

# Roles of Monoamine Neurotransmitters in the Regulation of Hypothalamic-Pituitary-Adrenal Axis(HPA)(II)—Role of 5-Hydroxytryptamine(Serotonin) in Controlling the Circadian Rhythmicity of Corticosterone in Rat

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## INTRODUCTION

Corticotropin-releasing factor(CRF) secreted from hypothalamic neurosecretory cells(neuroendocrine transducer cells) stimulates the release of ACTH from the pituitary gland, which in turn activates the adrenal cortex and results in an elevation of circulating glucocorticoid hormones.

The hypothalamic neurosecretory cells are regulated through synaptic connections by signals from neurons using various neurotransmitters.

In recent years efforts have been directed toward and progress has been made in identifying some of the neurotransmitters that control the release of CRF.

Among them, monoaminergic neural systems in the hypothalamic control of pituitary function have been widely studied. Several lines of evidence suggest the existence of serotonergic control, but the conclusions from studies on the possible role of serotonin in the regulation of hypothalamo-pituitary-adrenal (HPA) activity (circadian rhythm of corticosterone secretion, feedback sensitivity and stress-responsiveness of the HPA system) are not in agreement.

The present experiments were designed to test whether using various different pharmacologic approaches to stimulate or eliminate the central serotonergic system have any consistent effect on the circadian rhythmicity of the HPA system and to investigate whether a correlation exists between the concentrations of 5-HT in the rat brain and the concentrations of plasma corticosterone changed by administration of drugs affecting serotonergic system.

## MATERIALS AND METHODS

In all experiments male Sprague-Dawley rats (SNU animal house, Seoul, Korea) weighing 160~250g were used. Animals were housed five to a cage in a constant-temperature room(20~25°C) with a 12-h light cycle(lights on 7.00~19.00 hours) and given commercial rat chow and tap water ad libitum. Animals were allowed to acclimatize to the condition of a quiet laboratory for 1hr before experimental procedures were started. Great care was taken to minimize the disturbance to the animals during the transfer. Experiments were performed between 10h and 12h(morning study group) or 16hr and 18hr(evening study group) each day.

Rats were killed by decapitation and blood collected from the severed neck blood vessels into heparinized tubes. Care was taken to ensure that the rats following in line did not view

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the decapitation of the preceding animals.

Plasma corticosterone was estimated by the spectrofluorimetric method of Zanker and Bernstein(1958). Fluorimetric readings were made on an Perkin-Elmer Spectrophotofluorometer (Model 1000, England) at an excitation wavelength of 468nm and an emission wavelength of 520nm. Corticosterone standard was obtained from the Sigma Chemical Company Ltd(USA).

Brains were quickly removed and chilled and the hypothalamus dissected out. 5-hydroxytryptamine concentrations were measured in hypothalamus and the remainder whole brain by the spectrofluorometric method of modified Curzon and Green(1970).

Drugs were dissolved in normal saline for injection except 5-methoxy-N-N-dimethyl tryptamine(5-MeODMT). 5 MeODMT was dissolved in absolute ethanol, then diluted with saline to a final ethanol concentration of 2% v/v. Control animals were injected with an equal volume of normal saline.

Drugs used were L-tryptophan(Sigma, Mo. U.S.A.), 5-hydroxytryptophan(5HTP, Sigma), Pargyline HCl(Sigma), Parachlorophenylalanine(PCPA, Sigma), 5-methoxy-N-N-dimethyltryptamine(5-MeODMT, Sigma), 5,7-dihydroxytryptamine(5,7-DHT, Sigma).

All drugs were injected intraperitoneally except for 5,7-DHT, which was injected stereotactically into the lateral ventricle in a volume of 10 $\mu$ l at a rate of 1 $\mu$ l/min. The coordinate were 2mm from the midline, 1mm posterior to bregma and 3.5mm below the skull surface.

All animals were killed 1hr after injection of drug except for PCPA and 5,7-DHT. Rats were killed 24hr after last dose of PCPA and 5,7-DHT was intraventricularly injected to rats, and the rats were killed 7 days after injection of 5,7 DHT. Statistical analysis was carried out using the student's t-test, one way analysis of variance and least squares linear regression.

## RESULTS

### 1. Effect of Serotonin precursor, L-tryptophan:

L-tryptophan(100mg/kg) caused a significant rise both in the morning plasma corticosterone levels(199%,  $P < 0.001$ ) and in the hypothalamic serotonin contents(162%,  $P < 0.001$ ). The effect of L-tryptophan was significantly potentiated by 1hr prior injection of a irreversible monoamine oxidase inhibitor, pargyline(70mg/kg)(Table 1, Fig. 1).

