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The Time-Dependent Effect of the Antidepressant Drug Paroxetine on the Synthesis of 5-Hydroxytryptamine in the Rat Brain

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The effect of paroxetine on the day-night variations in the synthesis of 5-hydroxytryptamine was determined in the rat brain in an effort to gain an insight into the mechanism of action of this drug. This was done by determining its effect on the activity of tryptophan hydroxylase, the rate-limiting enzyme in the biosynthesis of 5-hydroxytryptamine in serotonergic neurons. The enzyme activity was determined in two brain regions, cortex and the brainstem, at two time points of 12 h light/12 h dark cycle, namely, mid-light and mid-dark. The results obtained showed that the activity of tryptophan hydroxylase was significantly greater in control animals during the dark than light phase both in the cortex and brainstem. They also demonstrate that the rate of synthesis of 5-hydroxytryptamine was affected by paroxetine in a time-dependent manner. It was therefore concluded that these time-dependent changes observed in paroxetine effect may influence the activity of serotonergic input into the suprachiasmatic nucleus and hence the regulation or expression of certain circadian rhythms. This action may help correct or compensate for abnormalities present in depressive illness.

Key words: Antidepressants, circadian rhythms, 5-hydroxytryptamine, paroxetine, tryptophan hydroxylase

INTRODUCTION

Paroxetine is a well established and clinically effective antidepressant [1,2]. It is an inhibitor of monoamine reuptake at the nerve endings with greater selectivity for 5-hydroxytryptamine (5HT) [2,3]. However, its mechanism of action remains to be firmly established though it is believed to be related to the antagonism of 5-hydroxytryptamine reuptake. The long-term administration of antidepressants is associated with changes in the synthesis and release of monoaminergic neurotransmitters and in the number and responsiveness of their receptors [4,5,6]. Apart from these biochemical changes, it has also been suggested that the clinical efficacy of antidepressants may result from their ability to resynchronize abnormal physiological rhythms which are believed by some to be associated with depressive illness [7,8,9].

In the present study, the effect of paroxetine on the day-night variations in the synthesis of 5HT in the rat brain was assessed to gain insight into the mechanism of action of this drug. This was done by determining the effect of the drug on the activity of tryptophan hydroxylase (EC 1.14.16.4, L-tryptophan; tetrahydropteridine: oxygen oxidoreductase). Since this enzyme is rate-limiting in the biosynthesis of 5HT in serotonergic neurons [10], it is expected to play an important role in the regulation of 5HT concentration at this level. Circadian variation has been reported in the activity of tryptophan hydroxylase [11,12]; concentration of 5HT [13,14]; concentration of 5-hydroxyindole acetic acid (5-HIAA), the main metabolite of 5HT [15]; and release of 5HT in the neurons [16,17]. All these rhythms peak during the dark (activity phase), except for that of 5HT concentration which peaks during the light (rest) span. It should however be pointed out that circadian variation in the activity of tryptophan hydroxylase has not been confirmed by others [18,19].

The activity of tryptophan hydroxylase was determined by assay of accumulated 5-hydroxytryptophan (5HTP) after inhibition of aromatic amino acid decarboxylase by 3-hydroxybenzylhydrazine [20]. Under normal

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physiological conditions, 5HTP is undetectable because it is rapidly decarboxylated to 5HT by aromatic amino acid decarboxylase (AAD). Inhibition of AAD thus leads to rapid accumulation of 5HTP which can be measured. The initial rate of accumulation gives an index of the *in vivo* activity of tryptophan hydroxylase or turnover of 5HT in the neurons. The levels of 5HTP were measured at two time points during the 24 h light/dark cycle, namely, the mid-dark and mid-light times. These times were selected arbitrarily, though bearing in mind that, as indicated above, the activity of tryptophan hydroxylase is higher during the dark than the light phase. It was hoped that changes, if any, brought about by paroxetine on the activity of tryptophan hydroxylase would most likely be detectable at these time points. Measurements were done after single acute as well as chronic dosing of paroxetine and compared to saline treatments in controls. The brainstem and the cerebral cortex were chosen since they represent two regions of the brain having different capacities of 5HT synthesis. The brainstem is richer in serotonergic neurons, has higher activity of tryptophan hydroxylase and hence higher serotonin concentration than the cerebral cortex, an area mainly endowed with nerve terminals [21,22,23].

