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Drug concentrations in mouse brain at pharmacologically active doses of fluoxetine enantiomers

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Abstract—The i.p. injection of *R*-fluoxetine into mice at doses of 1–10 mg/kg led to higher concentrations of the desmethyl metabolite, *R*-norfluoxetine, in whole brain than was true for *S*-fluoxetine. *R*-Norfluoxetine, but not *S*-norfluoxetine, concentrations predominated over those of the parent drug at 7–24 hr after injection of the corresponding fluoxetine enantiomer. The more rapid N-demethylation of *R*-fluoxetine, and the relative inactivity of *R*-norfluoxetine as a serotonin uptake inhibitor compared with *S*-norfluoxetine, may explain the earlier report that *R*-fluoxetine is less potent than *S*-fluoxetine in antagonizing *p*-chloroamphetamine depletion of brain serotonin in mice. In the present study, a 10 mg/kg, i.p., dose of *S*-fluoxetine completely prevented *p*-chloroamphetamine given 24 hr later from depleting brain serotonin, whereas *R*-fluoxetine offered no protection at this time.

Fluoxetine is a selective inhibitor of serotonin uptake *in vitro* and *in vivo* and is widely used as a drug for treating mental depression [1, 2]. Fluoxetine is a racemate, and both enantiomers have equal potency in blocking the serotonin uptake carrier *in vitro* [2, 3]. Fluoxetine is metabolized by N-demethylation to norfluoxetine [4–6]. Although *S*-norfluoxetine is essentially equally effective as *S*-fluoxetine as a serotonin uptake inhibitor, recently it has been found that *R*-norfluoxetine unexpectedly is relatively weak as a serotonin uptake inhibitor [2, 3, 7, 8].

Several studies of brain or blood concentrations of norfluoxetine and fluoxetine in rats have been published [2, 4, 6, 9, 10], but few if any data are available for mice. The present experiments were done to determine brain concentrations of parent drug and of N-demethylated metabolite in mice treated with fluoxetine enantiomers, especially for comparison with earlier published data showing pharmacological effects of fluoxetine enantiomers in mice [11].

Methods

Male ICR mice weighing 18–22 g were purchased from Harlan–Sprague–Dawley, Inc., Cumberland, IN, and were housed in a temperature- and light-controlled room with food and water available *ad lib*. Enantiomers of fluoxetine (synthesized as the HCl salts in the Lilly Research Laboratories and provided for these studies by D. W. Robertson and J. Krushinski) or (±)*p*-chloroamphetamine HCl (Regis Chemical Co., Morton Grove, IL) were injected i.p. into mice that were decapitated at time intervals thereafter. Whole brains were removed, frozen on dry ice, and stored at −60° prior to analysis. Fluoxetine and norfluoxetine concentrations were determined by liquid chromatography with ultraviolet absorbance detection [12]. Serotonin was determined by liquid chromatography with electrochemical detection. Statistical analyses were done by analysis of variance followed by Tukey's test.

Results

Figure 1 shows brain concentrations of fluoxetine and of

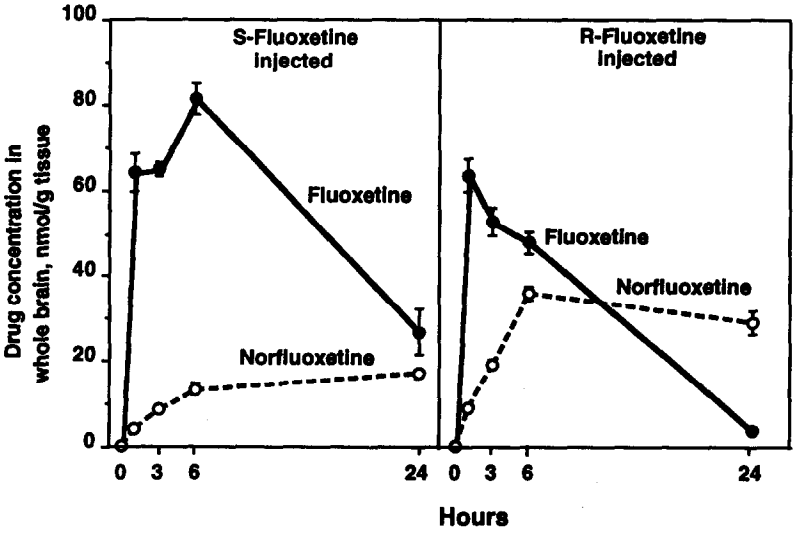

Fig. 1. Time course of whole brain concentrations of fluoxetine and its N-demethylated metabolite, norfluoxetine, after injection of 10 mg/kg, i.p., doses of *S*- or *R*-fluoxetine HCl into mice. Mean values ± SEM for five mice per group are shown.

