ELSEVIER

European Journal of Pharmacology 337 (1997) 111–114

ejp

Short communication

Evaluation of monoamine oxidase B inhibition by fluoxetine (Prozac): An in vitro and in vivo study

Jogeshwar Mukherjee *, Zhi-Ying Yang

Department of Radiology, MC 1037, Franklin McLean Institute, The University of Chicago, 5841 S. Maryland Avenue, Chicago, IL 60637, USA

Received 10 April 1997; revised 31 July 1997; accepted 1 August 1997

Abstract

Inhibition of monoamine oxidase B was investigated both in vitro and in vivo in rats by using the radioligand, *N*-(6-[^{18}F]fluorohexyl)-*N*-methylpropargylamine ([^{18}F]FHMP). Binding affinities of five compounds, deprenyl, clorgyline, fluoxetine, norfluoxetine and citalopram were studied. Fluoxetine and norfluoxetine showed affinities of 17 and 13 μM for monoamine oxidase B, respectively. Acute doses of fluoxetine and norfluoxetine (20 mg/kg) also significantly inhibited (10 to 15%) the binding of the radioligand in vivo while citalopram showed lower affinity (140 μM) for monoamine oxidase B and little effect in vivo. The in vivo effects of the various drugs were directly comparable to their in vitro affinities for binding to monoamine oxidase B in the correlation plot of percent control in vivo binding of [^{18}F]FHMP and binding affinity, $-\log IC_{50}$ ($R^2 = 0.989$). These results provide evidence for a potential role of monoamine oxidase B inhibition in the therapeutic effects of Prozac. © 1997 Elsevier Science B.V.

Keywords: Fluoxetine; Norfluoxetine; Monoamine Oxidase B; *N*-(6-[^{18}F]fluorohexyl)-*N*-methylpropargylamine

1. Introduction

Three classes of antidepressant drugs are known: (1) the tricyclic antidepressants, (2) the monoamine oxidase inhibitors, and (3) the selective serotonin uptake inhibitors. Fluoxetine (Prozac) belongs to the class of selective serotonin uptake inhibitors, and has been shown to be highly efficacious with little of the side effects that are known to occur with the other two classes of antidepressants (Harvey et al., 1992). Fluoxetine and its active metabolite, norfluoxetine, inhibit serotonin uptake with affinities of 20 and 44 nM, respectively, and they have lower (micromolar) affinities for the inhibition of dopamine and norepinephrine uptake sites (Wong et al., 1993). Fluoxetine thus exerts its therapeutic effect as a result of the inhibition of serotonin uptake sites (Wong et al., 1995). It has also recently been shown that fluoxetine has an inhibitory effect on monoamine oxidase A and B (particularly monoamine oxidase B, $IC_{50} = 80$ μM) and it has been suggested that this action of fluoxetine may also contribute to its antidepressant properties (Leonardi and Azmitia, 1994). We report here our findings on the in vitro and in vivo inhibition of monoamine oxidase B by fluoxetine and norfluoxetine using a fluorine-18 labeled radioligand, *N*-(6-[^{18}F]fluorohexyl)-*N*-methylpropargylamine ([^{18}F]FHMP) that binds to monoamine oxidase B.

2. Materials and methods

In vitro binding affinities of five compounds ((*R*)-deprenyl and clorgyline (from Research Biochemicals International), citalopram, fluoxetine and norfluoxetine were gifts) to monoamine oxidase B in rat (Sprague–Dawley) brain homogenates were carried out by incubating various concentrations (0.01 nM to 0.1 mM) of the compounds along with the radioligand, [^{18}F]FHMP (a fluorinated analog of *N*-hexyl-*N*-methylpropargylamine (Yu et al., 1993), that binds selectively to monoamine oxidase B; Mukherjee et al., manuscript in preparation). Rat brains were isolated and homogenized with a Tekmar Tissumizer (15 s at half-maximum speed) in a 100-fold (w/v) dilution of a 50 mM Tris HCl buffer, pH 7.4, containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM NaEDTA, and 0.1 mM Na ascorbate. The homogenate was centrifuged at $12\,000 \times g$ for 15 min at 4°C. The pellet was

