinson's disease. *In vivo* imaging of Complex I offers a unique method for evaluation of these changes in living human brain. Previous carbon-11 labeled rotenoids showed promising results, but were prepared as mixtures of stereoisomers at the 5'-position. We report here the stereospecific syntheses of (-)-6',7'-[^{11}C]dihydroroten-12α-ol ((-)-[^{11}C]DHROL), a modified rotenoid with *in vitro* affinity for Complex I. *O*-[^{11}C]methylation of the appropriate desmethyl precursor provided (-)-[^{11}C]DHROL in an average radiochemical yield, corrected to end of bombardment, of 27% (n = 4) and >99% radiochemical purity. In mice, (-)-[^{11}C]DHROL gave a high and uniform brain uptake similar to that obtained with prior radiolabeled rotenoids. Further *in vivo* evaluation of (-)-[^{11}C]DHROL in rats with unilateral quinolinic acid-induced striatal lesions showed significant losses of radioligand binding after neurotoxin treatment (lesion/unlesioned ratio of 0.66). As this reduction of *in vivo* radioligand binding is very similar to that obtained previously with the mixture of [^{11}C]DHROL isomers, the stereochemistry at the 5'-position of [^{11}C]DHROL does not significantly influence the *in vivo* applications of this radiotracer.

KEY WORDS: carbon-11; tomography, emission computed; mitochondria; rotenone; ubiquinone; quinolinic acid

INTRODUCTION

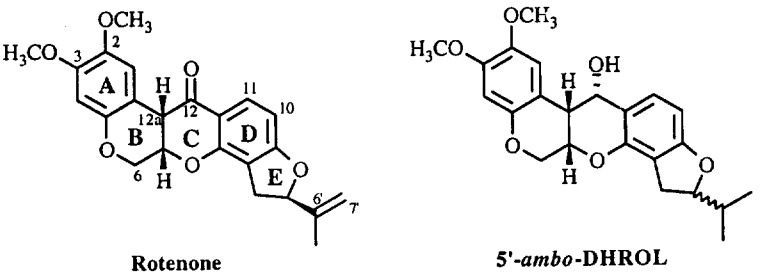
It is now well established that deficits in cellular oxidative metabolism are associated with neurodegenerative disorders such as Alzheimer's, Parkinson's and

CCC 0362–4803/99/070641–12$17.50

Received 18 December 1998
Revised 2 February 1999
Accepted 8 February 1999

Huntington's diseases (1-3). In Parkinson's disease, for example, losses of mitochondrial Complex I (NADH-ubiquinone oxidoreductase) are evident in the neurons of the substantia nigra (4). Such defects in Complex I would lead to compromised oxidative metabolism, production of reactive oxygen species (ROS) and eventually apoptosis (5,6). The gradual and progressive neuronal loss associated with such an oxidative stress event cascade agrees well with the delayed onset and slow progression of neurodegenerative disorders (2). This suggests a possible causal relationship for mitochondrial dysfunction in neurodegenerative processes rather than simply another symptom of neuronal degradation. Thus a focus of research in our laboratories has been the development of radiotracers for *in vivo* studies of various components of oxidative metabolism (7-9) and ROS production (10).

Rotenone (Figure 1) is a naturally occurring reversible noncompetitive Complex I inhibitor. Although rotenone can and has been labeled with carbon radioisotopes (7,11), its use as an *in vivo* radioligand may be limited by formation of radiolabeled metabolites. We thus designed 6',7'-dihydroroten-12-ol (DHROL), where reduction of the exocyclic 6',7'-double bond and the 12-oxo removes two primary sites of metabolism. *In vitro* binding studies using [^{3}H]dihydrorotenone and rat brain tissues showed that DHROL (prepared as the mixture of isomers at the 5'-position, termed 5'-*ambo*-DHROL) exhibited a binding affinity 6- to 16-fold lower than (-)-rotenone (DHROL IC_{50} = 77-113 nM; rotenone, IC_{50} = 8-20 nM). *In vivo* studies with 5'-*ambo*-[^{11}C]DHROL demonstrated losses of radioligand binding in a rat model of unilateral quinolinic-acid induced striatal lesions (9). Finally, chromatographic analysis of radioactivity in mouse brain homogenates revealed no radiolabeled metabolites (Charalambous and Kilbourn, unpublished data).