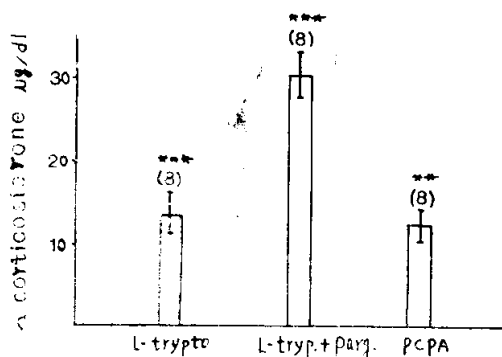
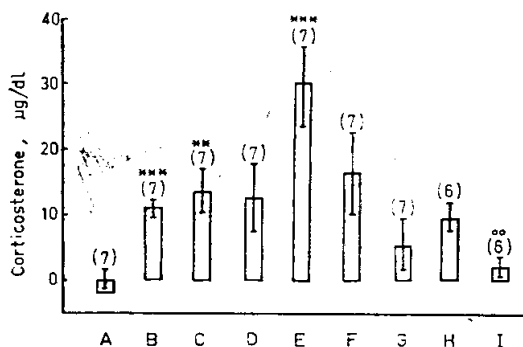


Fig. 1. Effect of L-tryptophan, L-trypt.+Pargyline and PCPA on the morning plasma corticosterone levels. All the data are presented as an increase of plasma corticosterone above the resting level. Vertical bars represent 1 SEM. The number of determinations is given in parenthesis. \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

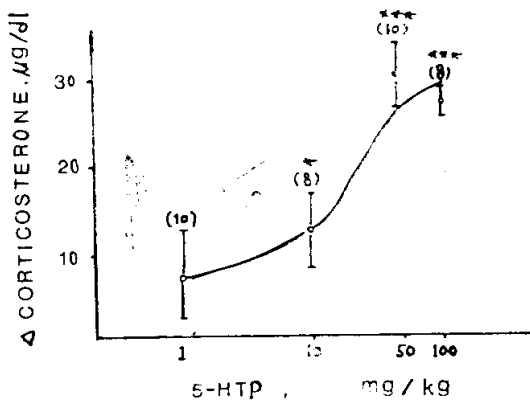
2. Effect of Serotonin immediate precursor, 5-hydroxytryptophan(5-HTP): 5-HTP(1mg ~100 mg/kg) caused a dose-related elevation of morning plasma corticosterone and brain serotonin levels in intact rats(Fig.3, Table 1, and Table 2). Also,5-HTP(10mg and 50mg/kg) produced a large rise in the concentrations of evening corticosterone and brain serotonin(Table 2, Fig. 2).

3. Effect of Serotonin synthesis inhibitor, p-chlorophenylalanine(PCPA): p-chlorophenylalanine (300mg/kg/day for 2 days) caused a marked depletion of morning and evening brain serotonin and raised the morning plasma corti-

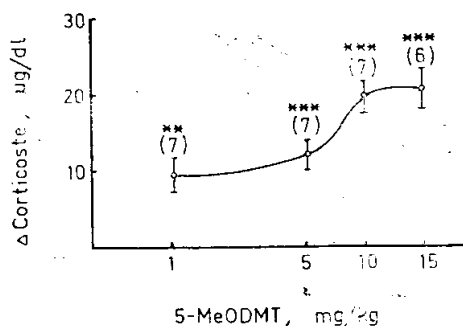


**Fig. 2.** Effect of 5HTP, PCPA, 5-MeODMT and 5,7-DHT on the evening plasma corticosterone levels. Vertical bars are 1 SEM. Parenthesis (No. of experiments). \*Significantly different from saline control (\*\* $P < 0.01$ , \*\*\* $P < 0.001$ ). °Significantly different from sham-operated group(° $P < 0.01$ ). A: PCPA, B: PCPA+5-MeODMT (50mg/kg), C: PCPA+5-HTP (100mg/kg), D: 5-HTP (10mg/kg), E: 5-HTP (50mg/kg), F: 5-MeODMT (5mg/kg), G: 5-MeODMT (10mg/kg), H: Sham. Operation, I: 5,7-DHT (100  $\mu$ g).

corticosterone levels (191%,  $P < 0.001$ ) and decreased the evening levels to 94% of control (Table 1, Table 2, Fig. 1, and Fig. 2). Fall in evening plasma corticosterone levels by PCPA was reversed by following administration of 5-HTP (100mg/kg) and 5-methoxy-N-N-dimethyl tryptamine (5mg/kg) (Fig. 2).