* Corresponding author. Tel.: (1-937) 296-7211; Fax: (1-937) 296-4265
e-mail: j-mukherjee@uchicago.edu

resuspended in the same volume of buffer, centrifuged a second time, and resuspended in fresh buffer at a concentration of 50 mg of tissue/ml. Each assay tube contained 0.10 ml of this stock solution. Binding was initiated by addition of the tissue homogenate, and the tubes were incubated for one hour at 37°C. The binding was terminated by filtration using a Brandel filtration apparatus, followed by washings with cold 50 mM Tris–HCl buffer (4×1 ml). Non-specific binding was determined in the presence of 10 μM (*R*)-deprenyl. The filters were counted in a well-counter for fluorine-18 activity. The data was analysed using Ligand and IC_{50} values for the various drugs were obtained (Munson and Rodbard, 1980). The binding curves were displayed using GraFit.

For in vivo studies, groups ($n = 4$) of Sprague–Dawley rats (250 g) were administered with the five compounds at the following doses: (*R*)-deprenyl 10 mg/kg, clorgyline 10 mg/kg, citalopram 20 mg/kg, fluoxetine 20 mg/kg and norfluoxetine 20 mg/kg (acute doses of fluoxetine 20 mg/kg and norfluoxetine 20 mg/kg were chosen based on published reports (Caccia et al., 1990)). All compounds (including saline, for control rats) were administered intraperitoneally under anesthesia (brief exposure to vapors of diethyl ether), 90 min prior to injection of the radioligand and the rats were allowed free access to food and water during the interval. The radioligand, [^{18}F]FHMP, 88 μCi (specific activity 1 Ci/μmol), was administered intravenously into each rat under anesthesia. The rats were subsequently allowed to recover and had free access to food and water. All rats were sacrificed 90 min after the radioligand injection (since a maximal ratio between brain regions and blood levels of [^{18}F]FHMP was seen at approximately 90 min) and the various brain regions (striata, cortex, thalamus, rest of cerebrum and cerebellum) were isolated into tared vials and counted for fluorine-18 activity in order to provide a percent of injected dose of [^{18}F]FHMP/g of wet tissue for each group of rats. A correlation of in vivo binding of [^{18}F]FHMP (expressed as percent control) and binding affinity (expressed as $-\log IC_{50}$) of the various inhibitors was generated.