MATERIALS AND METHODS

Treatment of animals

Male Wistar rats were housed 4 per cage in specially constructed cabinets and maintained on a 12 h light/12 h dark cycle (LD 12:12) [24]. The experiments were performed during the normal working hours of the laboratory and thus some animals had to be phase-shifted to accommodate the need to conduct procedures during the daytime hours. An automatic timer switched the light on at 04h00 and off at 16h00. At least 14 days were allowed to entrain the animals to the new light/dark cycle before experimentation. When the animals had to be injected with the drug or sacrificed during the dark phase, the laboratory was illuminated only by photographic red light [25]. The animals had free access to both food and water and were left undisturbed except for treatment and minimal animal caretaking activities.

The effect of acute single paroxetine treatment was determined by administering a dose of 20 mg/kg intraperitoneally (i.p.) to different groups of animals at either the mid-light (10h00) or mid-dark (22h00) times. Control rats received only normal saline. Fifteen (15) min later, 75 mg/kg of 3-hydroxybenzylhydrazine (NSD 1015) was injected i.p. The rats were sacrificed by decapitation 60 min after NSD 1015. The brain areas were dissected out and the amount of 5HTP assayed.

Paroxetine was also administered chronically i.p. at a dose of 10 mg/kg once daily for 14 days to rats weighing 180-300 g (mean wt 200 ± 10 g) at the start of the experiment. Control rats received only normal saline. The rats continued to gain weight normally compared to controls studied under the experimental regimen. At the end of the 14 day study period, NSD 1015 (75 mg/kg) was given i.p. 24 h after the last paroxetine dose. The rats were sacrificed 1 h after NSD 1015 injection, their brains removed immediately and rinsed with ice-cold 0.32 M sucrose solution. Dissection of the brainstem and cerebral cortex was carried out rapidly on a glass plate chilled on ice as described by Glowinski and Iversen [26].

The dissection procedure was rapid and reproducible as indicated by the wet weights of the brain regions. The brain regions were immediately stored frozen in liquid nitrogen until assayed, which was usually the same day but not later than one week. The brain regions were weighed frozen and homogenized to form a 10% w/v homogenate in 0.1 M perchloric acid containing a known concentration of dihydroxy-benzylamine (DHBA) as the internal standard. The contents were chilled on ice during homogenization. The homogenate was shaken for 30 min in a whirl mixer and centrifuged at 0°C and 15,000 g using an ultracentrifuge. The clear supernatant was filtered through 0.2 µm pore size filters. It was chilled on ice and assayed immediately. Protein content was subsequently measured according to the method described by Read and Northcote [27].

HPLC analysis

High performance liquid chromatography (HPLC) technique was used for the assay. The chromatographic analysis was carried out using hypersil ODS column (25 cm × 4.6 mm × 5 µm) connected to LDC pump (Constametric, model III); amperometric detector with working glassy carbon and reference Ag/AgCl electrodes (Bioanalytical Systems, Inc., model LC-4A); LDC/Milton Roy Integrator (model CI-10); LDC/Milton Roy Printer (model SEK); autosampler (Magnus Scientific, model M7110) and sample injection valve (Rheodyne Inc., model 7010) fitted with 100 or 50 µl injection loop. The mobile phase was made in distilled water with 70 mM sodium dihydrogen phosphate, 250 µM octane sulphonic acid sodium salt, 100 µM EDTA and 6-8% methanol. The pH was adjusted to 4.0 with 1.0 M perchloric acid, vacuum-filtered and degassed with helium. The detector was set at potential +0.65 V and range 5 nA or 10 nA and the pump flow rate was 1.7 ml/min at 2,000 psi. For assay of L-tryptophan, the detector was set at potential +0.75 V and range 10 nA or 50 nA. Retention times of authentic standards were used to identify 5HTP and L-tryptophan. Accurately known concentrations of 5HTP and L-tryptophan were used as standards for calibration.