**Fig. 3.** Log dose response curve for rise in morning plasma corticosterone levels after i.p. 5-HTP. Bars represent 1 SEM. The number of determinations is given in parenthesis. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

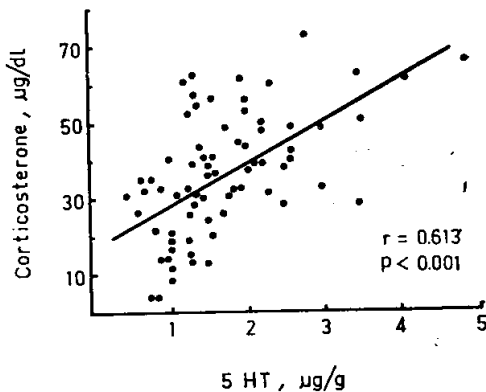


**Fig. 4.** Log dose response curve for rise in morning plasma corticosterone levels after i.p. 5-MeODMT. Bars represent 1 SEM. The number of determinations is given in parenthesis. \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

**4. Effect of direct Serotonin agonist, 5-methoxy-N-N-dimethyltryptamine (5-MeODMT):** 5-MeODMT (1~15mg/kg) produced a dose related elevation of morning plasma corticosterone levels (Fig. 4). Evening plasma corticosterone levels were increased by injection of 5-MeODMT (5mg, 10mg/kg, i.p.) (Fig. 2).

**5. Effect of Serotonin neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT):** 5,7-DHT (100 $\mu$ g) lowered brain 5-HT contents and plasma corticosterone concentrations to a similar extent (about 20%).

**6. Correlation between plasma corticosterone concentration and brain serotonin concentrations:**



**Fig. 5.** Correlation between plasma corticosterone and hypothalamic 5-HT concentrations after administration of several drugs affecting 5-HT system.  $y = 11.006x + 16.989$ ,  $r = 0.6125$ ,  $p < 0.001$ .

**Table 1.** Morning brain 5-hydroxytryptamine contents after various treatment

Treatment	No. of animals	5-HT levels, $\mu\text{g/g}$	
		hypothalamus	Remainder brain
Saline control	10	0.976 $\pm$ 0.048	0.571 $\pm$ 0.024
L-tryptophan (100mg/kg)	8	1.583 $\pm$ 0.141***	0.501 $\pm$ 0.016
L-tryptophan+Pargyline (70mg/kg)	8	3.375 $\pm$ 0.392***	2.122 $\pm$ 0.183***
5-HTP, 1mg/kg	10	0.914 $\pm$ 0.111	0.422 $\pm$ 0.024
10mg/kg	8	1.478 $\pm$ 0.134**	0.789 $\pm$ 0.029***
50mg/kg	10	2.080 $\pm$ 0.211***	2.217 $\pm$ 0.133***
100mg/kg	8	2.360 $\pm$ 0.172***	2.122 $\pm$ 0.122***
PCPA(300mg/kg)	8	0.262 $\pm$ 0.027***	0.209 $\pm$ 0.025***

Rats were killed between 10:00 and 12:00. Values are mean $\pm$ S.E.M.

Significantly different from saline control: \*P<0.05 \*\*P<0.01 \*\*\*P<0.001

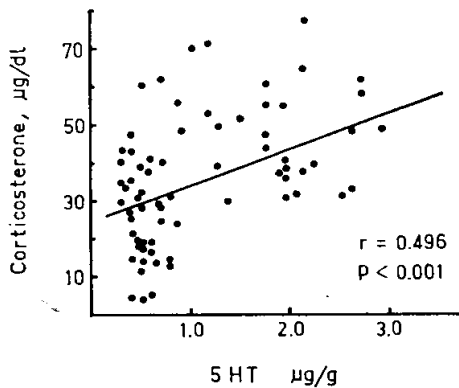
**Table 2.** Evening brain 5-HT contents