Rotenone **5'-*ambo*-DHROL**

Figure 1. Rotenoid structures including the common numbering system for rotenone.

Recently, the 5'-isomers of rotenone have been reported to exhibit a 10-fold difference in inhibitory potency in a bovine heart mitochondrial assay system, with the (-)-isomer being the more active (12). However, there is no information in the literature as to whether this stereoselectivity extends to other rotenoids, including a 12-hydroxy analog such as DHROL. We report here the stereospecific synthesis of (-)-[^{11}C]DHROL and subsequent evaluation of this radiotracer in a rat striatal lesion model to determine the stereoselectivity of binding to Complex I.

MATERIALS AND METHODS

Chemistry. ^{1}H NMR spectra, 2-dimensional ^{1}H-^{1}H Correlation Spectroscopy (COSY) and Nuclear Overhauser Effect (NOE) data were obtained with Brüker (300 or 500 MHz) NMR instruments. Chemical shifts are reported in δ values (parts per million) relative to an internal reference of tetramethylsilane (δ 0.0). Abbreviations used in NMR analyses are as follows: br d = broad doublet, br s = broad singlet, d = doublet, dd = doublet of doublets, m = multiplet, s = singlet, t = triplet, td = triplet of doublets. Electron ionization (EI) mass spectra were obtained on a V.G. Analytical 70-250S spectrometer and are within ± 0.0015 mass units of the calculated value. Melting points were performed on a Mel-Temp apparatus (Laboratory Devices, Cambridge, MA) and are uncorrected. Starting materials for chemical syntheses were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). Chromatography columns for HPLC analyses and purification were purchased from Phenomenex (Torrance, CA). Specific rotations for optically active compounds were measured in chloroform (unless otherwise noted) using a Perkin-Elmer 241 polarimeter and are an average of at least three readings. The specific rotations of commercially available (-)-rotenone (Aldrich) in this assay system were $[\alpha]_D^{23}$ -93° (c = 1.0) and $[\alpha]_D^{23}$ -116° (c = 2.1).

[^{11}C]Methyl trifluoromethanesulfonate ([^{11}C]methyl triflate) was prepared by the method of Jewett (13). All syntheses with carbon-11 were done in a remote apparatus which was manipulated from outside the shielded hot cell. Radiochemical yields were calculated based on the theoretical amount of [^{11}C]CO_2 produced at end of bombardment (EOB) and are decay corrected. Determinations of chemical and

radiochemical purity and specific activity were performed by comparisons of HPLC retention times and peak intensities with standard compounds of known concentration. Specific activity is reported at end of synthesis (EOS).

