Pharmacology Biochemistry & Behavior Vol 24, pp 281–284, 1986 © Ankho International Inc Printed in the U S A 0091-3057/86 $3 00 + 00

Fluoxetine Enantiomers as Antagonists of p-Chloroamphetamine Effects in Rats

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Received 2 April 1985

FULLER, R W AND H D SNODDY *Fluoxetine enantiomers as antagonists of p-chloroamphetamine effects in rats* PHARMACOL BIOCHEM BEHAV **24**(2) 281–284, 1986 —The dextrorotatory enantiomer of fluoxetine was slightly more potent than the levorotatory enantiomer in antagonizing the depletion of brain serotonin by p-chloroamphetamine in rats The time course of the depletion of brain serotonin at times out to 24 hr after the injection of p-chloroamphetamine was determined with or without simultaneous administration of one of the fluoxetine enantiomers The dextrorotatory enantiomer prevented the depletion of brain serotonin at any time after p-chloroamphetamine The levorotatory enantiomer prevented the initial depletion of brain serotonin at 2 and 4 hr, but by 8 hr brain serotonin concentration was decreased and by 24 hr the depletion of serotonin was almost as great as in rats treated with p-chloroamphetamine alone The elevation of serum corticosterone that occurred acutely after injection of a low dose of p-chloroamphetamine was significantly antagonized by both enantiomers of fluoxetine, the dextrorotatory enantiomer being slightly more potent In contrast, the lowering of DOPAC (3,4-dihydroxyphenylacetic acid) concentration in rat brain by p-chloroamphetamine was not antagonized by either enantiomer of fluoxetine, indicating this effect is not secondary to serotonin release by p-chloroamphetamine The results are consistent with other evidence that both enantiomers of fluoxetine are potent inhibitors of serotonin uptake, the dextrorotatory enantiomer being longer-acting than the levorotatory enantiomer in rats

Serotonin p-Chloroamphetamine Fluoxetine Corticosterone

FLUOXETINE is a potent and selective inhibitor of serotonin uptake [15] that has been widely used as a pharmacologic tool in animal studies [3] Fluoxetine is clinically effective in the treatment of mental depression [1,13] and acts synergistically with L-5-hydroxytryptophan in the treatment of intention myoclonus [14] Fluoxetine has been used as the racemate, and until recently no information was available on the stereospecificity in fluoxetine's inhibition of serotonin uptake Wong *et al* [16] reported that both enantiomers of fluoxetine are potent inhibitors of serotonin uptake but that the dextrorotatory enantiomer is slightly more potent *in vitro* and *in vivo* and has a longer duration of action than the levorotatory enantiomer Previously, we had shown that fluoxetine (as the racemate) antagonizes effects of p-chloroamphetamine (PCA) that are dependent on the serotonin uptake carrier, namely the prolonged depletion of brain serotonin [7] and the acute elevation of serum corticosterone [9] This paper describes a comparison of the fluoxetine enantiomers in blocking these effects of PCA in rats

METHOD

Male Wistar rats (HSD/[WI]BR), 150–200 g, were obtained from Harlan Sprague-Dawley, Inc , Cumberland, IN The rats received IP injections of (±)-p-chloroamphetamine hydrochloride (Regis Chemical Company, Morton Grove, IL) after pretreatment with one of the enantiomers of fluoxetine hydrochloride, synthesized in the Lilly Research Laboratories We are grateful to Dr B G Jackson for supplying these enantiomers Rats were decapitated, and whole brains were excised, frozen on dry ice, and stored at −15° until assayed Trunk blood samples were allowed to clot, then serum obtained by centrifugation was stored at −15° prior to analysis Serotonin and DOPAC (3,4-dihydroxyphenylacetic acid) concentrations in brain were measured by high performance liquid chromatography with electrochemical detection Corticosterone concentration in serum was measured spectrofluorometrically [12]

RESULTS

Figure 1 shows that the depletion of serotonin concentration in whole brain of rats treated with PCA was antagonized in a dose-dependent manner by the enantiomers of fluoxetine The dextrorotatory enantiomer was slightly more potent than was the levorotatory enantiomer

In other experiments, the enantiomers of fluoxetine were found not to affect serotonin concentration by themselves At 1, 2 and 4 hr after injection of the dextrorotatory enantiomer (10 mg/kg IP), brain serotonin concentration was 3 25±0 09, 3 52±0 07 and 3 53±0 03 nmoles/g, respectively, not significantly different from the control value of 3 32±0 09 nmoles/g At 1, 2 and 4 hr after injection of the levorotatory enantiomer (10 mg/kg IP), brain serotonin concentration was 3 56±0 15, 3 28±0 09 and 3 59±0 05 nmoles/g, respectively

Figure 2 shows the time course of brain serotonin depletion by PCA given alone or simultaneously with one of the fluoxetine enantiomers (10 mg/kg) After PCA alone, brain