Treatment	No. of animals	5-HT levels $\mu\text{g/g}$	
		hypothalamus	Remainder brain
Saline control	8	1.211 $\pm$ 0.288	0.428 $\pm$ 0.039
5-HTP, 10mg/kg	7	1.132 $\pm$ 0.101	0.726 $\pm$ 0.05***
50mg/kg	7	2.363 $\pm$ 0.501*	1.525 $\pm$ 0.196***
PCPA, 300mg/kg	7	0.435 $\pm$ 0.066***	0.208 $\pm$ 0.006***
+5HTP(100mg/kg)	7	1.100 $\pm$ 0.121	0.621 $\pm$ 0.105
Sham operated control	6	1.024 $\pm$ 0.120	0.384 $\pm$ 0.062
5,7-DHT	6	0.837 $\pm$ 0.061 $^{\circ}$	0.284 $\pm$ 0.070

Rats were sacrificed between 17:00~18:00. Values represent mean $\pm$ S.E.M.

Significantly different from saline control (\*P<0.05, \*\*P<0.01, \*\*\*P<0.001).

Significantly different from sham-operated group( $^{\circ}$ P<0.01).



**Fig. 6.** Correlation between plasma corticosterone and 5-HT concentrations in remainder whole brain after administration of several drugs affecting 5-HT system.

$$y = 9.746x + 25.2, r = 0.496, p < 0.001.$$

There was a moderate-positive correlation between plasma corticosterone concentrations and hypothalamic serotonin concentration changed by several serotonin drugs ( $r = 0.6125, p < 0.001$ ; Fig. 5). As compared to this result, there was a weaker correlation between plasma corticosterone concentrations and remainder whole brain serotonin concentrations ( $r = 0.4955, p < 0.001$ ; Fig. 6).

## DISCUSSION

It is now firmly established that the hypothalamus is the focal point at which neural stim-

uli converge to influence the secretion of ACTH, and the median eminence is regarded as the final common path through which information is transmitted to the anterior pituitary. The origins of the neurons which exert pharmacological effects on the CRF neurons are at present not defined although tracts inhibiting and stimulating ACTH release have been found ascending in the spinal cord and brain stem (Gann et al., 1978).

Considerable progress has been made in the past ten years on the elucidation of the role of neurotransmitters in regulating hypothalamo-pituitary-adrenal (HPA) function. But it is still uncertain which transmitters and which pathways play important regulatory roles. In the rat hypothalamus *in vitro*, secretion of CRF has been shown to be stimulated by acetylcholine and serotonin and inhibited by norepinephrine and  $\gamma$ -aminobutyric acid (Buckingham and Hodges, 1977; Jones et al., 1976). Several lines of evidence suggest the existence of serotonergic control *in vivo*, but there is considerable controversy concerning the role of brain 5-HT in the regulation of HPA activity (circadian rhythmicity, feedback sensitivity and stress responsiveness).

Some studies have suggested that a serotonergic neural pathway stimulates ACTH release (Naumenko, 1969; Krieger and Rizzo, 1969; Krieger and Krieger, 1970; Scapagnini et al., 1971; Popova et al., 1972; Krieger, 1975; Fuller et al., 1976; Krieger and Condon, 1978; Knych and Eisenberg, 1979, 1980; Steiner and Grahame Smith, 1980).

However, other studies have led to the suggestion that there are inhibitory serotonergic neural pathways influencing HPA activity (Westermann et al., 1962; Vermes and Telegdy, 1972; Vernikos-Danellis et al., 1973; Berger et al., 1974; Vermes et al., 1974; Pavel et al., 1977).

At least two studies (Rotsztein et al., 1977; Karteszi et al., 1981) found that neither the circadian rhythm of HPA activity nor the stimulated secretion of ACTH in response to adrenalectomy depended on central serotonergic control.

Thus conflicting results could be due in part to experiments carried out at different times of day and to using different species. Another likely possible explanation for the conflicting results is that peripheral administration of relatively nonspecific drugs affecting serotonergic system not only alters brain 5-HT, but also affects other neurotransmitter systems and whole body.

The aim of the present study was to elucidate one aspect of the central control of CRF-ACTH secretion, namely, does 5-hydroxytryptamine play an essential role in controlling the circadian rhythm of HPA system of rat.

Rats have a diurnal plasma corticosterone with a trough early in the morning and a peak in the late afternoon (Critchlow, 1963; Rinne and Sonninen, 1964; Lee et al., 1982). Our experiments were performed in the morning (low level) and in the late afternoon (peak level).