(-)-Roten-12-ol [1]. To a stirred suspension of $LiAlH_4$ (1.09 g of 95% $LiAlH_4$ powder, 27.29 mmol) in 30 mL of dry tetrahydrofuran, at 0 °C, was added commercially available natural rotenone (5.18 g, 13.13 mmol). The mixture was heated at reflux for 3 hours, cooled to room temperature and quenched with distilled H_2O. The resulting aqueous mixture was extracted with CH_2Cl_2 (3 x 75 mL) and the combined organic extracts were dried ($MgSO_4$), filtered and the solvent removed by rotary evaporation to provide a quantitative yield of **1** as an off-white solid which was used without further purification. mp 68-75 °C; $[\alpha]_D^{23}$ -157° (c = 1.0); ^{1}H NMR ($CDCl_3$): 7.08 (d, 1H, J = 8.1 Hz, C_{11}-H), 6.63 (s, 1H, C_1-H), 6.51 (s, 1H, C_4-H), 6.49 (d, 1H, J = 8.1 Hz, C_{10}-H), 5.24 (t, 1H, J = 8.8 Hz, $C_{5'}$-H), 5.12 (s, 1H, $C_{7'}$-H), 4.95 (br s, 2H, C_{12}-H and $C_{7'}$-H), 4.86 (ddd, 1H, J = 11.4, 6.1 and 5.3 Hz, C_{6a}-H), 4.65 (dd, 1H, J = 11.0 and 10.2 Hz, $C_{6\alpha}$-H), 4.26 (dd, 1H, J = 9.9 and 5.2 Hz, $C_{6\beta}$-H), 3.89 (s, 3H, C_2-OCH_3), 3.88 (s, 3H, C_3-OCH_3), 3.43 (br dd, 1H, J = 5.2 and 4.8 Hz, C_{12a}-H), 3.33 (dd, 1H, J = 15.7 and 9.7 Hz, $C_{4'\alpha}$-H), 2.99 (dd, 1H, J = 15.7 and 8.1 Hz, $C_{4'\beta}$-H), 1.82 (s, 3H, $C_{8'}$-CH_3), 1.71 (br s, 1H, C_{12}-OH); EIMS m/z (relative intensity): 396 (M^+, 5), 378 (7), 192 (100). Exact mass calculated for $C_{23}H_{24}O_6$ (M^+) 396.1573, found 396.1579.

(-)-6',7'-Dihydroroten-12α-ol [(-)-DHROL]. The crude **1** (403 mg, 1.018 mmol) was dissolved in 20 mL of ethyl acetate and hydrogenated overnight at atmospheric pressure over 40 mg of 5% platinum on activated carbon. TLC (silica, methanol / CH_2Cl_2, 5:95) showed a spot of Rf corresponding to starting material. However, filtration through Celite, solvent removal and purification *via* centrifugally accelerated radial chromatography (chromatotron; silica, CH_2Cl_2) provided 301 mg (74%) of a clear, colorless oil with a ^{1}H NMR spectrum corresponding to DHROL. mp 81-86 °C; $[\alpha]_D^{23}$ -181° (c = 1.1); ^{1}H NMR ($CDCl_3$): 7.06 (d, 1H, J = 8.1 Hz, C_{11}-H), 6.73 (s, 1H, C_1-H), 6.50 (s, 1H, C_4-H), 6.44 (d, 1H, J = 8.1 Hz, C_{10}-H), 4.95 (br d, 1H, J = 3.5 Hz, C_{12}-H), 4.86 (td, 1H, J = 11.4 and 5.2 Hz, C_{6a}-H), 4.65 (dd, 1H, J = 11.3 and 9.9 Hz, $C_{6\alpha}$-H), 4.58 (td, 1H, J = 8.8 and 7.0

Hz, $C_{5'}$-H), 4.26 (dd, 1H, J = 9.5 and 5.1 Hz, $C_{6\beta}$-H), 3.89 (s, 3H, C_2-OCH_3), 3.87 (s, 3H, C_3-OCH_3), 3.42 (br dd, 1H, J = 5.1 and 4.9 Hz, C_{12a}-H), 3.17 (dd, 1H, J = 15.6 and 9.2 Hz, $C_{4'\alpha}$-H), 2.88 (dd, 1H, J = 15.6 and 8.6 Hz, $C_{4'\beta}$-H), 2.00 (sextuplet, 1H, J = 6.7 Hz, $C_{6'}$-H), 1.72 (br s, 1H, C_{12}-OH), 1.07 (d, 3H, J = 6.6 Hz, $C_{7' \text{ or } 8'}$-CH_3), 1.01 (d, 3H, J = 6.8 Hz, $C_{7' \text{ or } 8'}$-CH_3); EIMS m/z (relative intensity): 398 (M^+, 5), 380 (9), 192 (100). Exact mass calculated for $C_{23}H_{26}O_6$ (M^+) 398.1729, found 398.1741.