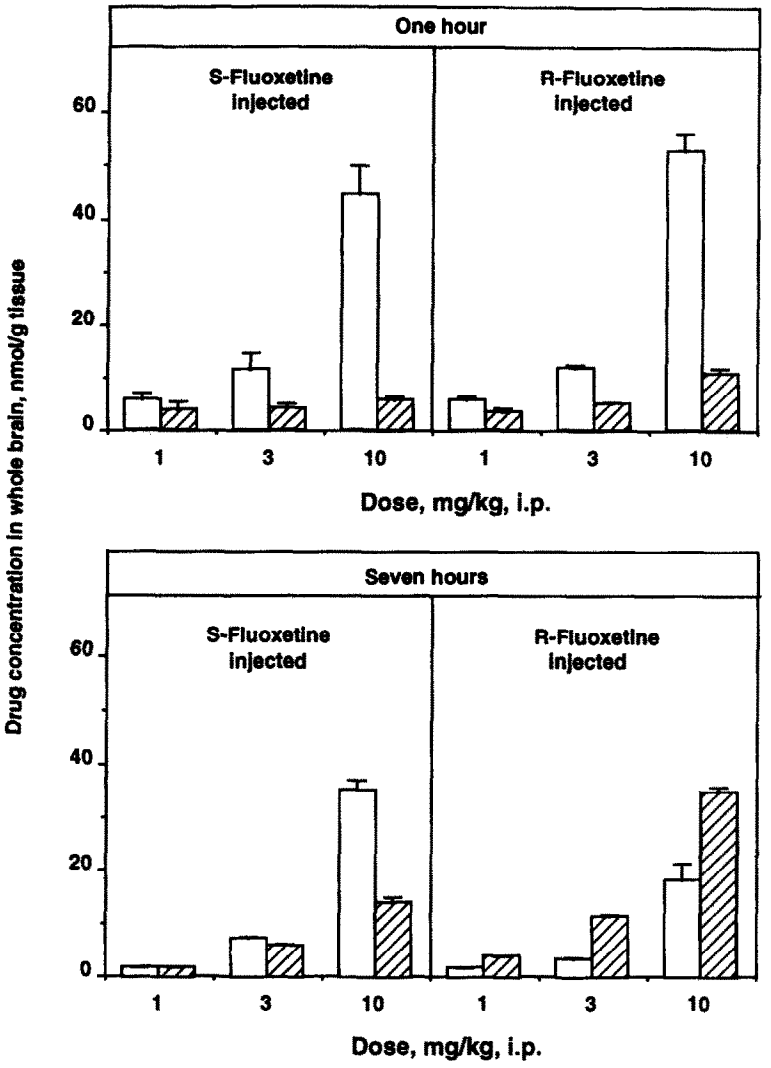

Fig. 2. Concentrations of fluoxetine (open bars) and norfluoxetine (shaded bars) in whole brain of mice 1 hr (top panel) or 7 hr (bottom panel) after the injection of *S*- or *R*-fluoxetine HCl at doses of 1, 3 or 10 mg/kg, i.p. Mean values ± SEM for five mice per group are shown.

its N-demethylated metabolite, norfluoxetine, at times up to 24 hr after the i.p. injection of *S*- or *R*-fluoxetine at a dose of 10 mg/kg. Concentrations of parent drug were equivalent for the two enantiomers at 1 hr. In both cases, brain concentrations of norfluoxetine were low but detectable at 1 hr. After 1 hr, *S*-fluoxetine concentrations were higher than those of *R*-fluoxetine. In contrast, concentrations of norfluoxetine were higher for the *R*-enantiomer than for the *S*-enantiomer at all times and predominated in the case of the *R*-enantiomer over those of the parent drug at 24 hr.