RESULTS

A typical chromatogram obtained during routine assay of 5HTP and L-tryptophan in the brainstem and cerebral cortex is presented in Figure 1. This particular example shows the result of NSD 1015 (75 mg/kg, i.p.) injected 15 min after L-tryptophan (25 mg/kg i.p.). The conditions of the experiment are detailed under "Materials and Methods" except that 2.9 mM sodium acetate (trihydrate) was incorporated in the mobile phase to improve the separation of l-tryptophan. The chromatographic method of assay was highly sensitive since concentrations of 5HTP and L-tryptophan in the nanogram range could be measured accurately. The assays were also reproducible as confirmed by low standard errors of the mean. As shown in Figure 1, there was good resolution of the various peaks.

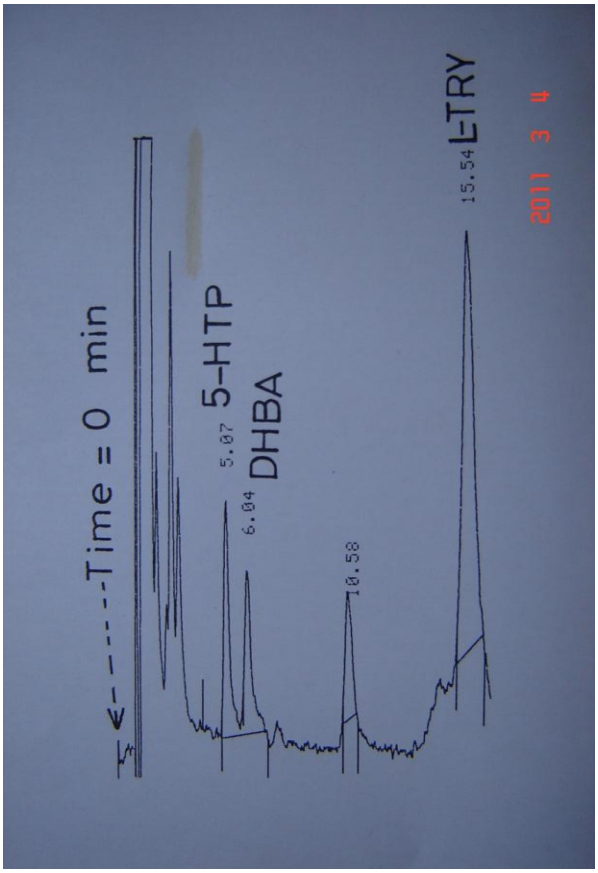

Figure 1. Typical chromatogram of rat cortical extracts following an intraperitoneal dose of NSD 1015 (75 mg/kg i.p.) 15 min after L-tryptophan (25 mg/kg i.p.). 5-HTP: 5-hydroxy-tryptophan; DHBA: dihydroxybenzylamine; L-TRY: L-tryptophan. The retention times are indicated in minutes.

Preliminary experiments showed that the amount of 5HTP formed was dependent on time up to 60 min as well as dose of NSD 1015 below 50 mg/kg (Figure 2 and 3). In the experiments described herein, the rats were sacrificed exactly 60 min after the 75 mg/kg injection of NSD 1015.

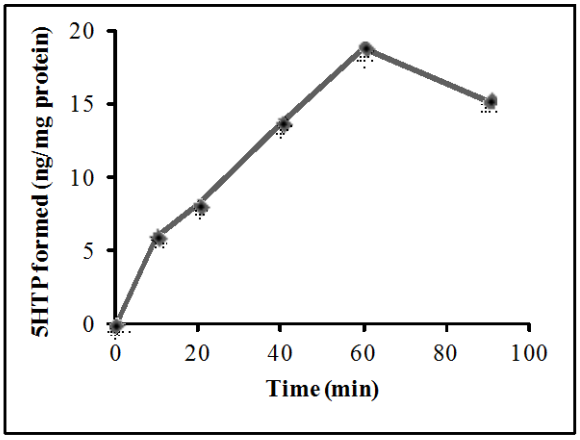

Figure 2. **Mean ± SEM (n=5 per group) 5-HTP accumulation versus time in the rat brainstem after a single peripheral dose of NSD 1015 (100 mg/kg i.p.).** The amount of 5-HTP formed is expressed as ng/mg protein of a 10% homogenate of the brainstem.