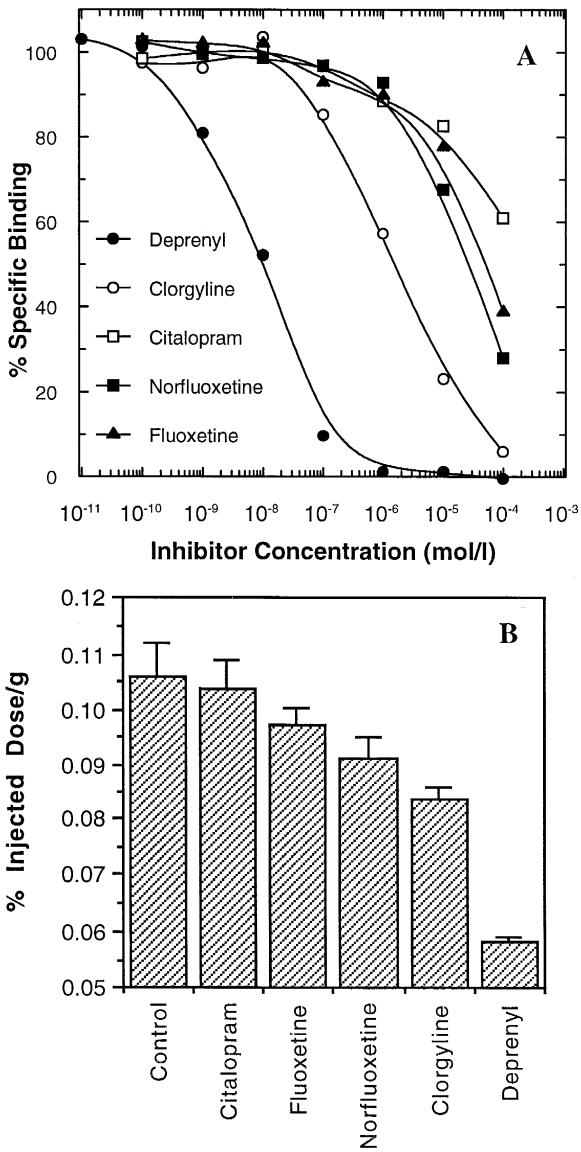

Fig. 1. (A) In vitro binding of the compounds to monoamine oxidase B measured by using [^{18}F]FHMP in rat brain homogenates. (B) In vivo binding of [^{18}F]FHMP in the rat brains (cerebrum and cerebellum) shown as percent of injected dose/g of wet tissue. Rats were pretreated either with saline (control) or the various drugs (citalopram, fluoxetine, norfluoxetine, clorgyline and (*R*)-deprenyl) administered i.p. 90 min prior to the radioligand. All rats were sacrificed 90 min post-i.v. injection of the radioligand, [^{18}F]FHMP.

3. Results

The in vitro binding profiles of the various compounds are shown in Fig. 1a. As expected, (*R*)-deprenyl which is a potent monoamine oxidase B inhibitor (Knoll and Magyar, 1972) showed the highest affinity ($IC_{50} = 6.8$ nM). Clorgyline, which is a monoamine oxidase A selective agent exhibited a significantly lower affinity ($IC_{50} = 1.2$ μM). This is indicative of the selective labeling of monoamine oxidase B sites by the radioligand, [^{18}F]FHMP, which is similar to the reported alkylated analogous of *N*-methyl-propargylamines (Yu et al., 1993). Both, fluoxetine and norfluoxetine had approximately similar affinities ($IC_{50} =$ 17 and 13 μM, respectively), whereas citalopram exhibited weaker binding ($IC_{50} = 0.14$ mM). The in vivo effects of the various compounds on the binding of [^{18}F]FHMP in the rat brains are shown in Fig. 1b. The effect of (*R*)-deprenyl on the binding of [^{18}F]FHMP was dramatic (binding was reduced to 54.7% compared to controls), consistent with the high affinity of (*R*)-deprenyl for monoamine oxidase B in vitro. There was no measurable effect of citalopram compared to controls on the binding of [^{18}F]FHMP which is also consistent with the lower affinity of citalopram for monoamine oxidase B in vitro. However, the remaining three compounds, clorgyline, norfluoxetine

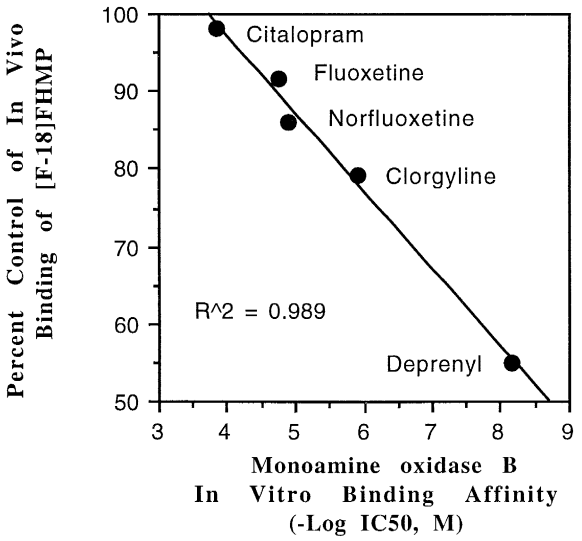

Fig. 2. Correlation of the percent control of in vivo binding of [^{18}F]FHMP versus monoamine oxidase B binding affinities ($-\log IC_{50}$) of the various inhibitors ($y = 137.2 - 10.000x$, $R^2 = 0.989$).

and fluoxetine reduced the binding of [^{18}F]FHMP in all the brain regions significantly (79.2%, 85.8% and 91.5%, respectively) compared to controls. This in vivo effect is directly comparable to their in vitro affinities for binding to monoamine oxidase B as can be seen in the correlation plot ($R^2 = 0.989$) shown in Fig. 2.