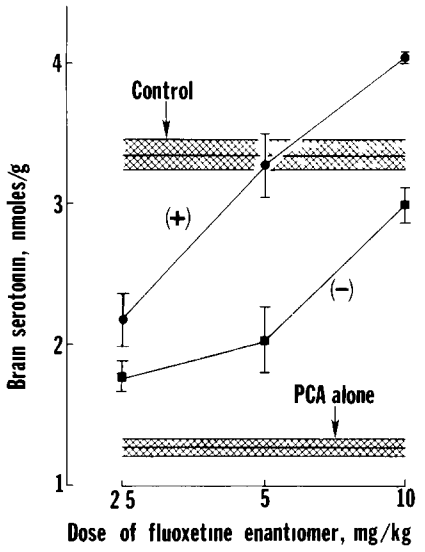

FIG 1 Dose-dependent antagonism of p-chloroamphetamine-induced depletion of brain serotonin by the dextrorotatory (●) and levorotatory (■) enantiomers of fluoxetine Mean and standard error range for control rats is shown by the shaded area at the top Mean and standard error range for rats treated with PCA alone (10 mg/kg IP of PCA HCl 4 hr before rats were killed) is shown by the shaded area at the bottom Lines connect points representing rats treated with PCA 4 hr before they were killed The fluoxetine enantiomers were injected IP at the mg/kg doses shown on the bottom axis (log scale) 1 hr before PCA All data represent mean and standard errors for 5 rats per group All groups that received the fluoxetine enantiomers had significantly ($p<0$ 01) higher serotonin concentration than rats receiving PCA alone

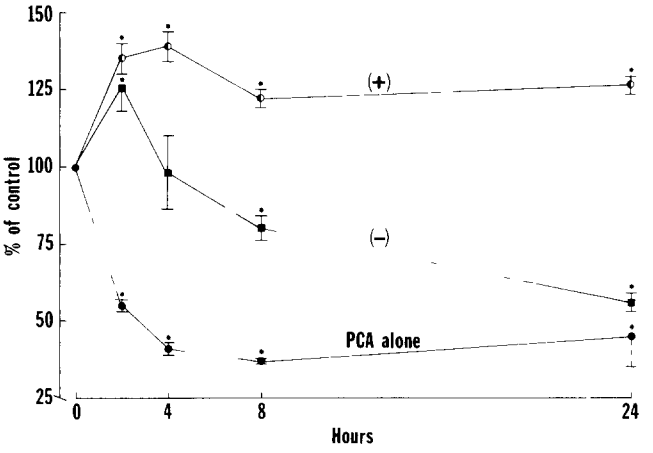

FIG 2 Serotonin concentration in rat brain at various times after the injection of PCA alone (●) (10 mg/kg IP of PCA HCl) or together with the dextrorotatory (◐) or levorotatory (■) enantiomers of fluoxetine (each at 10 mg/kg ip) Mean values ± standard errors for 5 rats per group are expressed as percent of the control mean Asterisks indicate significant differences from the control group ($p<0$ 05)

TABLE 1

DOSE-DEPENDENT ANTAGONISM OF THE PCA-INDUCED ELEVATION OF SERUM CORTICOSTERONE CONCENTRATION IN RATS BY THE ENANTIOMERS OF FLUOXETINE

Treatment group	Serum corticosterone μg/100 ml
Control	4 6 ± 0 5
PCA alone	47 5 ± 1 7*
PCA + (+) enantiomer (1 mg/kg)	28 8 ± 4 5*†
PCA + (+) enantiomer (3 mg/kg)	26 9 ± 5 6*†
PCA + (+) enantiomer (10 mg/kg)	20 2 ± 4 6*†
PCA + (−) enantiomer (1 mg/kg)	45 6 ± 3 1*
PCA + (−) enantiomer (3 mg/kg)	39 1 ± 2 7*†
PCA + (−) enantiomer (10 mg/kg)	25 7 ± 7 3*†

*Significant difference from control group ($p<0$ 05)

†Significant difference from group with PCA alone ($p<0$ 05)

Serum corticosterone concentration was measured 1 hr after the IP administration of PCA HCl (2 5 mg/kg) Fluoxetine enantiomers were injected IP 1 hr before PCA Mean values ± standard errors for 5 rats per group are shown

TABLE 2

INABILITY OF FLUOXETINE ENANTIOMERS TO ANTAGONIZE THE PCA-INDUCED LOWERING OF BRAIN DOPAC CONCENTRATION IN RATS

Treatment group	DOPAC in brain, nmoles/g
Control	0 55 ± 0 02
PCA alone	0 28 ± 0 02*
PCA + (+) enantiomer (1 mg/kg)	0 30 ± 0 01*
PCA + (+) enantiomer (3 mg/kg)	0 25 ± 0 01*
PCA + (+) enantiomer (10 mg/kg)	0 26 ± 0 01*
PCA + (−) enantiomer (1 mg/kg)	0 27 ± 0 02*
PCA + (−) enantiomer (3 mg/kg)	0 28 ± 0 01*
PCA + (−) enantiomer (10 mg/kg)	0 23 ± 0 01*

*Significant difference from control group ($p<0$ 05)