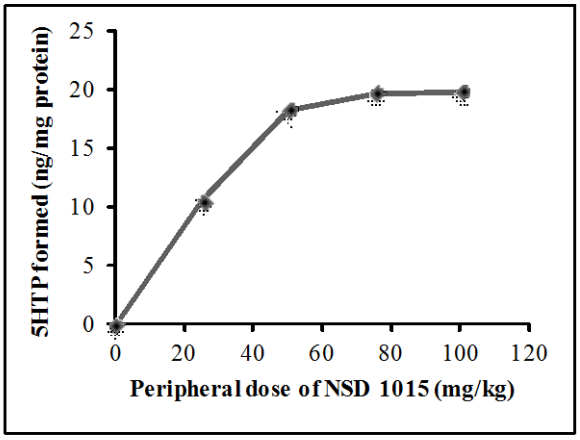

Figure 3. **Mean ± SEM (N=3 per group) effect of varying peripheral doses of NSD 1015 on 5HTP accumulation in the rat brainstem.** The amounts 5HTP formed are expressed as ng/mg protein of a 10% homogenate of the brainstem.

Figure 4 reveals that there was a greater accumulation of 5HTP in the brainstem than cortex following administration of the various peripheral doses of L-tryptophan i.p. This confirms the literature reports that the brainstem has greater capability of synthesizing 5HT than does the cerebral cortex [21,22,23]. The amount of 5HTP accumulated during the 60 min-interval following NSD 1015 after the dark and light period and saline treatment of the control rats is shown in Figure 5.

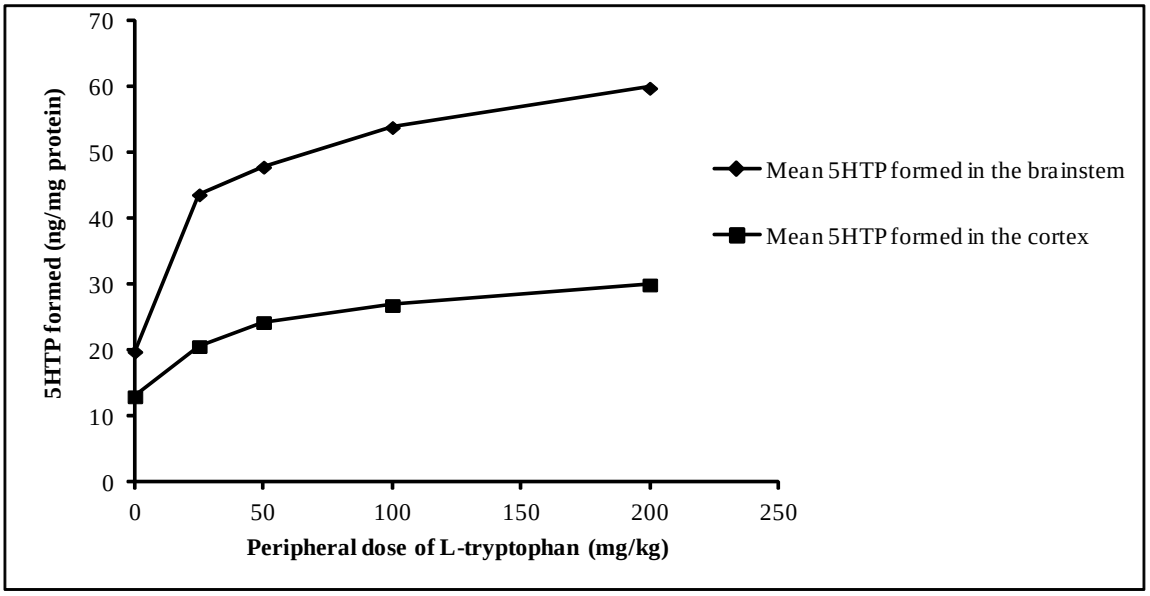

Figure 4. The accumulation (mean ± SEM; n=3 per group) of 5HTP in the brainstem and cortex following different peripheral doses of L-tryptophan. The concentration of 5HTP formed is expressed as ng/mg protein of a 10% homogenate of brainstem or cortex per 60 min.