4. Discussion

Fluoxetine has been shown to compete in vivo at sites labeled with [^{3}H]paroxetine (an agent known to bind at the serotonin uptake sites) as well as inhibit serotonin uptake (Scheffel and Hartig, 1989; Wong et al., 1993; Wong et al., 1995). This action of fluoxetine as well as other antidepressants such as citalopram, paroxetine and sertraline on the serotonin uptake sites has been suggested to account for their therapeutic effect (Harvey et al., 1992; Gurevich and Joyce, 1996). However, the late onset of action of these drugs has raised questions on the direct involvement of the serotonin uptake blockade in alleviating depression and if there are other secondary mechanisms which may also be involved in their therapeutic action (Cooper et al., 1996).

Inhibition of the in vivo binding of [^{18}F]FHMP by the monoamine oxidase inhibitors, (*R*)-deprenyl and clorgyline related well to their affinities for monoamine oxidase B sites (Knoll and Magyar, 1972; Saura et al., 1992). However, citalopram had little effect on the in vivo binding of [^{18}F]FHMP. Norfluoxetine had a slightly greater effect than fluoxetine as can be seen in the correlation plot of monoamine oxidase B binding affinity versus in vivo potency of the various drugs, shown in Fig. 2. Since norfluoxetine is a major metabolite of fluoxetine (Caccia et al., 1990; Wong et al., 1993), this inhibition of monoamine oxidase B by norfluoxetine accompanied by its inhibition of the serotonin uptake sites (Wong et al., 1993), may be very significant therapeutically. In this limited study, it is interesting to compare the two antidepressant drugs, citalopram and fluoxetine. Clearly, the ability of citalopram to interact with monoamine oxidase B is significantly smaller compared to that observed with fluoxetine.

Monoamine oxidase B is distributed in several serotonergic and dopaminergic brain regions and high concentrations of this enzyme have been found in the cell bodies of raphe and in glia cells in rat as well as human brain (Konradi et al., 1988; Saura et al., 1992). Dopamine is a substrate for monoamine oxidase B and is known to play a significant antidepressant role (Kapur and Mann, 1992). Thus, by inhibiting monoamine oxidase B, Prozac could potentially enhance dopaminergic neurotransmission. Additionally, although serotonin has been known to be a poor substrate for monoamine oxidase B (Yu, 1986), inhibition of this enzyme in serotonergic regions by Prozac could potentially have indirect therapeutic effects (Saura et al., 1992).

Using fluorine-19 magnetic resonance spectroscopic methods, it has been shown that the human brain deposits significant levels of fluoxetine and norfluoxetine, and that brain to blood ratio can be as high as 20 in subjects who received doses of fluoxetine in the range of 20 to 40 mg per day (Karson et al., 1993). The brain concentrations of fluoxetine/norfluoxetine were found to range up to 10.7 μg/ml, which is approximately equivalent to a concentration of 35 μM (Karson et al., 1993). It has also been shown that fluoxetine as well as its metabolite, norfluoxetine, are present in subcellular components of the rodent brain, such as the nuclei, mitochondria and synaptosomes (Caccia et al., 1990). Thus, with high subcellular concentrations and micromolar affinities for monoamine oxidase B, both fluoxetine and norfluoxetine have the potential to exert a significant inhibitory effect on the enzyme. Our findings, using a single acute dose of fluoxetine and norfluoxetine, on the in vivo inhibition of binding of [^{18}F]FHMP to monoamine oxidase B provide direct evidence to the monoamine oxidase inhibition hypothesis of Prozac. These findings should therefore assist in the development of the unique 'Prozac-like drugs' that take into account both the serotonin uptake inhibition as well as monoamine oxidase inhibition characteristics of Prozac.

