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Monoamine Oxidase A Inhibition by Fluoxetine: An In Vitro and In Vivo Study

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KEY WORDS fluoxetine (Prozac); norfluoxetine; monoamine oxidase A; ^{18}F-fluoroclorgyline

ABSTRACT Monoamine oxidase A (MAO-A) inhibition was investigated both in vitro and in vivo in rat brains by using the radioligand, ^{18}F-fluoroclorgyline (*N*-[3-(2′,4′-dichlorophenoxy)-2-^{18}F-fluoropropyl]-*N*-methylpropargylamine). In vitro binding affinities of six compounds, clorgyline, Ro 41–1049, deprenyl, fluoxetine, norfluoxetine and citalopram, were studied. Fluoxetine and norfluoxetine showed in vitro affinities of 36.5 and 68 μM for MAO-A, respectively. Fluoxetine and norfluoxetine also significantly inhibited (more than 20%) the binding of the radioligand in vivo while citalopram and deprenyl showed very poor affinities in vitro for MAO-A and had no effect in vivo. The in vivo effects of the various drugs were directly comparable to their in vitro affinities for binding to MAO-A as seen in the correlation plot of percent control in vivo binding of ^{18}F-fluoroclorgyline and binding affinity, -log IC_{50} ($R^2 = 0.979$). An acute dose of 20 mg/kg of fluoxetine inhibited binding of ^{18}F-fluoroclorgyline by more than 20%, while lower doses had some significant effects. These results provide evidence on the in vitro and in vivo inhibition of monoamine oxidase A by fluoxetine. **Synapse 31:285–289, 1999.**

INTRODUCTION

One of the current hypothesis for depressive illness suggests an impairment of serotonin neurotransmission (Cooper et al., 1996). Treatments known to increase the "serotonergic tone," such as the chronic administration of selective serotonin reuptake inhibitors (SSRIs), alleviate the illness (Richelson, 1994). The SSRIs have proved to be an important development in the treatment of depression because of both their greater practical ease of use and their postulated primary action on a single binding site (selective blockade of the reuptake of serotonin into the presynaptic neuron, Wong et al., 1995). Compared to other SSRIs, fluoxetine (Prozac) is more widely prescribed and its effects extend beyond depression, thus raising questions about its basic pharmacology in relation to that of other SSRIs (Stanford, 1996). Additionally, the delayed onset of therapeutic action of the SSRIs has raised new questions about the importance of serotonin transporter inhibition alone (Cooper et al., 1996; Richelson, 1994). There have been recent reports on the potential role of fluoxetine's effects in serotonin 5HT2C receptor inhibition (Ni and Miledi, 1997), depolarization-induced calcium uptake (Lavoie et al., 1997), inhibition of neuronal sodium ion channels (Pancrazio et al., 1998), and blockade of nicotinic acetylcholine receptors (Garcia-Colunga et al., 1997). We and others have investigated the ability of fluoxetine to inhibit the enzymes, monoamine oxidase (MAO) A and B as a potential secondary therapeutic mechanism of fluoxetine (Leonardi and Azmitia, 1994; Holt and Baker, 1996; Mukherjee and Yang, 1997). Our previous work showed significant MAO-B inhibition in vivo in rats pretreated with an acute dose of fluoxetine (Mukherjee and Yang, 1997). Since MAO-A is known to metabolize both serotonin and dopamine (Fowler and Tipton, 1984), we have now investigated the in vitro and in vivo inhibition of MAO-A by fluoxetine and norfluoxetine using a fluorine-18 labeled radioligand [^{18}F]fluoroclorgyline (*N*-[3-(2′,4′-dichlorophenoxy)-2-[^{18}F]fluoropropyl]-*N*-methylpropargylamine) that binds selectively to MAO-A.

MATERIALS AND METHODS

Binding affinity of the various compounds were measured in vitro by using [^{18}F]fluoroclorgyline (a fluorinated analog of clorgyline, prepared by reacting [^{18}F]fluoride with *N*-[3-(2′,4′-dichlorophenoxy)-2-mesyloxypropyl]-*N*-methylpropargylamine; Mukherjee et al.,

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manuscript in preparation) in rat brain homogenates. Rat brains (from Sprague-Dawley rats, 150 g) were isolated and homogenized with a Tekmar Tissumizer (15 seconds 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*g* for 15 minutes at 4 °C. The pellet was 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.

