

INHIBITION OF RAT LIVER TRYPTOPHAN PYRROLASE ACTIVITY BY FLUOXETINE

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ABSTRACT:

The present study has demonstrated the effectiveness of acute administration of fluoxetine to inhibit rat liver tryptophan pyrrolase activity. The maximum inhibition of basal liver tryptophan pyrrolase activity at 2h after administration was observed with 1 mg/kg dose for the total enzyme and apoenzyme activities and that significant inhibition of these two activities was evident with a dose of the drug as small as 0.5 mg/kg. Serum free tryptophan concentrations were also increased using 10 mg/kg dose of fluoxetine. In view of the role of tryptophan depletion and thus 5-HT in pathophysiology of depression, it is strongly suggested that the inhibition of liver tryptophan pyrrolase activity may be a major mechanism of antidepressant action.

INTRODUCTION

The neurotransmitter serotonin [5-hydroxytryptamine, 5-HT] has been implicated in various cognitive and behavioral functions (Beryly and Risch, 1985). Disruption of the serotonergic systems may be one of the factors involved in the etiology of mental disorders such as schizophrenia and depression (Harding *et al.*, 1993; Joyce, 1993). Fluoxetine is a non-tricyclic compound that has been effective in the treatment of depression (Fuller *et al.*, 1991). On the other hand fluoxetine has also been reported to be effective in reducing ethanol intake in rats and humans (Slywka and Hart, 1993). It has been proposed that its therapeutic activity is due to enhanced serotonergic neurotransmission. A central serotonin deficiency has been demonstrated in the Sardinian alcohol preferring SP rats in discrete areas of the brain (Devoto *et al.*, 1998) and in whole brain of C57BL alcohol-preferring mouse (Badawy and Evans, 1976). S P rats are also found to have increased liver tryptophan pyrrolase activity and decreased brain tryptophan concentrations in whole brain (Bano *et al.*; 1998).

It has been shown previously that large number of antidepressant drugs increase rat brain tryptophan concentration by inhibiting liver tryptophan pyrrolase activity (Bano *et al.*, 1995). Tryptophan pyrrolase inhibition (and hence 5-HT synthesis) by antidepressants inhibition should increase circulating tryptophan availability to the brain which may be important in the treatment of depression.

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It is therefore interesting to establish the involvement of hepatic tryptophan metabolism in relation to the mechanism of action of fluoxetine.

MATERIALS AND METHODS

Locally bred male Wistar rats (150-200 g body weight) were housed five per cage at $22\pm 1^\circ\text{C}$; under natural light-dark cycles and were maintained on solid lab chow and water ad libitum under standard housing conditions. Fluoxetine was administered i.p. in doses of 0-10 mg/kg body weight, and appropriate control rats received an equal volume of 0.9% NaCl and were killed after 2h. In the case of time course of i.p. of fluoxetine (10 mg/kg), rats were killed at different time intervals until 5h. Rats were killed between 13.30 and 14.30h. Livers were perfused in situ with ice cold saline before freezing at -20°C until analysis. Sera were also frozen rapidly following isolation.

Enzymatic and other Determinations

Liver tryptophan pyrroase activity was determined in homogenates either in the absence (holoenzyme activity) or in the presence (total enzyme activity of added) $2\ \mu\text{M}$ haematin, as described previously (Bano *et al.*, 1996). Free serum tryptophan concentrations were determined by modification (Bloxam and Warren, 1974) of the method of Denckla and Dewey (1967). Statistical analysis of results was performed by student's t-test

RESULTS

Effects of fluoxetine on the basal activity of rat liver tryptophan pyrrolase activity:

The time course of the effects of i.p. administration of fluoxetine (10 mg/kg) on the basal pyrrolase activity is shown on figure 1. The activity of the holoenzyme remained unaltered throughout. The total enzyme activity was significantly decreased by 38, 44, 41, 31% ($P<0.001$) at 0.5, 1, 2, 3h respectively. Activity of the apoenzyme was decreased by 50-78 percent ($P<0.001$) at the above time intervals respectively. Pyrrolase activity then returned to the control (zero time) values at 5h.

The results in figure 2 show that maximum inhibition of basal liver tryptophan pyrrolase activity at 2h after i.p. administration of fluoxetine was observed with the 1 mg/kg dose (44% and 78% for the enzyme and apoenzyme activities respectively; $P<0.001$) and that significant inhibition of these two activities (of 38% and 61% respectively; $P<0.001$) was evident with a dose of the drug as small as 0.5 mg/kg.

The results in Table 1 show that 3.5h after administration of 10 mg/kg dose of fluoxetine serum free tryptophan concentrations were significantly increased by 51 percent. Table 1 also shows that the serum total tryptophan concentrations were not changed, however tryptophan binding to serum proteins (expressed as the percentage free serum tryptophan) was significantly decreased.