(-)-*O*-Desmethyl-6',7'-dihydroroten-12α-ol [2]. (-)-DHROL (254 mg, 0.6382 mmol) and sodium methanethiolate (125 mg, 1.783 mmol) were dissolved in 4 mL of *N,N*-dimethylacetamide and heated at 80-95 °C for 26 h. The reaction mixture was diluted to 50 mL with distilled H_2O and extracted with CH_2Cl_2 (2 x 50 mL) to remove unreacted (-)-DHROL. The aqueous mixture was then acidified with 5% HCl extracted with CH_2Cl_2 (3 x 50 mL) and the combined organic extracts were dried, filtered and concentrated by rotary evaporation. This provided a dark oil which was purified by chromatotron (silica, 0-2% methanol / CH_2Cl_2) to yield 48 mg (20%) of **2** as a white solid which proved, by integration of the OCH_3 signals in the ^{1}H NMR spectrum, to be >94% the 2-*O*-monodesmethyl compound (approx. 16:1, 2-desmethyl/3-desmethyl). mp (mixture) 177-197 °C (dec); $[\alpha]_D^{23}$ -213° (c = 0.31, 10% DMSO in methanol); ^{1}H NMR (DMSO, major product): 8.86 (s, 1H, C_2-OH), 7.19 (s, 1H, C_1-H), 7.01 (d, 1H, J = 8.0 Hz, C_{11}-H), 6.27 (d, 1H, J = 8.1 Hz, C_{10}-H), 6.18 (s, 1H, C_4-H), 5.53 (s, 1H, C_{12}-OH), 5.00 (m, 1H, C_{12}-H), 4.76 (m, 1H, C_{6a}-H), 4.44 (m, 2H, $C_{6\alpha}$-H and $C_{5'}$-H), 4.10 (br d, 1H, J = 10.4 Hz, $C_{6\beta}$-H), 3.62 (s, 3H, C_3-OCH_3), 3.34 (m, 2H, C_{12}-OH and C_{12a}-H), 2.97 (dd, 1H, J = 15.6 and 9.1 Hz, $C_{4'\alpha}$-H), 2.72 (dd, 1H, J = 15.6 and 8.3 Hz, $C_{4'\beta}$-H), 1.85 (sextuplet, 1H, J = 6.5 Hz, $C_{6'}$-H), 0.93 (d, 3H, J = 6.4 Hz, $C_{7' \text{ or } 8'}$-CH_3), 0.89 (d, 3H, J = 6.5 Hz, $C_{7' \text{ or } 8'}$-CH_3); EIMS m/z (relative intensity): 384 (M^+, 3), 366 (4), 178 (100). Exact mass calculated for $C_{22}H_{24}O_6$ (M^+) 384.1573, found 384.1579.

(-)-6',7'-[^{11}C]Dihydroroten-12α-ol [(-)-[^{11}C]DHROL]. Based on the procedure of Charalambous, *et al.* (8) reported for [^{11}C]DHROL, approximately 1.0 mg of **2** was dissolved in 100 µL of dry *N,N*-dimethylformamide (DMF) and placed under N_2 in a 1.1 mL V-vial. To this was added 1 mole-equivalent of tetrabutylammonium hydroxide and the reaction vessel was placed in the remote

radiochemical synthesis apparatus. This precursor was then methylated by bubbling [^{11}C]methyl triflate, with N_2 as the carrier gas, through the DMF solution at room temperature until radioactivity accumulation in the reaction vial was maximal (approx. 3 min.). Purification of the crude radiolabeled mixture by semi-preparative scale HPLC (Ultremex C8, 10 x 250 mm, acetonitrile/methanol/water, 40:30:30, 3.5 mL/min, t_R = 9.3 min) and formulation with 10% ethanol in sterile isotonic saline provided (-)-[^{11}C]DHROL in a radiochemical yield of 27 ± 4 % (mean ± S.D., n = 4) corrected to EOB. HPLC analysis (Ultremex 5μ C8, 4.6 x 250 mm; acetonitrile/methanol/water, 40:30:30; 1.0 mL/min; UV = 254 nm; t_R = 5.5 min) of (-)-[^{11}C]DHROL *versus* a standard solution of unlabeled (-)-DHROL determined a radiochemical purity of >99% and a specific activity of 947 ± 376 Ci/mmole (mean ± S.D., n = 4) at EOS. This corresponded to a (-)DHROL concentration of 5-7 μg/mL. Total synthesis time was approximately 35 min.