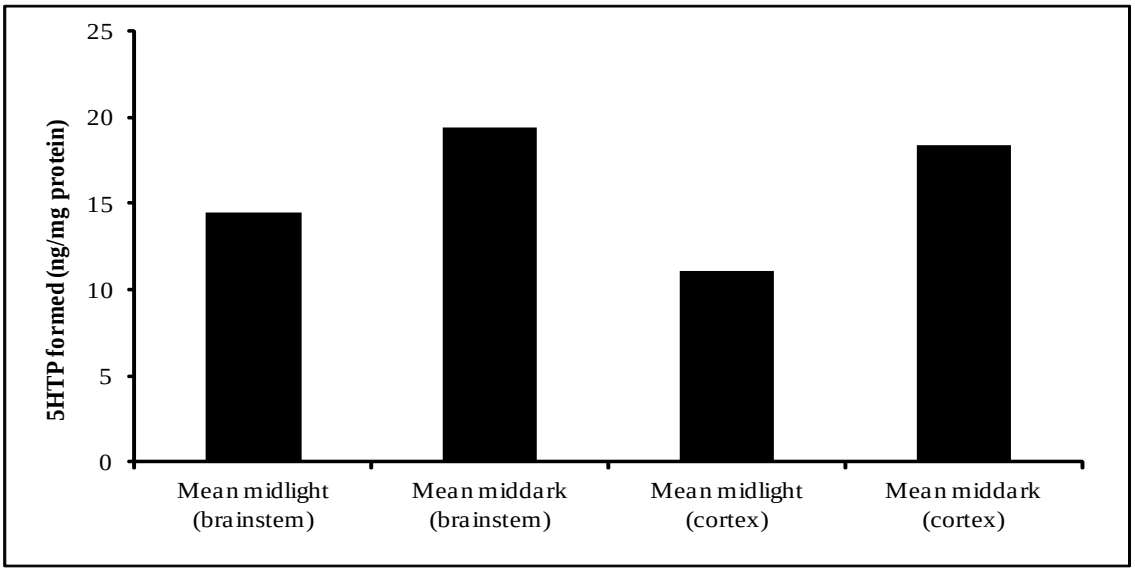

Figure 5. Differences between mid-dark and mid-light accumulation of 5HTP in the rat brainstem and cerebral cortex in control animals. The results are expressed as 5HTP formed in ng/mg protein of a 10% homogenate per 60 min (n=5 or 6 per group).

Acute (single) dosing of paroxetine (20 mg/kg) led to decreased 5HTP formation in the brainstem which was statistically significant ($p<0.01$) only in the mid-dark injection time (Figure 6).

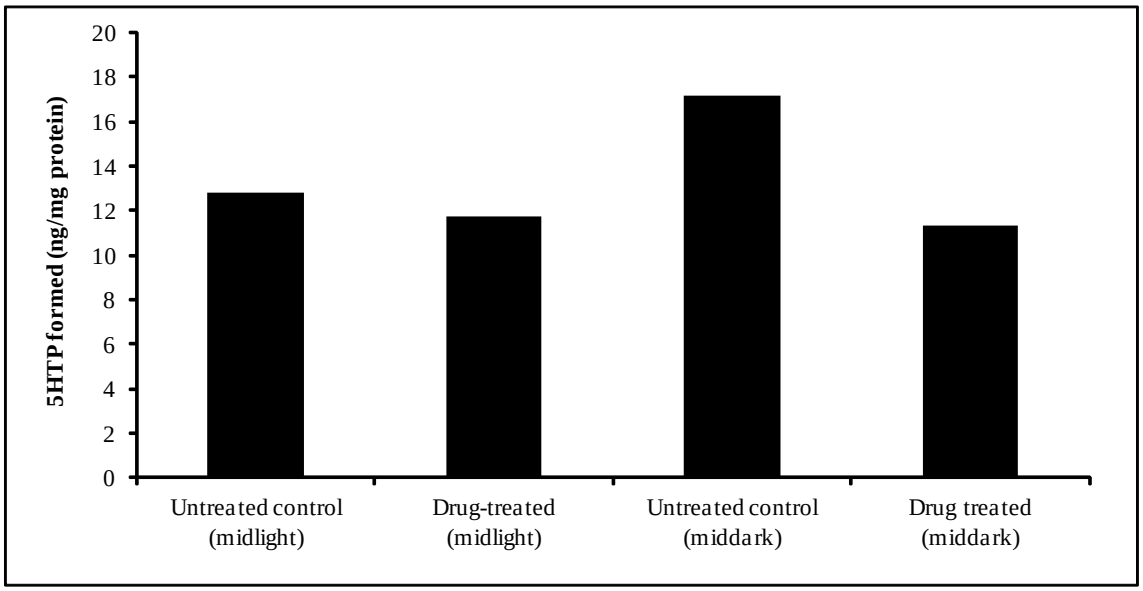

Figure 6. Effect of a single dose of paroxetine (20mg/kg i.p.) on 5HTP accumulation in the rat brainstem at mid-light and mid-dark of LD 12:12. The results are expressed as 5HTP formed in ng/mg protein of a 10% homogenate per 60 min.