In vitro binding affinities of six compounds [(*R*)-deprenyl, clorgyline and Ro 41–1049 from Research Biochemicals Int. Natick, MA; citalopram, fluoxetine and norfluoxetine were gifts] to MAO-A 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]fluoroclorgyline. Binding was initiated by addition of the tissue homogenate, and the tubes were incubated for 1 hour at 37 °C. The binding was terminated by filtration using a Brandel filtration apparatus (Brandel, Inc., Gaithersburg, MD), followed by washings with cold 50 mM Tris-HCl buffer (3 x 3 ml). Non-specific binding was determined in the presence of 10 µM clorgyline. 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 Rodberg, 1990). The binding curves were displayed using GraFit.

For in vivo studies, groups (n = 2–4) of Sprague-Dawley rats (150 g) were administered with the six compounds at the following doses: clorgyline 10 mg/kg, Ro 41–1049 10 mg/kg, (*R*)-deprenyl 10 mg/kg, citalopram 20 mg/kg, fluoxetine 20 mg/kg, and norfluoxetine 20 mg/kg. All compounds (including saline, for control rats) were administered intraperitoneally under anesthesia (brief exposure to vapors of diethyl ether), 90 minutes prior to injection of the radioligand and the rats were allowed to recover and had free access to food and water during the interval. The radioligand, [^{18}F]fluoroclorgyline, 90 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 75 minutes after the radioligand injection 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]fluoroclorgyline/g of wet tissue for each group of rats. A correlation of in vivo binding of [^{18}F]fluoroclorgyline (expressed as percent control) and binding affinity (expressed as -log IC_{50}) of the various inhibitors was generated.

In order to evaluate the effect of different doses of fluoxetine, rats were divided into four different groups. To the first group, saline was administered and the remaining three groups were administered with a dose of 2.5, 10, and 20 mg/kg of fluoxetine, intraperitonealy. The dosing was done 90 minutes prior to the administration of [^{18}F]fluoroclorgyline, and the rats were awake and had free access to food and water during the time interval. The radioligand, [^{18}F]fluoroclorgyline, 100 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 75 minutes after the radioligand injection and the brain regions (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]fluoroclorgyline/g of wet tissue for each group of rats.

RESULTS

The in vitro binding profiles of the various compounds are shown in Figure 1a. Clorgyline, which is a potent MAO-A inhibitor (Johnston, 1968) showed the highest affinity (IC_{50} = 39 nM) followed by the selective MAO-A inhibitor, Ro 41–1049 (Saura et al., 1992), which exhibited an order of magnitude weaker affinity (IC_{50} = 0.42 µM), compared to clorgyline. Deprenyl, which is a MAO-B selective agent, exhibited very low affinity (IC_{50} >100 µM). This is indicative of the selective labeling of MAO-A sites by the radioligand, [^{18}F]fluoroclorgyline. This selectivity of [^{18}F]fluoroclorgyline for MAO-A sites is similar to that reported for clorgyline and some of its derivatives (Ohmono et al., 1991). Both, fluoxetine and norfluoxetine exhibited significant affinities (IC_{50} = 36.5 and 68 µM, respectively), whereas citalopram exhibited very weak binding (IC_{50} >100 µM).

The in vivo effects of the various compounds on the binding of [^{18}F]fluoroclorgyline in the rat brains are shown in Figure 1b. The effect of clorgyline on the binding of [^{18}F]fluoroclorgyline was dramatic (binding was reduced to 30.3 % compared to controls), consistent with the high affinity of clorgyline for MAO-A in vitro. In vivo inhibition by the MAO-A reversible inhibitor, Ro 41–1049 was also high but somewhat lower than clorgyline (down to 40.3% of controls). There was no measurable decrease by citalopram and (*R*)-deprenyl compared to controls on the binding of [^{18}F]fluoroclorgyline, which is also consistent with the very weak affinity of the two compounds for MAO-A in vitro. However, fluoxetine and norfluoxetine reduced the binding of [^{18}F]fluoroclorgyline in all the brain regions significantly compared to controls (77.4 and 91.7%, respectively). This in vivo effect is directly comparable to their in vitro affinities for binding to MAO-A as can be seen in the correlation plot (MAO-A in vitro binding affinities vs. percent control of in vivo binding of [^{18}F]fluoroclorgyline; R^2 = 0.979) shown in Figure 2a.