***In vivo* brain uptake and distribution of radioactivity.** Determinations of regional brain retention of radioactivity for (-)-[^{11}C]DHROL were performed as previously reported for 5'-*ambo*-[^{11}C]DHROL (8).

***In vivo* striatal uptake of (-)-[^{11}C]DHROL.** Unilateral quinolinic acid-induced lesions of the left striatum of male Sprague-Dawley rats (150-200 g, Charles River, n = 7) were performed using previously reported procedures (9). Control animals (n = 4) were not injected. Studies of radiotracer binding using (-)-[^{11}C]DHROL and [^{14}C]iodoantipyrine ([^{14}C]IAP, DuPont) were performed two weeks after neurotoxin injection as described previously (9). Radioactivity concentrations in the neurotoxin injected sides were compared with those in the uninjected sides and are expressed as asymmetry ratios (ratio of radioactivity uptake in the left *versus* right striata).

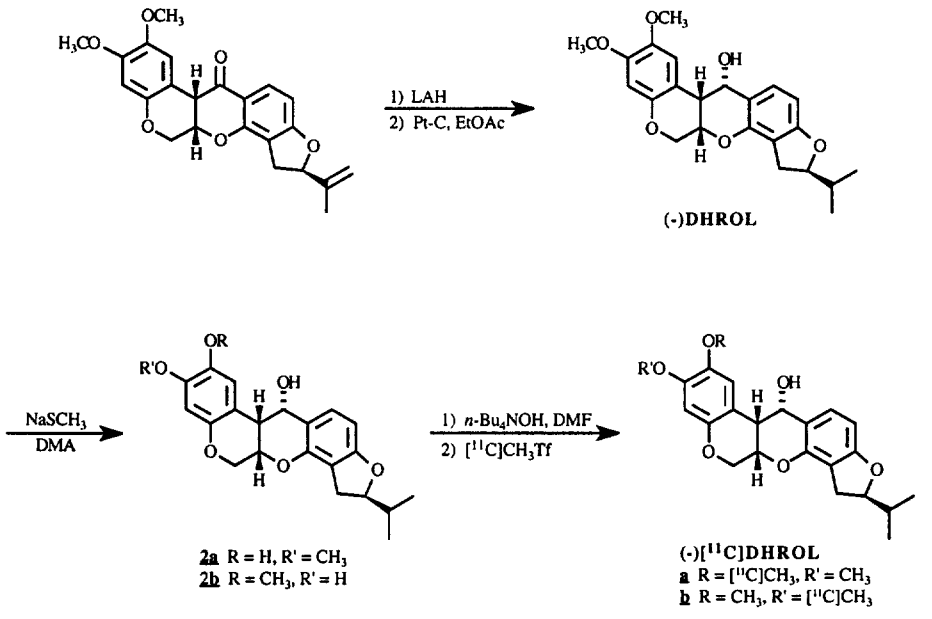
Statistical analyses. Regional differences in radiotracer distributions within animals were analyzed using the two-tailed, paired Student's *t*-test. The statistical significance of asymmetry ratios between treated and control groups was assessed using the unpaired, one-tailed Student's *t*-test assuming unequal variance. A p value of < 0.05 was considered significant. The correlation coefficient, r^2, between

(-)-[^{11}C]DHROL and [^{14}C]IAP was calculated by linear least squares regression analysis.