Acknowledgements

Financial support was provided by US Department of Energy DE-FG02-94ER61840. We like to thank Dr. E. Cook, Dr. Robert Lew and Dr. Lewis Seiden for samples of fluoxetine, norfluoxetine and citalopram, Dr. Malcolm Cooper and Dr. John Metz for helpful discussions and

Robert Mintzer and Nicholas Yasillo for technical assistance.

References

Caccia, S., Cappi, M., Fracasso, C., Garattini, S., 1990. Influence of dose and route of administration on the kinetics of fluoxetine and its metabolite norfluoxetine in the rat. Psychopharmacology 100, 509–514.

Cooper, J.R., Bloom, F.E., Roth, R.H., 1996. The Biochemical Basis of Neuropharmacology, 7th ed. Oxford University Press, New York, NY, pp. 487–495.

Gurevich, E.V., Joyce, J.N., 1996. Comparison of [^{3}H]paroxetine and [^{3}H]cyanoimipramine for quantitative measurement of serotonin transporter sites in human brain. Neuropsychopharmacology 14, 309–323.

Harvey, R.A., Champe, P.C., Mycek, M.J., Gertner, S.B., Perper, M.M., 1992. Pharmacology. J.B. Lippincott, New York, NY, pp. 119–126.

Kapur, S., Mann, J.J., 1992. Role of the dopaminergic system in depression. Biol. Psychiatry 32, 1–17.

Karson, C.N., Newton, J.E.O., Livingston, R., Jolly, J.B., Cooper, T.B., Sprigg, J., Komoroski, R.A., 1993. Human brain fluoxetine concentrations. J. Neuropsychiatry Clin. Neurosci. 5, 322–329.

Knoll, J., Magyar, K., 1972. Some puzzling pharmacological effects of monoamine oxidase inhibitors. Adv. Biochem. Psychopharmacol. 5, 393–408.

Konradi, C., Svoma, E., Jellinger, K., Riederer, P., Denney, P., Thibault, J., 1988. Topographic immunocytochemical mapping of monoamine oxidase A, monoamine oxidase B and tyrosine hydroxylase in human post mortem brain stem. Neuroscience 26, 791–802.

Leonardi, E.T.K., Azmitia, E.C., 1994. MDMA (Ecstasy) inhibition of MAO type A and type B: Comparisons with fenfluramine and fluoxetine (Prozac). Neuropsychopharmacology 10, 231–238.

Munson, P.J., Rodbard, D., 1980. LIGAND: A versatile approach for the characterisation of ligand binding systems. Anal. Biochem. 107, 220–239.

Saura, J., Kettler, R., Da Prada, M., Richards, J.G., 1992. Quantitative enzyme radioautography with [^{3}H]Ro 41-1049 and [^{3}H]Ro 19-6327 in vitro: Localization and abundance of MAO-A and MAO-B in rat CNS, peripheral organs, and human brain. J. Neurosci. 12, 1977–1999.

Scheffel, U., Hartig, P.R., 1989. In vivo labeling of serotonin uptake sites with [^{3}H]paroxetine. J. Neurochem. 52, 1605–1612.

Wong, D.T., Bymaster, F.P., Reid, L.R., Mayle, D.A., Krushinski, J.H., Robertson, D.W., 1993. Norfluoxetine enantiomers as inhibitors of serotonin uptake in rat brain. Neuropsychopharmacolgy 8, 337–344.

Wong, D.T., Bymaster, F.P., Engleman, E.A., 1995. Prozac (fluoxetine, Lilly 110140), the first selective serotonin uptake inhibitor and an antidepressant drug; Twenty years since its first publication. Life Sci. 57, 411–441.

Yu, P.H., 1986. Monoamine oxidase. In: Boulton, A.A., Baker, G.B., Yu, P.H. (Eds.), Neuromethods: Neurotransmitter Enzymes, vol. V. Humana Press, Clifton, NJ, pp. 235–272.

Yu, P.H., Davies, B.A., Boulton, A.A., 1993. Effect of structural modification of alkyl N-propargylamines on the selective inhibition of monoamine oxidase B activity. Biochem. Pharmacol. 46, 753–757.