DISCUSSION

Previous studies have demonstrated the effectiveness of a single dose of the eight antidepressants to inhibit tryptophan pyrrolase activity in rat liver (Bano *et al.*, 1995). Pyrrolase inhibition by antidepressants should increase circulating tryptophan availability to the brain which

may be important in the treatment of depression, in view of previous evidence for decreased plasma tryptophan levels in major depression (Cowen *et al.*, 1989) and for the reversal of antidepressant-induced remission by rapid depletion of plasma tryptophan (Delgado *et al.*, 1990). Recently opposite region-specific effects of acute and chronic treatments of fluoxetine on the 5-HT synthesis rate in the rat brain, i.e. increased synthesis in acute experiments and decreased synthesis in chronic experiments have been reported. The decrease in the 5-HT synthesis rate after the chronic treatment was found to be due to feedback inhibition of increased extracellular 5-HT levels. However as far as the acute are concerned, it was also reported (Muck-Seler *et al.*, 1996) that acute administration of higher doses of fluoxetine increases the plasma levels of free tryptophan, secondarily to its displacement from albumin-binding sites. This latter increase in free tryptophan is conformed in present investigation, using a 10 mg/kg dose of fluoxetine. With this latter dose, the major effect on tryptophan disposition appears to be that of displacement of bound tryptophan. Had a lower dose been used, the pyrrolase inhibition and its influence on total tryptophan would have been more evident.

Table I
Effect of fluoxetine on serum tryptophan concentration

Treatment	Serum tryptophan		% Free
	Free [$\mu\text{g/ml}$]	Total [$\mu\text{g/ml}$]	
Saline	1.06 \pm 0.05	19.63 \pm 0.58	5.40 \pm 0.25
Fluoxetine	1.60 \pm 0.11**	18.86 \pm 0.31	8.48 \pm 0.68**

Experiment details are as described in the Material and Method section. Measurements were performed at 3.5h after intraperitoneal administration of drugs (10 mg/kg each) or an equal volume (2 ml/kg) of 0.9% NaCl. Values are means \pm S.E.M. for each group of 6 rats. Significance of the difference is indicated by **P<0.01.

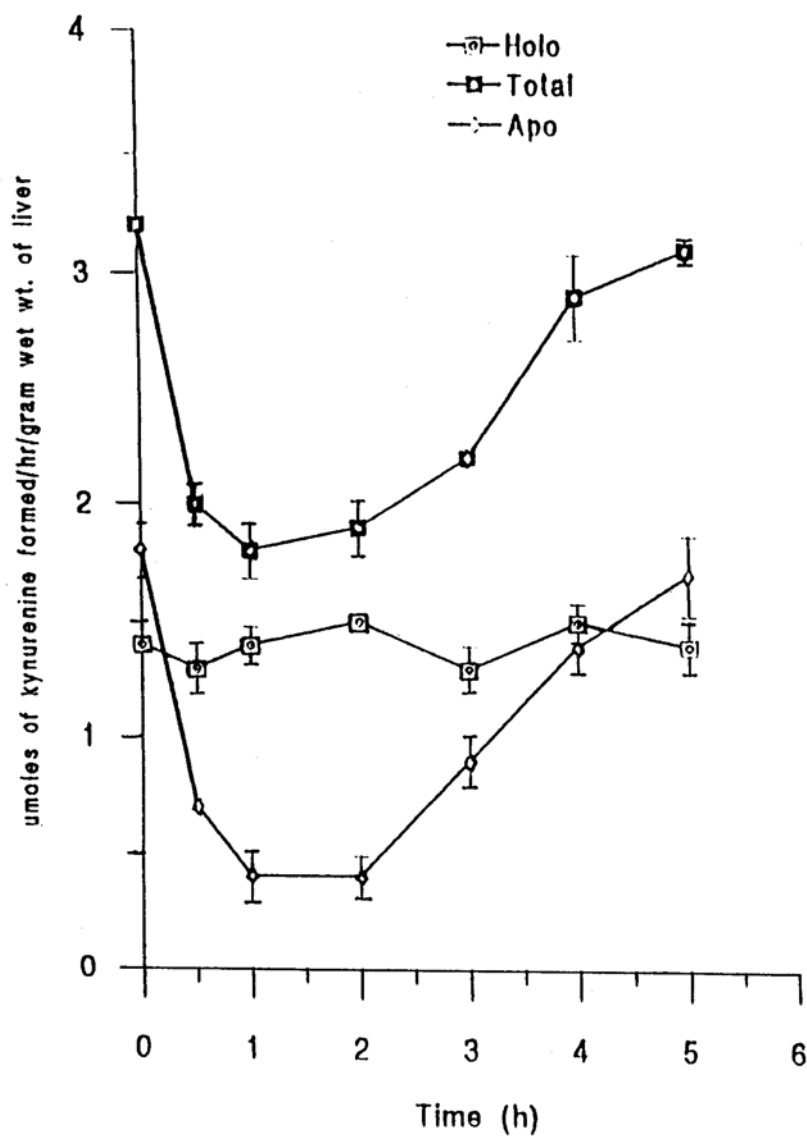


Fig. 1: Time course of the effects of i.p. administration of fluoxetine (10 mg/kg) on the basal activity of rat liver tryptophan pyrrolase.