RESULTS AND DISCUSSION

Separation of the mixture of 5'-*ambo*-*O*-desmethyl-DHROL isomers synthesized previously (8) was attempted using a preparative scale Chirex (*S*)-valine/(*R*)-1-(α-naphthyl)ethylamine HPLC column. Although isomerically enriched samples could be isolated, this procedure proved cumbersome due to the small amounts of material which could be separated at one time and to the presence of several minor contaminants. It was therefore necessary to undertake the stereospecific synthesis of 2-*O*-desmethyl-(6a*S*,12*S*,12a*S*,5'*R*)6',7'-dihydroroten-12-ol for use as a precursor for (-)-[^{11}C]DHROL.

Preparation of (-)-[^{11}C]DHROL. As shown in Scheme 1, natural rotenone was subjected to $LiAlH_4$ reduction of the 12-oxo to the corresponding alcohol followed by selective catalytic hydrogenation of the 6',7'-alkene, using 5% platinum on activated carbon, to give (-)-DHROL. This compound has been reported previously in the literature (14), but had not been fully characterized: no polarimetry data were given and no attempt was made to verify the absolute stereochemistry. Assignment of ^{1}H NMR chemical shifts for the protons at C6, C6a, C12 and C5' was problematic due both to the complexity of the spectrum and to inconsistencies in the coupling constants among adjacent protons. Definitive connectivity and assignment of signals was determined using a 2-dimensional COSY spectrum. The $J_{6a,12a}$ and $J_{12,12a}$ coupling constants were both approximately 5 Hz, consistent with a *cis* ring fusion and the 12α-hydroxyl configuration (15). NOE experiments demonstrated an effect between the 5' and 4'α protons but not between 5' and 4'β. Likewise, an NOE was observed between 4'β and 6'. For the 7',8'-methyl protons, a stronger NOE was observed with 4'β than with 4'α. These data, along with the known absolute configuration of the starting material, (-)-rotenone (16), and large negative optical rotation of -181°, confirm the 6a*S*,12*S*,12a*S*,5'*R* configuration of (-)-DHROL.



Scheme 1. Synthesis of (-)-6',7'-dihydroroten-12α-ol ((-)-DHROL), (-)-2-/3-*O*-desmethyl-6',7'-dihydroroten-12α-ol (**2**)and (-)-[^{11}C]DHROL.

Selective monodemethylation of (-)-DHROL with sodium thiomethoxide in dimethylacetamide provided predominantly (>94%) the 2-*O*-desmethyl compound with no evidence of epimerization at the 5'-position as assayed by both ^{1}H NMR spectroscopy and polarimetry. The mixture of phenolic precursors was used without separation for the synthesis of (-)-[^{11}C]DHROL. The ratio of 2-[^{11}C]methoxy to 3-[^{11}C]methoxy labeled (-)-[^{11}C]DHROL produced from this mixture should be no lower than that of the precursor (16:1). That is, any difference in reactivity toward methylation between the 2-hydroxy and 3-hydroxy precursors would favor 2-methylation for the same reasons this position is favored in the demethylation reaction (17). Specifically, due to steric considerations, the 2-methoxy (or 2-hydroxy) substituent is out of plane with the rest of the molecule. This makes it both more accessible for reaction and disallows delocalization of the oxygen electrons into

the aromatic ring. Thus, the small amount of 3-*O*-labeled (-)-[^{11}C]DHROL (< 4%) should in no way detract from the utility of this radiotracer.

Labeling reactions were analogous to the previous report by Charalambous *et al.* (8) and provided similar results. (-)-[^{11}C]DHROL was synthesized at room temperature using [^{11}C]methyl triflate in a radiochemical yield of 27 ± 4 % (mean ± S.D., n = 4) corrected to EOB, >99% radiochemical purity and with a specific activity at EOS of 947 ± 376 Ci/mmole (mean ± S.D., n = 4). The actual concentration of (-)-DHROL in these preparations was 5-7 μg/mL. In the striatal lesioning experiments described hereafter, this corresponded to a dose in rats of 7-9 μg/kg. No toxicity data exists for DHROL, however the LD_{50} for *iv.* injection of rotenone in rats is 6 mg/kg, 650-850 times higher than the injected dose of (-)-DHROL. Based on the 6 to 16-fold lower binding affinity of DHROL *vs.* rotenone, the LD_{50} for DHROL is probably at least 4000-fold higher than the injected radiotracer dose. Thus, specific activity should not be an issue in these, or future, *in vivo* experiments.