The only conditions known to be associated with alterations in the circadian periodicity of plasma corticosterone level are; prolonged reversal (Sharp et al., 1961) or alteration of the sleep-wake schedule (Orth et al., 1967); diffuse CNS disease (Perkoff et al., 1959) or focal hypothalamic-limbic system disease (Krieger and Krieger, 1966) and discrete hypothalamic lesions in animals (Golichich and Halberg, 1965; Slusner, 1964).

These observations suggest that the CNS plays a dominant role in determining this daily variation in plasma corticosteroid levels. The present study indicated that the rise in the brain 5-HT contents 1 hr after injection of serotonin precursors (L-tryptophan and 5-HTP) was accompanied by a rise in the plasma corticosterone

concentrations and 5HTP (1~100mg/kg) caused a dose-related elevation whereas L-tryptophan (over 100mg/kg) caused a significant elevation of the concentrations of brain 5-HT and corticosterone. L-Tryptophan, in contrast to L-5HTP, would not lead to serotonin formation at sites that do not form serotonin physiologically. However, only a very small percentage of administered tryptophan is converted to serotonin and the degree of serotonin receptor stimulation attainable by giving L-tryptophan is less than that with 5HTP, and other nonspecific mechanisms may influence corticosterone secretion. Our results concerning the effect of 5HT precursors were supported by several studies (Okada et al., 1972; Popova et al., 1972; Fuller et al., 1976; Steiner and Grahame-Smith, 1980) but in disagreement with Pavel et al., (1977) and Vernikos-Danellis(1980).

p-Chlorophenylalanine (PCPA) inhibits tryptophan hydroxylase irreversibly and lowers serotonin concentration in brain. Maximum depletion occurs within about 24 hrs and depletion persists for at least 1 week (Koe and Weissman, 1968). We observed that PCPA(300mg/kg/day for 2 days) treatment raised the morning corticosterone and decreased the evening levels, which may be opposite effect, and following treatment of 5HTP and 5-MeODMT prevented the decrease of evening levels. There have been numerous studies showing that PCPA abolishes or attenuates the normal diurnal rhythm of circulating corticosterone levels in rats (Scapagnini et al., 1971, 1972; Van Delft et al., 1973; Vernikos-Danellis et al., 1973; Rotsztejn et al., 1977). Szafarczyk et al., (1979) observed that PCPA treatment increased corticosterone while decreasing ACTH: this disparity suggests that this amino acid may influence corticosterone rhythmicity in ways other than preventing the usual physiologic signals to rhythmicity. De Schaepestryver et al., (1969) Vernikos-Danellis et

al., (1977) and Pavel et al., (1977) reported that PCPA caused adrenocortical activation. One possible explanation for these conflicting data is that PCPA has to be given at high doses to deplete serotonin and probably influences catecholamine, amino acid availability and other physiologic process (Vernikos-Danellis et al., 1973, 1977), 5-Methoxy-N,N-Dimethyltryptamine (5-MeODMT) are indole compounds structurally related to serotonin that are potent serotonin agonists and elevated plasma corticosterone levels in a dose-related fashion and prevented fall in the evening corticosterone concentration caused by PCPA treatment.

5, 6-and 5, 7-dihydroxytryptamine(DHT) are serotonin neurotoxins that result in very long-lasting depletion of brain serotonin and do not pass blood-brain barrier (Baumgarten et al., 1978). The present study showed that intraventricular injection of 5, 7-dihydroxytryptamine lowered the evening corticosterone levels. Kepic et al. (1979) reported that i.v. injection of 5, 7-dihydroxytryptamine abolished the normal diurnal changes in plasma corticosterone in rats, although Kriger(1975) had found that injection of 5, 6-DHT did not prevent diurnal rhythmicity of plasma corticoids in rats.

The results obtained in the present experiments demonstrate a direct positive correlation between the concentrations of hypothalamic 5-HT and plasma corticosterone caused by pharmacologically manipulating serotonin system.

Caution should be exercised in drawing absolute conclusions from any one of several data since the techniques used to manipulate brain 5-HT levels suffer from several disadvantages and in assuming that a single action of the drug was responsible for the observed effects (Vernikos-Danellis et al., 1977).

In conclusion, our present data strongly suggest that 5-HT is a key neurotransmitter involved in the regulation of the circadian

rhythmicity of HPA system.

### SUMMARY

Various pharmacologic studies have supported a stimulatory role of brain serotonin neurons in the hypothalamic regulation of circadian rhythmicity of pituitary-adrenocortical system in rats.