Fluoxetine was administered intraperitoneally at zero time and pyrrolase activity was determined at the various times shown as described in the Material and Methods section either in the absence (holoenzyme activity), or in the presence (total enzyme activity) of added ($2 \mu\text{M}$) haematin. The apoenzyme activity was obtained by difference. Values are means (S.P.M. Shown by vertical bars except where error lies within size of symbol) for each group of 4 rats.

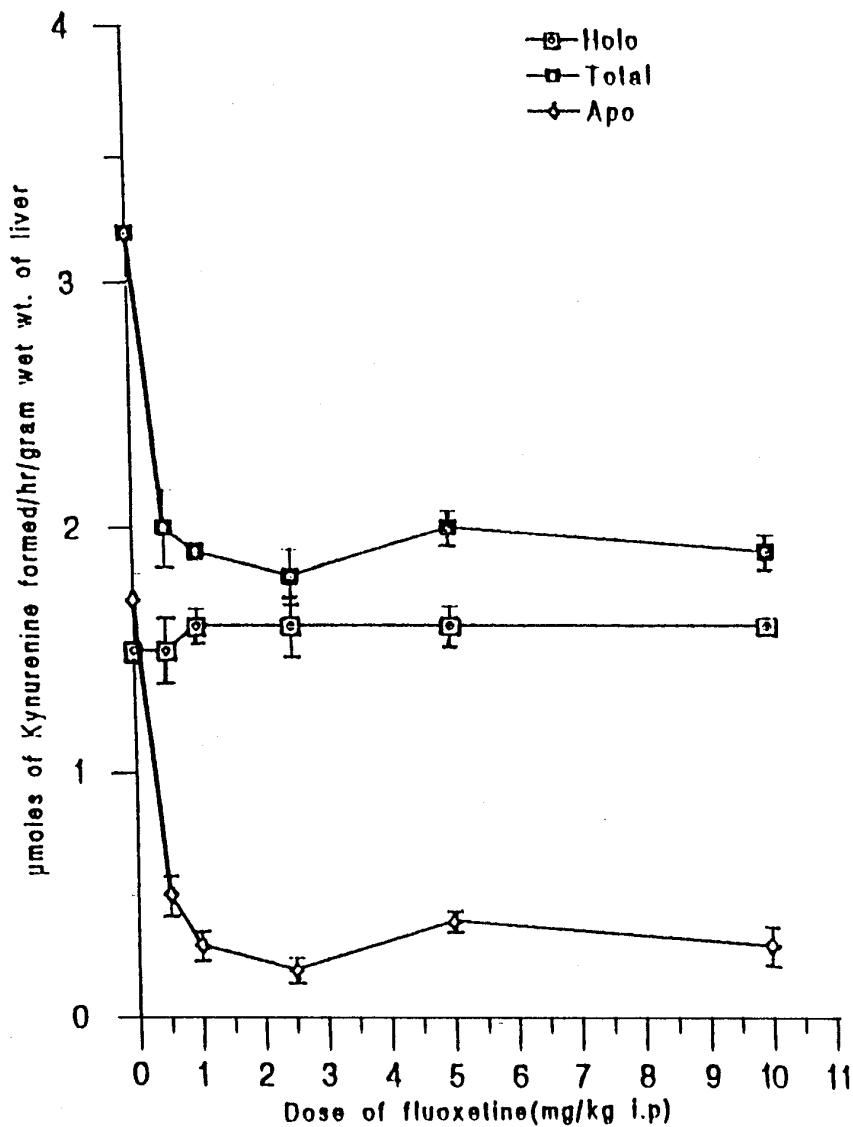


Fig. 2: Effects of i.p. administration of various doses of fluoxetine on the basal activity of rat liver tryptophan pyrrolase.

Rats were injected fluoxetine 0-10 mg/kg or an equal volume (2 ml/kg) of 0.9% NaCl and were killed 2h later. Liver tryptophan pyrrolase activity was determined as described in the Material and Methods section either in the absence (holoenzyme activity), In the presence (total enzyme activity) of added (2 μ M) haematin. The apoenzyme activity was obtained by difference. Values are means (S.E.M. shown by vertical bars except where error lies within size of symbol) for each group of 4 rats.

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