Fourteen-day paroxetine dosing gave rise to different results in the brainstem. Chronic administration of paroxetine caused a statistically significant decrease in the amount of 5HTP

formed ($p<0.02$) only in the mid-light time. No significant effect was observable at mid-dark time (Figure 7).

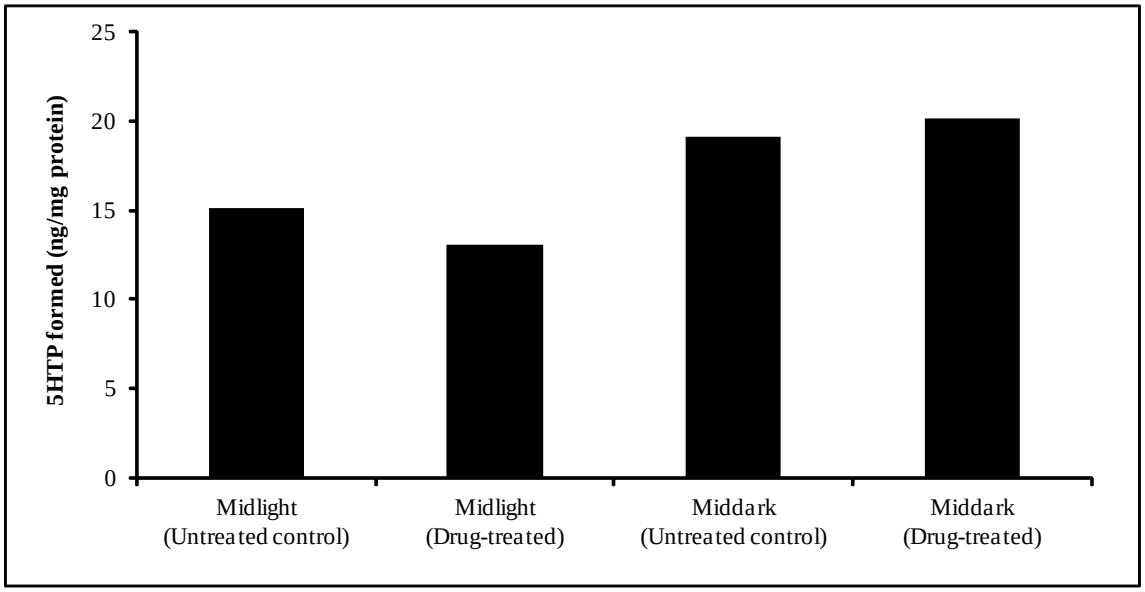

Figure 7. Effect of chronic administration of paroxetine (10 mg/kg once daily × 14 days) on 5HTP accumulation in the rat brainstem at mid-light versus mid-dark of LD 12:12. The results are expressed as 5HTP formed in ng/mg protein of a 10% homogenate per 60 min.

In the cerebral cortex, however, no statistically significant effect could be detected following chronic paroxetine dosing at either the mid-light or the mid-dark times (Figure 8).