DOPAC concentration in whole brain was measured 4 hr after the IP administration of PCA HCl (10 mg/kg) Fluoxetine enantiomers were injected IP 1 hr before PCA Mean values ± standard errors for 5 rats per group are shown

serotonin concentration was significantly decreased within 2 hr, reached a minimum concentration at 8 hr, and remained decreased at 24 hr This time course of serotonin depletion by PCA has previously been reported [11] In rats that received the dextrorotatory enantiomer of fluoxetine at the same time as PCA, brain serotonin concentration was not decreased but instead was slightly increased at all time points Such an increase has been observed previously with PCA and some of its analogs and has been attributed to an unmasking of monoamine oxidase inhibition when the depletion of serotonin concentration was blocked by uptake inhibition [5,6] In rats that received the levorotatory

enantiomer of fluoxetine at the same time as PCA, brain serotonin concentration was slightly increased at 2 hr but declined thereafter Within 8 hr there was a significant decrease in serotonin concentration, and by 24 hr the concentration of serotonin was almost as low as in rats receiving PCA alone

Table 1 shows the elevation of serum corticosterone concentration by PCA, as influenced by pretreatment with the enantiomers of fluoxetine Both enantiomers caused significant and dose-related (albeit incomplete) antagonism of the effect of PCA With the levorotatory enantiomer, the lowest dose did not antagonize PCA, and the effect at the middle dose was less than that of the dextrorotatory enantiomer

Table 2 shows that PCA decreased whole brain concentrations of DOPAC, a metabolite of dopamine This effect of PCA was not antagonized by either enantiomer of fluoxetine

DISCUSSION

The ability of fluoxetine enantiomers to antagonize the depletion of brain serotonin by PCA agrees with earlier findings with the racemate This antagonism is believed to be due to antagonism of the active accumulation of PCA by the amine carrier on the cell membrane of serotonin neurons [4], and other inhibitors of serotonin uptake produce similar antagonism [2] The dextrorotatory enantiomer of fluoxetine was slightly more potent than the levorotatory enantiomer This finding is consistent with the report of Wong *et al* [16] that the levorotatory enantiomer of fluoxetine has a shorter duration of action than the dextrorotatory enantiomer *in vivo* The ability of serotonin uptake inhibitors to antagonize brain serotonin depletion at times up to 24 hr after PCA is an index of uptake inhibition throughout the period of time from PCA injection until the measurement of brain serotonin concentration Thus the inhibition of serotonin uptake during the 4 hr after PCA injection was slightly less with the levorotatory enantiomer of fluoxetine, consistent with its shorter duration of action as a serotonin uptake inhibitor *in vivo* [16]

A clear indication of this shortened duration of action is revealed in Fig 2 In this experiment, the enantiomers of fluoxetine were injected at the same time as PCA, and brain serotonin concentration was measured at various time intervals later The dextrorotatory enantiomer of fluoxetine prevented any serotonin depletion from occurring The slight increase in serotonin concentration may be due to an unmasking of the monoamine oxidase-inhibiting action [5,6] of PCA The levorotatory enantiomer of fluoxetine antagonized serotonin depletion by PCA at early times but did not antagonize the depletion of serotonin at longer times after PCA This pattern of effect is similar to that reported earlier with chlorimipramine [11], a potent and selective inhibitor of serotonin uptake that has a short duration of action *in vivo* because of its metabolism to chlordesipramine (which inhibits norepinephrine uptake, not serotonin uptake) Apparently the continuous transport of PCA into serotonin neurons via the uptake carrier is required for the depletion of serotonin to be maintained As we have shown previously, an uptake inhibitor can be injected after the injection of fluoxetine, at a time when serotonin stores are depleted, and the depletion of serotonin is terminated, serotonin levels returning rapidly to normal [8] A short-acting uptake inhibitor can initially prevent the depletion of serotonin by PCA, but if PCA remains in the brain longer than the uptake inhibitor does, then serotonin levels become depleted at longer times as inhibition of the accumulation of PCA into serotonin neurons diminishes That phenomenon is suggested to account for the loss of protection against PCA-induced serotonin depletion with time after injection of chlorimipramine [9] or the levorotatory enantiomer of fluoxetine (Fig 2)

The enantiomers of fluoxetine antagonized the acute increase in serum corticosterone concentration that occurs following PCA administration This increase is attributed to central serotonergic activation due to release of serotonin by PCA, that release occurring secondarily to accumulation of PCA by serotonin neurons [9] Prevention of PCA accumulation by fluoxetine prevents the release of serotonin The slightly greater potency of the dextrorotatory enantiomer in antagonizing corticosterone elevation by PCA is consistent with the greater potency of that enantiomer in blocking serotonin depletion by PCA The lack of complete antagonism of PCA-induced elevation of serum corticosterone may have been related to the transient increase in serum corticosterone that occurs with fluoxetine itself [10]

The lowering of brain DOPAC by PCA is probably due directly to monoamine oxidase inhibition, but in any case is apparently not an effect that is secondary to the uptake carrier-dependent release of serotonin The enantiomers of fluoxetine had no effect on the ability of PCA to decrease brain DOPAC concentrations Those findings indicate that effects of PCA not dependent on the serotonin uptake carrier are not influenced by fluoxetine pretreatment

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