Figure 2 shows brain concentrations of parent drug at 1 hr and at 7 hr for comparison with earlier pharmacological results with the fluoxetine enantiomers. Robertson *et al.* [11] had reported that *S*-fluoxetine (ED_{50} 1.2 mg/kg, i.p.) is more potent than *R*-fluoxetine (ED_{50} 2.1 mg/kg, i.p.) inantagonizing brain serotonin depletion by *p*-chloroamphetamine (PCA) in mice. In those experiments, PCA was injected 1 hr after the fluoxetine enantiomers, and mice were killed 7 hr after injection of the fluoxetine enantiomers. Those experiments measured inhibition of the uptake carrier on brain serotonin neurons throughout the period of time PCA was present, inasmuch as the uptake carrier has to be blocked continuously to prevent serotonin depletion by PCA [13, 14]. The data in Fig. 2 revealed that brain concentrations of parent drug at 1 hr after fluoxetine injection were similar over the dose range studied for both enantiomers, but at 7 hr the parent *R*-fluoxetine was present at lower concentrations than the parent *S*-fluoxetine, at the two higher doses studied, due to more extensive N-demethylation.

Figure 3 compares the abilities of *S*- and *R*-fluoxetine to antagonize brain serotonin depletion by PCA in an experiment in which the enantiomers were given 1, 6, 24 or 48 hr prior to PCA. To minimize the time after PCA injection during which uptake had to be inhibited, the mice were killed only 2 hr after PCA, even though a submaximal degree of serotonin depletion occurs at this time. Particularly at these early times after PCA injection, when carrier-dependent depletion of serotonin is prevented, a

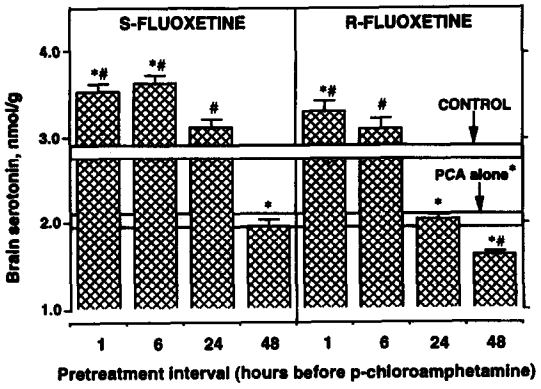

Fig. 3. Comparative abilities of *S*- and *R*-fluoxetine given at 1, 6, 24 or 48 hr before PCA to antagonize the depletion of brain serotonin 2 hr after PCA. The fluoxetine enantiomers were given as hydrochloride salts at a dose of 10 mg/kg i.p., and PCA HCl was given at a dose of 20.6 mg/kg, i.p. Horizontal shaded areas indicate standard error ranges around mean values for the control group or for rats treated with PCA alone. Vertical bars indicate groups that received PCA after pretreatment with *S*- or *R*-fluoxetine. Mean values ± SEM for five mice per group are shown. Key (*) significant difference from the control group ($P < 0.05$) and (#) significant difference from the group treated with PCA alone ($P < 0.05$).

significant increase in brain serotonin concentration results from inhibition of monoamine oxidase by PCA [15]. The left panel in Fig. 3 shows that *S*-fluoxetine completely prevented brain serotonin depletion when given 1, 6 or 24 hr before PCA. There was no protection when *S*-fluoxetine was given 48 hr before PCA. The right panel in Fig. 3 shows that *R*-fluoxetine had a shorter duration of action; it prevented brain serotonin depletion when given 1 or 6 hr but not when given 24 or 48 hr before PCA.