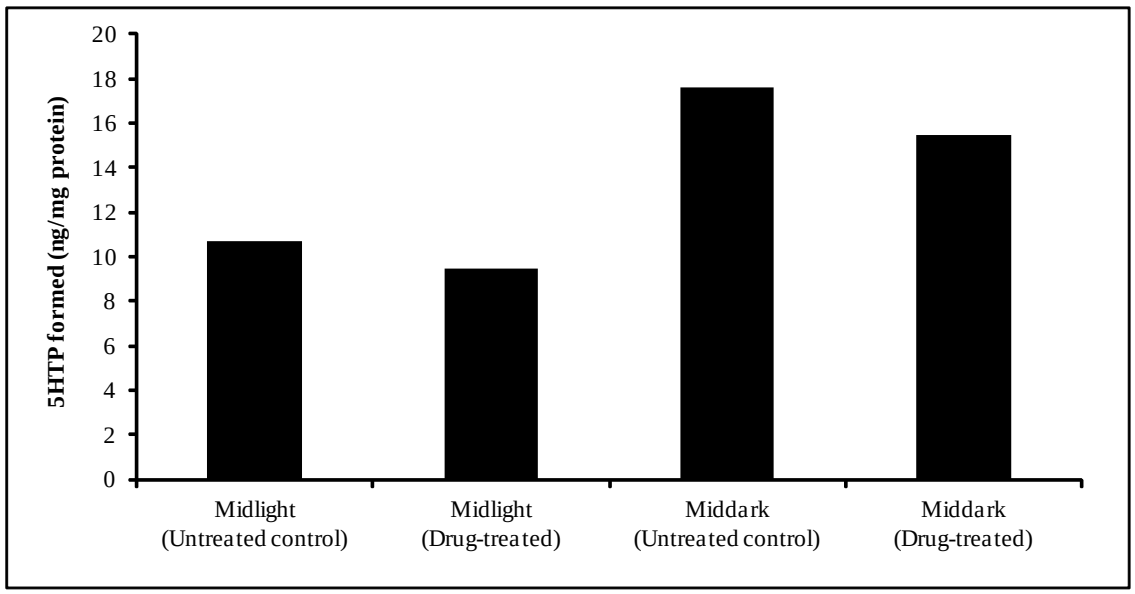

Figure 8. Effect of chronic paroxetine administration (10 mg/kg once daily × 14 days) on 5HTP accumulation in the rat cerebral cortex at mid-light versus mid-dark of LD 12:12. The results are expressed as 5HTP formed in ng/mg protein of a 10% homogenate per 60 min.

DISCUSSION

The 5HTP accumulation in control animals was greater during the dark than light phase both in the cortex and brainstem. These results are consistent with the literature quoted earlier, thus confirming the presence circadian variation of tryptophan hydroxylase activity in the brain [11,12,28]. Acute paroxetine dosing decreased 5HTP accumulation in the brainstem. This effect is similar to that of several other antidepressants, for example, the tricyclics, which also inhibit 5HT re-uptake at nerve endings [29,30]. The decrease in 5HT synthesis has been attributed to a negative feedback mechanism which is triggered by the resultant increase in 5HT concentration in the synaptic cleft [31].

Our results showed that acute paroxetine dosing led to a significant decrease in 5HTP accumulation in the brainstem only when the drug was timed at mid-dark. With chronic drug administration, the statistically significant effect was shifted to mid-light (rest span). In the cerebral cortex, however, no significant effect was detectable with the dose used. This finding suggests that the susceptibility of the enzyme tryptophan hydroxylase to paroxetine may vary according to brain region. Moreover, the brainstem data suggest a time-dependent difference in response to paroxetine by serotonergic neurons.

The drug effect, on acute administration, is apparently more pronounced during the dark than light phase but the reverse occurs on chronic dosing. These results are partly similar to what we reported elsewhere on the effect of two other antidepressants, mianserin and clomipramine on 5HT turnover. While chronic mianserin decreased turnover at the two time points, clomipramine led to an increase [32]. A time dependent effect was also noted in this earlier report. A survey of the literature reveals similar conflicting findings about the effects of chronic antidepressant dosing on the turnover of brain monoamines [33,34]. Since all these drugs have proven antidepressant action, it is likely that other factors apart from, or in addition to, an effect on 5HT turnover may be important for their clinical efficacy.

Tryptophan hydroxylase is rate-limiting in the biosynthesis of 5HT. It is therefore probable that the time-dependent changes observed in paroxetine effect may influence the activity of serotonergic input to the suprachiasmatic nucleus and hence the regulation or expression of certain circadian rhythms. It has been established that serotonergic input into the suprachiasmatic nucleus plays an important role in the regulation of circadian rhythms [35,36,37]. This action may help correct or compensate for abnormalities present in depressive illness.

In conclusion, the results reported here show that the activity of tryptophan hydroxylase is higher during the dark than light phase. This finding is consistent with the presence of a circadian variation in the activity of this enzyme. The results also show that paroxetine decreases tryptophan hydroxylase activity and that the effect is dependent on both treatment time of day and brain region.

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