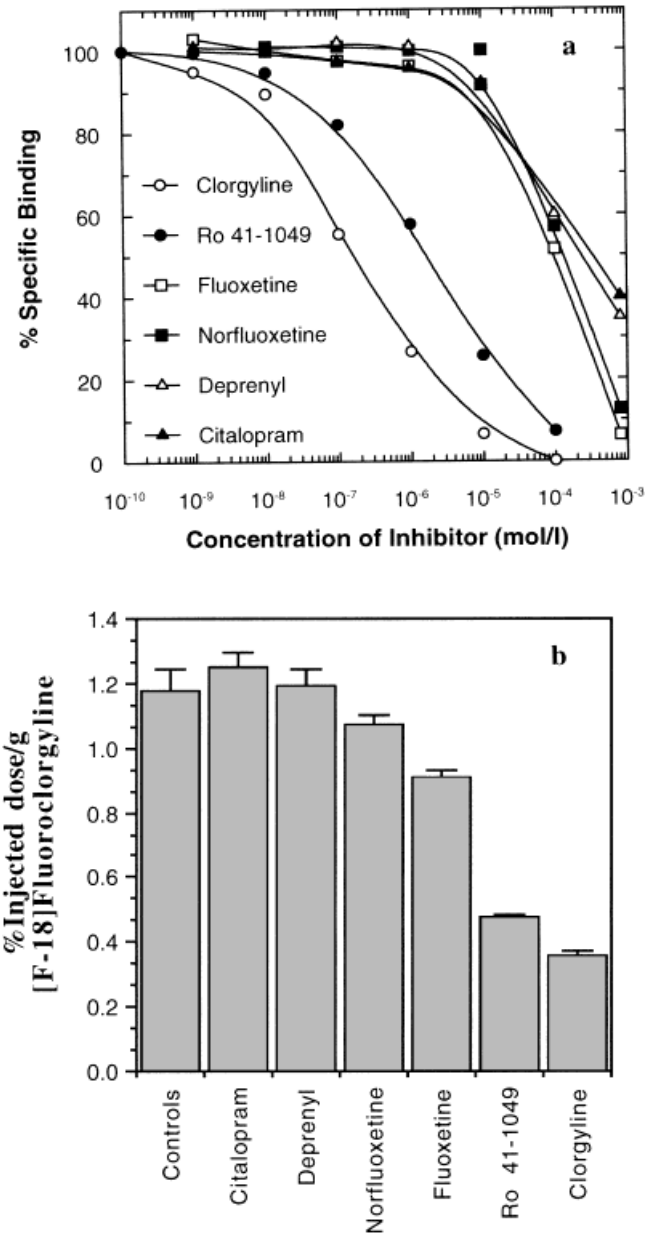

Fig. 1. **a:** In vitro binding affinity of the compounds for MAO-A measured by using [^{18}F]fluoroclorgyline in rat brain homogenates. **b:** In vivo binding of [^{18}F]fluoroclorgyline in the rat brains (within cerebrum) shown as percent of injected dose/g of wet tissue. Rats were pretreated either with saline (control) or the various drugs (citalopram, (*R*)-deprenyl, norfluoxetine, fluoxetine, Ro 41–1049 and clorgyline) administered i.p. 90 minutes prior to the radioligand. All rats were sacrificed 75 minutes post-i.v. injection of the radioligand, [^{18}F]fluoroclorgyline.