***In vivo* evaluation of (-)-[^{11}C]DHROL.** Isomerically pure (-)-[^{11}C]DHROL was first examined *in vivo* to compare the brain pharmacokinetics and regional distribution in mice with previous results obtained using 5'-*ambo*-[^{11}C]DHROL. The whole brain pharmacokinetics for (-)-[^{11}C]DHROL and 5'-*ambo*-[^{11}C]DHROL were very similar; with no difference in retention of radioactivity for these two radiotracers at 60 min. (-)-[^{11}C]DHROL also exhibited the expected uniform distribution across all brain regions (data not shown).

(-)-[^{11}C]DHROL was then evaluated in rats bearing a unilateral quinolinic acid-induced striatal lesion, a model of loss of neuronal mitochondrial Complex I binding sites (9,18) with minimal effects on regional cerebral blood flow (9). As shown in Figure 2A, there was consistent loss of (-)-[^{11}C]DHROL binding in the lesioned striata, with an average asymmetry ratio (lesioned/unlesioned striatum) of 0.66 ± 0.10, which was significantly ($p < 0.012$) lower than the left/right ratios found in controls (1.05 ± 0.20). The decreases in (-)-[^{11}C]DHROL binding did not correlate ($r^2 = 0.034$) with the smaller, but still significant ($p < 0.044$), changes in cerebral blood flow (striatal asymmetry ratio for [^{14}C]IAP binding of 0.85 ± 0.13).

Comparison of the results obtained with (-)-[^{11}C]DHROL to those previously reported with 5'-*ambo*-[^{11}C]DHROL showed essentially identical changes in both rotenoid binding (Figure 2B) and striatal blood flow (data not shown).

