European Journal of Pharmacology, 194 (1991) 133–134

ADONIS 001429999100213T

EJP 0293R

Rapid communication

[^{125}I]RTI-55: a potent ligand for dopamine transporters

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Received 28 January 1991, accepted 29 January 1991

Several binding ligands for dopamine transporters have been identified (see Madras et al., 1989 and Ritz et al., 1990 for references). Recently, we have identified a series of cocaine analogues that possess nanomolar potency for the transporter (Boja et al., 1990). One of these compounds, RTI-55 (3β-(4-iodophenyl)-tropane-2-carboxylic acid methyl ester), an iodinated analogue of cocaine and WIN 35,428, was selected for binding studies.

RTI-55 was synthesized by procedures analogues to those of Clarke et al. (1973) and obtained in radiolabeled form (specific activity of 2200 Ci/mmol) from New England Nuclear Corp (Boston, MA, USA). Ligand binding procedures were carried out as described by Boja et al. (1990) except that tissue concentrations were 0.1 mg (orig. wet weight) per 2.0 ml final assay volume, and that incubations were carried out at room temperature for 50 min, a time at which equilibrium was reached. For saturation studies, concentrations of unlabeled ligand were varied from 100 nM to 0.1 pM, and the concentration of [^{125}I]RTI-55 was 10 pM. Blanks were obtained using 50 μM (−)-cocaine.

Saturation analysis of indicated a two binding site model was statistically preferred over a one site model ($P < 0.005$) in male Sprague-Dawley rat striatum (Harlan Laboratories, Indianapolis, IN). Scatchard transformation of the data revealed a high affinity binding site with a K_d values of 0.11 ± 0.01 nM (mean ± S.E., $n = 6$) and a B_{max} of 0.16 ± 0.02 pmol/mg tissue (orig. wet weight) and a low affinity binding site with a K_d value of 2.57 ± 0.30 nM and a B_{max} of 0.57 ± 0.03 pmol/mg tissue. Specific binding in striatum was destroyed by exposing the tissue to 100 °C for 5 min and was not detected in cerebellar tissue. Specific binding was linear with increasing tissue concentration in the range of 0.006-0.2 mg tissue/ml.

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The binding of [^{125}I]RTI-55 had the pharmacological characteristics associated with the dopamine transporter. (−)-Cocaine exhibited an IC_{50} of 65.49 ± 6.62 nM (mean ± S.E., $n = 4$), which was about 100 times less than that of (+)-cocaine (7041.67 ± 537.19 nM). Other potent inhibitors of dopamine transport such as mazindol and GBR 12909 were also potent inhibitors of the binding ($IC_{50} = 6.72 \pm 0.86$ and 0.78 ± 0.04 nM respectively). However, haloperidol, a dopamine receptor blocker, desipramine, a norepinephrine transport blocker, and citalopram, a serotonin transport blocker were not potent inhibitors of [^{125}I]RTI-55 binding ($IC_{50} = 792 \pm 1$, 1591 ± 93 and 8708 ± 450 nM respectively).

[^{125}I]RTI-55 is perhaps the most potent ligand for the dopamine transporter utilized thus far. Its high specific activity and its availability in iodinated form has advantages such as the elimination of tissue quenching observed with tritium in autoradiographic experiments. It also can be used as an in vivo labeling ligand (in

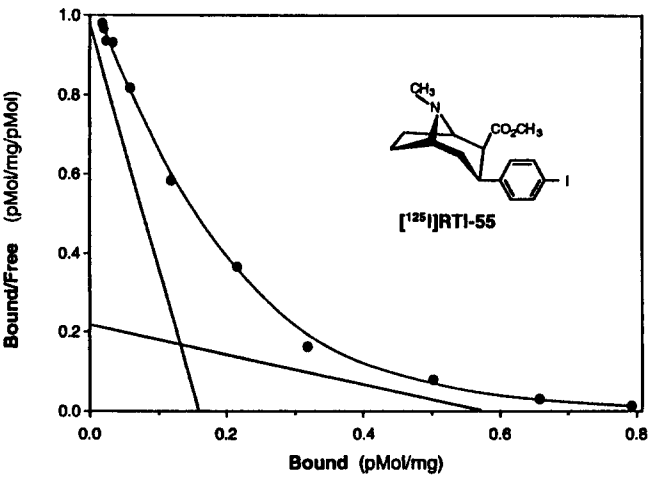

Fig. 1. Scatchard plot of saturation binding data obtained from a fixed concentration of [^{125}I]RTI-55 (10 pM) and 11 concentrations the unlabelled drug (100 nM-0.1 pM). The plots were drawn from values obtained by non-linear least squares analysis with LIGAND. Inset: Structure of [^{125}I]RTI-55.

preparation) and therefore may have application in PET and SPECT scanning.

Acknowledgements

This work was supported in part by NIDA Grant DA05477 to F. Ivy Carroll.

References

Boja, J.W., F.I. Carroll, M.A. Rahaman, A. Philip. A.H. Lewin and M.J. Kuhar, 1990, New, potent cocaine analogs: ligand binding and transport studies in rat striatum, European J. Pharmacol. 184, 329.

Clarke, R.L., S.J. Daum, A.J. Gambino, M.D. Aceto, J. Pearl, M. Levitt, W.R. Cumiskey and E.F. Bogado, 1973, Compounds affecting the central nervous system. 4. 3-Beta-phenyltropane-2-carboxylic esters and analogs, J. Med. Chem. 16, 1260.

Madras, B.K., R.D. Spealman, M.A. Fahey, J.L. Neumeyer, J.K. Saha and R.A. Milius, 1989, Cocaine receptors labeled by [^{3}H]2β-carbomethoxy-3β-(4-fluorophenyl)tropane, Mol. Pharmacol. 36, 518.

Ritz, M.C., J.W. Boja, D. Grigoriadis, R. Zaczek, F.I. Carroll, A.H. Lewin and M.J. Kuhar, 1990, [^{3}H]WIN 35,065-2: A ligand for cocaine receptors in striatum, J. Neurochem. 55, 1556.