Discussion

Fluoxetine was metabolized by N-demethylation in mice less extensively after i.p. administration than has been reported in rats [2]. After the same 10 mg/kg doses of the enantiomers in rats, brain concentrations of norfluoxetine predominate over those of fluoxetine within 4 hr [2] but in mice, brain concentrations of norfluoxetine rose more slowly. The difference between enantiomers was greater in mice than in rats. In rats, the two enantiomers produce similar brain concentrations of both fluoxetine and norfluoxetine, at all times measured up to 24 hr after i.p. injection [2]. In mice (Fig. 1), the concentration ratio of norfluoxetine/fluoxetine was higher at every time measured for the *R*-enantiomer than for the *S*-enantiomer ($P < 0.05$ by Tukey's test after analysis of variance), after the 10 mg/kg, i.p., doses. At 24 hr, this ratio was still below 1 for *S*-fluoxetine but had reached >7 for the *R*-enantiomer. This concentration ratio of norfluoxetine/fluoxetine was significantly higher ($P < 0.05$) after the 10 mg/kg i.p., dose than after the lower doses at 1 and 7 hr for *S*-fluoxetine and at 1 hr (but not 7 hr) for *R*-fluoxetine (Fig. 2). The data in Fig. 1 show that *R*-norfluoxetine concentrations were increasing rapidly during the first 6 hr after *R*-fluoxetine injection, whereas *R*-fluoxetine concentrations were declining. This increase apparently continued, because Fig. 2 shows that at 7 hr *R*-norfluoxetine concentrations predominated over those of *R*-fluoxetine, as was also true at 24 hr (Fig. 1).

The earlier report [11] that *R*-fluoxetine is less potent than *S*-fluoxetine in antagonizing PCA-induced depletion of brain serotonin in mice is understandable in light of the data in Fig. 2. *R*-Norfluoxetine is relatively inert as a serotonin uptake inhibitor, whereas *S*-norfluoxetine is essentially as potent as *S*- or *R*-fluoxetine in blocking the serotonin uptake carrier either *in vitro* or *in vivo* [2, 7, 8]. During the period that uptake inhibition needs to be blocked after PCA in order to prevent serotonin depletion, brain levels of *R*-fluoxetine were falling as the relatively inactive *R*-norfluoxetine was formed (Figs. 1 and 2). In contrast, the combined levels of *S*-fluoxetine and *S*-norfluoxetine (both active in blocking serotonin uptake) were maintained during the same time period.

Figure 3 shows that when PCA was injected 24 hr after *S*-fluoxetine, a time when the total of *S*-fluoxetine + *S*-norfluoxetine concentrations in brain was 43 nmol/g (Fig. 1), the depletion of brain serotonin was prevented completely, as it had been when PCA was injected at 1 or 6 hr after *S*-fluoxetine. In contrast, when PCA was injected 24 hr after the other enantiomer, *R*-fluoxetine, the depletion of brain serotonin was not prevented, even though it had been when PCA was injected at 1 or 6 hr after *R*-fluoxetine. Figure 1 shows that brain concentrations of *R*-fluoxetine were only 4 nmol/g at 24 hr. Although there were substantial quantities of *R*-norfluoxetine present, its relatively weak affinity for the serotonin uptake carrier meant that no protection against serotonin depletion by PCA was afforded.

Fluoxetine is used in racemic form for the treatment of mental depression in humans. Fluoxetine is N-demethylated in humans to norfluoxetine, and blood levels of norfluoxetine are comparable to those of fluoxetine at steady state [16]. Both enantiomers of fluoxetine and the *S*-enantiomer of norfluoxetine probably contribute to the serotonin uptake inhibition that is thought to underlie antidepressant efficacy. The relative rates of N-demethylation of fluoxetine enantiomers in humans have not been reported.

The data in this paper show that serotonin uptake was inhibited longer in mice after injection of *S*-fluoxetine, due to the persistence of parent drug and the metabolite, *S*-norfluoxetine, than it was after injection of *R*-fluoxetine which is N-demethylated more rapidly to a relatively inactive compound, *R*-norfluoxetine. In an experimental design in which duration of uptake inhibition is important, as used by Robertson *et al.* [11], *R*-fluoxetine appeared less potent as a serotonin uptake inhibitor *in vivo* than did *S*-fluoxetine.

Lilly Research Laboratories
Eli Lilly and Company
Indianapolis, IN 46285
U.S.A.

RAY W. FULLER*
HAROLD D. SNODDY

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* Corresponding author: Ray W. Fuller, Ph.D., Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285. Tel. (317) 276-4164; FAX (317) 276-5546.

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