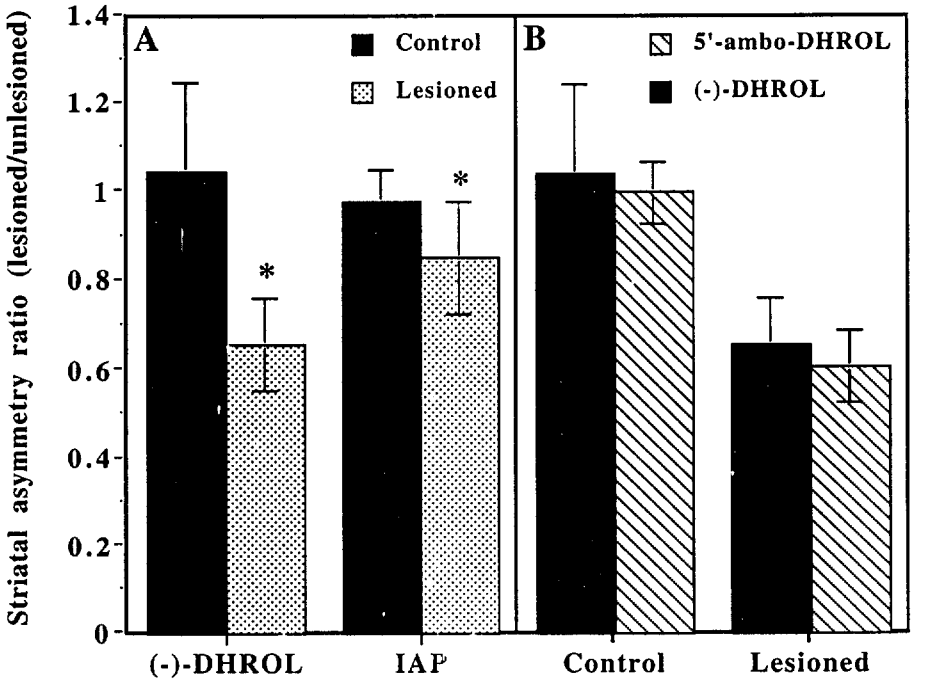


Figure 2. Striatal asymmetry ratios for control and lesioned male Sprague-Dawley rats. Studies were performed two weeks after unilateral quinolinic acid injections into the left striatum. Asymmetry ratio = (% inj. dose/g lesioned striatum)/(% inj. dose/g unlesioned striatum).

Panel A: Asymmetry ratios (mean ± S.D.) for (-)-[^{11}C]DHROL (30 min.) and [^{14}C]IAP (1 min.) in control and lesioned animals.

Panel B: Asymmetry ratios (mean ± S.D.) for (-)-[^{11}C]DHROL and 5'-*ambo*-[^{11}C]DHROL (9) at 30 min. post-injection of radiotracer.

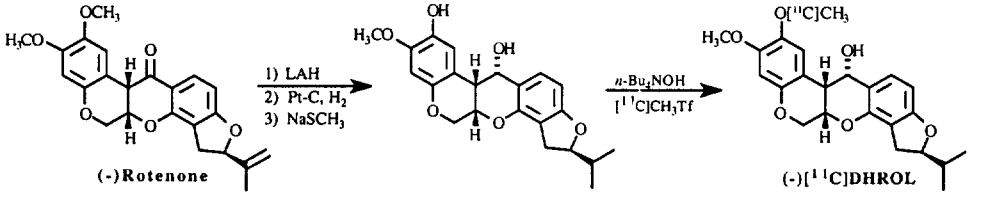
*$p < 0.05$ *vs.* controls

CONCLUSION

The synthesis of (-)-[^{11}C]DHROL allowed us to examine the effects of stereochemistry at the 5'-position of the rotenoid molecule on *in vivo* uptake and

specific binding of such radioligands to mitochondrial Complex I of the rodent brain. (-)-[^{11}C]DHROL exhibited good brain uptake and demonstrable *in vivo* specific binding in a rodent model of unilateral striatal mitochondrial loss.

The remarkable similarity of results between (-)-[^{11}C]DHROL and the previously prepared radioligand, 5'-*ambo*-[^{11}C]DHROL, was somewhat surprising given the large differences in activity reported by Ueno *et al.* (12) for the two 5'-isomers of rotenone. These results indicate that this stereoselectivity does not extend to the 12-hydroxy-6',7'-dihydro derivative in this *in vivo* experimental design. There are two interpretations of this observation. The DHROL molecule may be sufficiently different from rotenone that binding to Complex I is relatively insensitive to stereochemistry at the 5'-position. Alternatively, there may be only minimal non-specific distribution of [^{11}C]DHROL in brain tissues, and the residual [^{11}C]DHROL binding in quinolinic acid-lesioned striata may represent mitochondria in surviving terminals and cell bodies (19). Thus, the use of a stereospecific ligand (*e.g.* (-)-[^{11}C]DHROL) would not necessarily lead to an improved specific-to-nonspecific binding ratio *in vivo*. The fact that the *in vivo* data closely mirror the magnitude of *in vitro* estimates of mitochondrial loss in this quinolinic acid-induced lesion model (53% loss of 6',7'-[^{3}H]dihydrorotenone binding (9)) support this latter interpretation. Regardless of the origin, the lack of stereoselectivity for [^{11}C]DHROL binding in this rodent model is encouraging for the further application of this radiolabeled rotenoid as an *in vivo* radiotracer for Complex I.



ACKNOWLEDGMENTS

This work was funded through generous support by the National Institutes of Health grants NS 15655 and MH 47611. The authors thank Dr. Robert Koeppe for valuable assistance with statistical analyses.

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