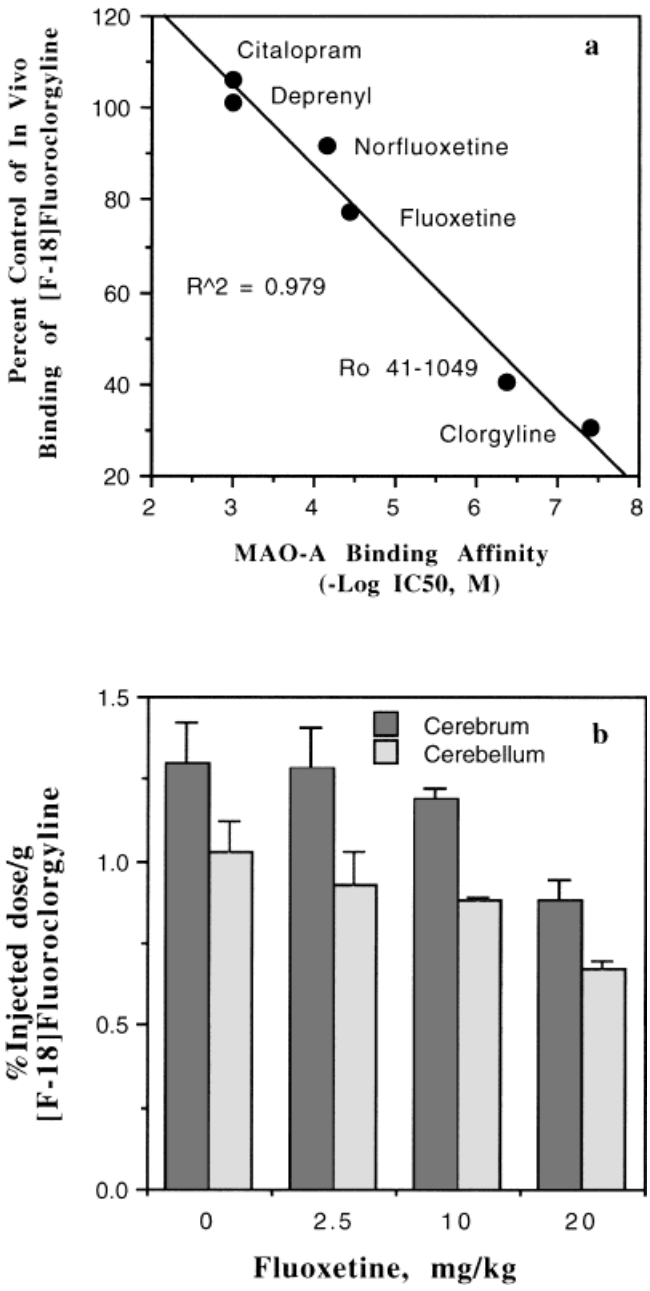

Fig. 2. **a:** Correlation of the percent control of in vivo binding of [^{18}F]fluoroclorgyline vs. MAO-A binding affinities (-log IC_{50}) of the various inhibitors ($y = 157.9 - 17.606x$, $R^2 = 0.979$). **b:** Dose effects of fluoxetine on the inhibition of [^{18}F]fluoroclorgyline in the rat brains (data on cerebrum and cerebellum shown separately).

The effect of three doses of fluoxetine (2.5, 10, and 20 mg/kg) administered acutely were examined in a group of rats (Fig. 2b). Lower doses (2.5 mg/kg) of fluoxetine show significant inhibition of [^{18}F]fluoroclorgyline binding in the cerebellum (a decrease of approximately 10%) whereas no significant decrease in the cerebrum was observed. At moderate doses (10 mg/kg) of fluoxetine, binding of [^{18}F]fluoroclorgyline was reduced by approximately 8% in cerebrum and 14% in cerebellum. At doses of 20 mg/kg, binding of [^{18}F]fluoroclorgyline showed a significant decrease (greater than 30%) both in the cerebrum and cerebellum.

DISCUSSION

Inhibition of the serotonin transporter has been postulated to be the primary mechanism through which the SSRIs such as fluoxetine exert their therapeutic effect (Gurevich and Joyce, 1996, Wong et al., 1995).

