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PRELIMINARY COMMUNICATION

INHIBITION OF [^{3}H] MPTP BINDING TO RAT BRAIN BY PARGYLINE.

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MPTP (1-Methyl-4-phenyl-1,2,5,6-tetrahydropyridine), a by-product in the synthesis of MPPP (1-Methyl-4-phenyl-4-propinoxy-piperidine), induces parkinsonism in man (1), rhesus monkey (2) and squirrel monkey (3).

Recently, some authors demonstrated by quantitative autoradiography that a high-affinity, saturable binding site exists for [^{3}H]-MPTP in rat brain (4).

We evaluated and report here some molecular properties that can be related to the mechanism of MPTP toxicity. Our studies confirm that [^{3}H]-MPTP binding sites are present in rat brain and suggest that inhibitors of monoamine oxidase (MAO) may interact with these sites. In our experiments brain tissue from male Sprague-Dawley rats was used. The tissue was homogenized using a polytron in 50 volumes (w/v) of ice-cold 0.05 M Tris HCl buffer (pH 7.0). Tissue pellets were prepared by centrifuging homogenates at 17,300 x g for 10 min. Tissue pellets were washed, centrifuged and resuspended twice with 50 volumes of ice-cold Tris HCl (0.05 M). The pellets were finally suspended in 50 volumes of buffer for the assay. Aliquots of homogenate were used for the samples. Proteins were determined according to Lowry et al (5).

The [^{3}H]-MPTP binding assay on brain homogenates was measured using an incubation volume of 500 µl consisting, in the routine assay, of the aliquots of homogenate and [^{3}H]-MPTP (85 Ci/mmole, New England Nuclear) at a final concentration of 5 nM. Each assay contained 50-70 µg tissue proteins. The assays were performed in 0.05 M Tris HCl (pH 7.0). Incubation was carried out at 4°C for 30 min. and then 7 ml of ice-cold Tris HCl buffer was added to each tube and the samples were immediately filtered through Whatman GF/B filters under low vacuum. The filtration was followed by two 7 ml rinses with ice-cold buffer. The filters were suspended in scintillation fluid and radioactivity was measured with a liquid scintillation spectrometer. The assay was performed in triplicate. Specific binding is defined as the difference between total binding and binding in the presence of 10 uM unlabeled MPTP (Aldrich).

When the brain homogenates were incubated with increasing concentrations of $[^3H]$-MPTP (0.5 to 280 nM), a specific binding was obtained. Saturation isotherm was reached by the specific binding but not by the non-specific one which increased linearly (data not shown) (Fig.1).

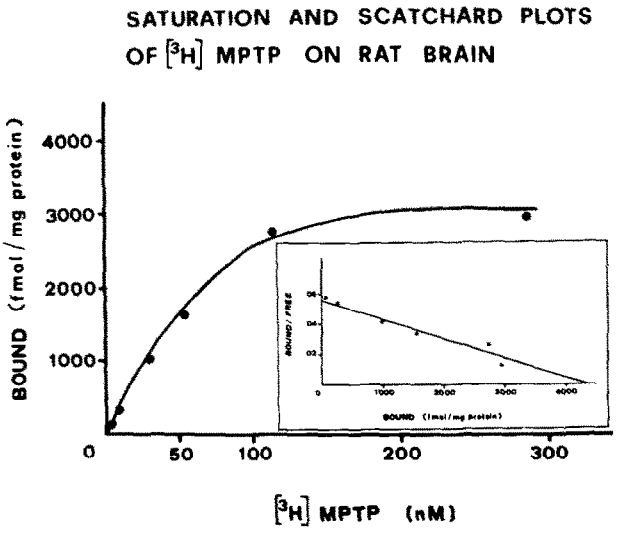

FIG.1. Saturation isotherm and Scatchard analysis (insert) of $[^3H]$-MPTP binding to rat brain homogenate. Data of 4 independent experiments are shown. Bound = fmoles of specifically bound $[^3H]$ MPTP per mg protein. Free = concentration of $[^3H]$ MPTP in the incubation medium.

Scatchard analysis resulted in one straight line, indicating an apparently homogenous population of binding sites. The apparent K_D was 87.7 nM and the Bmax was 4315 fmol/mg protein.

At a concentration of 1 µM, a variety of compounds such as dopamine, apomorphine, (+) butaclamol, (-) sulpiride, amphetamine, norepinephrine and verapamil failed to inhibit the $[^3H]$ MPTP binding to brain homogenates. On the contrary, pargyline, clorgyline and deprenyl displaced specific $[^3H]$ MPTP binding as shown in Fig.2.

It is noteworthy that pargyline and the selective inhibitor of type A MAO, clorgyline, inhibited specific $[^3H]$ MPTP binding at concentrations similar to those of unlabeled MPTP.

Moreover, other data from our lab demonstrated that MPTP decreases DOPAC formation both in vivo and in vitro, suggesting that this compound

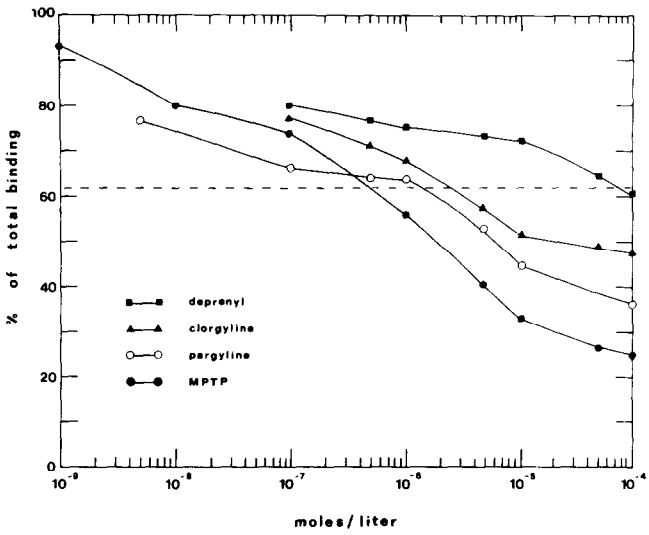

Fig.2. Inhibition of $[^3H]$ MPTP binding by different drugs. The concentration producing 50% inhibition of specific $[^3H]$ MPTP binding (IC_{50}) was: MPTP = .3 ± .01 μM; Pargyline = .52 ± .04 μM; Clorgyline = .66 ± .03 μM; Deprenyl = 9 ± .7 μM. In the above data approximately 74% of total binding was specific. These values are mean values (± SD) of at least three determinations.

inhibits MAO activity (M.P.Piccardi and G.U.Corsini, manuscript in preparation). In conclusion, these data suggest that the MPTP binding sites may be related to MAO activity and MAO activity may therefore be crucial for MPTP neurotoxicity.

REFERENCES

1) J.W.LANGSTON, P.BALLARD, J.W.TETRUD, I.IRWIN. Science, 219, 979 (1983).

2) R.S.BURNS, C.C.CHIUEH, S.P.MARKEY, M.H.EBERT, D.J.JACOBOWITZ, I.J.KOPIN. Proc.Natl.Acad.Sci.USA 80, 4546, (1983).

3) J.W.LANGSTON, L.S.FORNO, C.S.REBERT, I.IRWIN. Brain Research 292, 390, (1984).

4) C.M.WIECZOREK, B.PARSONS, T.C.RAINBOW. Eur.J.Pharmacol. 98, 453, (1984).

5) O.H.LOWRY, N.J.ROSENBROUGH, A.L.FARR, R.J.RANDALL. J.Biol.Chem. 193, 265, (1951).