The serotonin precursors (L-tryptophan and L-5-hydroxytryptophan), direct acting serotonin agonist (5-Methoxy-N-N-dimethyltryptamine) and monoamine oxidase inhibitor (Pargyline) all elevate both morning and evening plasma corticosterone levels in rats. The elevation of plasma corticosterone by L-tryptophan (100mg/kg) was potentiated by monoamine oxidase inhibitor, pargyline (70mg/kg). The elevation of plasma corticosterone by 5-hydroxytryptophan and 5-methoxy-N-N-dimethyl tryptamine showed a dose-related fashion both in the morning and in the evening.

Serotonin synthesis inhibitor (P-chlorophenylalanine) and serotonin neurotoxin (5,7-dihydroxytryptamine) caused a fall in plasma corticosterone level.

Daily injections of p-chlorophenylalanine (300 mg/kg/day) for 2 days raised the morning low and prevented the evening rise in plasma corticosterone.

The evening rise in plasma corticosterone was restored by the injection of 5-hydroxytryptophan and 5-methoxy-N-N-dimethyltryptamine.

There was a positive correlation between the concentrations of serotonin in the hypothalamus and the concentrations of plasma corticosterone caused by various pharmacologic manipulation.

Thus, our studies suggested that brain serotonergic neurons may play an important role in the regulation of the diurnal rhythmicity of adrenocortical function.

### ＝국문초록＝

## 시상하부—뇌하수체—부신계 조절에 대한 Monoamine 신경전달 물질의 역할에 관한 연구(II) —뇌 5-hydroxytryptamine(Serotonin) 의 Corticosteroid 일주기 변동에 미치는 영향

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CRF 분비를 조절하는 신경전달물질 특히 serotonin (5HT)의 역할에 대하여 많은 연구가 있어왔으나 아직 논란이 많이 일어나고 있다. 따라서 저자는 시상하부—뇌하수체—부신계 (hypothalamo-pituitary adrenal system; HPA)의 일주기변동에 미치는 serotonin (5-HT)의 역할을 알아보기 위하여 각종의 serotonin제를 변동시킬 수 있는 약물을 백서에 투여한 후 나타나는 결과를 분석 검토해 보았다.

1. Serotonin전구 물질인 L-tryptophan(100mg/kg)과 L-5-hydroxytryptophan (5HTP) (1-100mg/kg)을 투여하였을 때는 뇌 5-HT함량과 혈장 corticosterone함량의 증가가 나타났으며, 특히 5HTP의 증가는 용량에 비례해서 증가되었다. 또한 L-tryptophan의 효과는 MAO효소 억제제인 pargyline (70 mg/kg)의 투여에 의해서 현저히 상승되었다.

2. 5-HT (serotonin) 합성 억제제인 P-chlorophenylalanine(300mg/kg/day, 2일간 투여)을 투여했을 때는 뇌 5-HT 함량의 상당한 감소가 나타났으며, 혈장 corticosterone함량은 오전치는 상승되었으나 저녁치는 하강을 보였다. 저녁치의 하강은 5HTP (100mg/kg) 혹은 5-methoxy-N-N-dimethyltryptamine (5mg/kg)을 병용 투여함으로써 방지되었으며 오히려 저녁치의 증가가 나타났다.

3. 5-HT 수용체차극제인 5 Methoxy-N-N-dimethyltryptamine (1-15 mg/kg)을 투여할 시는 혈장 corticosterone농도가 용량에 비례해서 증가하였다.

4. Serotonin 신경독인 5,7-dihydroxytryptamine(5,7-DHT, 100µg base)을 뇌실로 투여할때는 뇌 5-HT 함량과 혈장 corticosterone함량의 감소가 나타났다.

5. 위의 serotonin제를 변동시킬 수 있는 약물을 투여한후에 나타나는 시상하부 serotonin 함량과 혈장 corticosterone함량 사이에는 유의있는 선 상관관계를 보였다 ( $r=0.6125$ ,  $P<0.001$ ,  $n=70$ ). 그러나 시상하부를

제외한 전뇌 serotonin 함량과 혈장 corticosterone 함량 사이에는 보다 약한 순 상관관계가 있었다. ( $r=0.4955$ ,  $P<0.001$ )

위의 결과를 종합해 볼 때, serotonin (5-HT)은 시상하부-뇌하수체-부신계의 일주기변동의 조절기전에 중요한 (자극적 역할) 위치를 차지하리라 생각된다.

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