Fluoxetine and its active metabolite, norfluoxetine, inhibit serotonin uptake with nanomolar affinities and they have lower (micromolar) affinities for the inhibition of dopamine and norepinephrine uptake sites (Wong et al., 1993). Recently, however, other secondary mechanisms which may potentially be involved in the therapeutic action of fluoxetine have been investigated (Garcia-Colunga et al., 1997; Lavoie et al., 1997; Ni and Miledi, 1997; Pancrazio et al., 1998).

Fluoxetine and norfluoxetine are present in significant quantities in the brain (Caccia et al., 1990) and by using fluorine-18 labeled fluoxetine, we have also measured large amounts of [^{18}F]fluoxetine bound to mitochondria and other subcellular components in the rat brain (Mukherjee et al., 1998). This intracellular localization of fluoxetine probably accounts for its prolonged pharmacokinetics and the high concentrations of fluoxetine in the brain. Using fluorine-19 magnetic resonance spectroscopy experiments, Karson and coworkers found concentrations of fluoxetine/norfluoxetine to range up to 10.7 μg/ml (a concentration of approximately 35 μM) in subjects receiving 20 to 40 mg/day of fluoxetine (Karson et al., 1993). Rodents treated with fluoxetine over a 7-day period at a dose of 20 mg/kg/day exhibited concentrations of fluoxetine/norfluoxetine to be around 700 μM (Holt and Baker, 1996). These are remarkably higher brain concentrations of fluoxetine than what is needed to inhibit the serotonin transporter (approximately 20–50 nM), and thus raise the prospects for potential secondary mechanisms of this unique drug.

Since MAO inhibition is one of the mechanisms of antidepressant therapy, the ability of fluoxetine and norfluoxetine to inhibit MAO-A could potentially provide another secondary mechanism of the antidepressant effect. Both fluoxetine and norfluoxetine inhibited binding of [^{18}F]fluoroclorgyline in vitro with affinities of 36.5 and 68 μM, which is consistent with previous reports (Holt and Baker, 1996; Leonardi and Azmitia, 1994). Inhibition of the in vivo binding of [^{18}F]fluoroclorgyline by the MAO inhibitors, (*R*)-deprenyl and clorgyline, related well to their affinities for MAO-A sites and citalopram had no effect on the in vivo binding of [^{18}F]fluoroclorgyline. Fluoxetine and norfluoxetine had significant (over 20%) in vivo inhibitory effects on the binding of [^{18}F]fluoroclorgyline and support the hypothesis of MAO-A inhibition by fluoxetine in vivo. These findings are also consistent with the previous in vivo results of MAO-A inhibition by fluoxetine, which were confounded because of dilution assays (Holt and Baker, 1996). Fluoxetine had a slightly greater effect than norfluoxetine as can be seen in the correlation plot of MAO-A binding affinity vs. in vivo potency of the various drugs, shown in Figure 2a. In this limited acute-dose study, of the three doses investigated, 20 mg/kg provided a maximal reduction in the binding of [^{18}F]fluoroclorgyline as seen in Figure 2b. The localization of [^{18}F]fluoroclorgyline in the rat brain (cerebellum and cerebrum) corresponded to the approximate distribution of MAO-A in the rat brain (Saura et al., 1992). The extent of [^{18}F]fluoroclorgyline inhibition by fluoxetine (20 mg/kg) was similar for both cerebrum and cerebellum.

The ability of fluoxetine and norfluoxetine to inhibit MAO-A accompanied by their significant inhibitory effects on MAO-B (Mukherjee and Yang, 1997) may be an important secondary mechanism, in addition to the inhibition of serotonin reuptake sites (Wong et al., 1993). Using acute doses of fluoxetine and gross dissection of the brain, we have observed significant in vivo inhibition (up to 15 to 25%) of the two enzymes. A more detailed quantitative autoradiographic study of the brain will be required in order to evaluate accurately the extent of regional inhibitory effects of MAO-A and MAO-B by fluoxetine.

Comparing the two antidepressant drugs, citalopram and fluoxetine, the ability of citalopram to inhibit MAO-A in vivo is significantly small or even absent compared to that observed with fluoxetine. Our results thus provide in vivo evidence on the moderate MAO-A inhibition by fluoxetine. In summary, these observations from our preliminary studies provide additional insights into the mechanism of action of fluoxetine and possibly its unique properties.

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