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Roles of Monoamine Neurotransmitters in Regulation of Hypothalamic PITUITITARY-ADRENAL AXIS (HPA) (Ⅲ)

Role of 5-hydroxytryptamine in Controlling the Stress-Induced Elevation of Corticosterone in Rat

Yoo Hun Suh, Jung Kyoo Lim and **Chan Woong Park**

Department of Pharmacology, College of Medicine, Seoul National University

=국문초록=

시상하부—뇌하수체—부신계 조절에 대한 Monoamine 신경전달물질의 역할에 관한 연구(III)—뇌 5-hydroxytyptamine(Serotonin)이 Stress시 Corticosteroid 변동에 미치는 영향

서울대학교 의과대학 약리학교실

서 유 헌·임 정 규·박 찬 웅

뇌내 여러 신경전달물질계가 시상하부—뇌하수체—부신계(HPA)를 복잡하게 조절하고 있다는 증거가 많이 나오고 있다. 그 중에서도 5-HT(Serotonin)계의 stress시의 역할에 대해서는 많은 연구가 있어왔으나 아직 논난이 많이 제기되고 있다. 따라서 저자는 5-HT가 stress시 HPA axis를 조절하는데 필수적인 역할을 하는가를 알아보기 위해서 1) Stress 전후에 5-HT의 steady state농도와 5-HT의 교체율(turnover rate) 및 합성율(synthesis rate)을 측정해 보았으며 2) 비교적 5-HT계에 특이하게 작용하는 여러 약물들을 주사한 후에 stress 반응을 측정해 보았다.

1) 1분간 ether stress 가한 직후에 시상하부와 다른 뇌부위에서의 5-HT와 5-HIAA 농도는 즉각적으로 의미있게 상승하였으나 혈장 corticosterone농도는 즉각상승을 보이지 않다가 2분후에 상승을 보였다.

2) Stress(30분 immobilization & 1분 ether stress)가한 백서에서는 시상하부와 다른 뇌부위에서 5-HT 합성율 혹은 교체율이 2배—4배까지 상승하였다.

3) 5-HT 합성 전구물질(L-tryptophan)과 수용체 자극제(5-MeODMT)를 투여하였을 때는 투여용량에 비례해서 혈장 corticosterone 함량이 상승하였다.

4) L-tryptophan과 MAO 억제제(pargyline) 혹은 L-tryptophan과 5-MeODMT의 병합 투여로 stress시 혈장 corticosterone의 상승이 더 높게 나타났다.

5) 5-HT 합성억제제(PCPA), 5-HT 신경독약(5,7-DHT)을 투여하고 stress를 가하였을 때는 시상하부와 다른 뇌 부위에서 5-HT의 하강이 별로 나타나지 않았으며, 동시에 혈장 corticosterone의 하강도 의미있게 나타나지 않았다. 그러나 midline raphe 핵을 파괴하였을 때는 5-HT와 corticosterone의 하강이 나타났다.

6) 비교적 특이하게 serotonin계에 작용하는 여러 약물들을 투여한 후에 나타나는 5-HT와 혈장 corticosterone 함량 사이에는 상당히 높은 양의 상관관계가 있었다($r>0.81$).

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이상의 결과를 종합해 볼때 serotonin(5-HT)은 시상하부—뇌하수체—부신계의 스트레스반응조절에 중요한 자극적 역할을 하리라고 생각된다.

INTRODUCTION

Several transmitters have been suggested to modulate the release of corticotropin-releasing factor (CRF: corticoliberin). Among them, roles of monoaminergic neural systems in the control of hypothalamo-pituitary adrenal (HPA) axis have been widely studied. Several lines of evidence suggest a possible role of 5-hydroxytryptaminergic neurons in the control of circadian rhythmicity and stress-induced activation of HPA axis, but there is considerable controversy concerning the role of brain 5-HT in the regulation of HPA activity.

Some have proposed the hypothesis that serotonin is a stimulatory neurotransmitter involved in the stress responsiveness of HPA (Fuller et al., 1976; Jones et al., 1976, 1979; Knych and Eisenberg, 1979, 1980; Krieger and Krieger, 1970, 1975; Meyer et al., 1978; Popova et al., 1972; Steiner and Grahame-Smith, 1980).

In the other hand, the results of several investigations have led to the opposite conclusion (Berger et al., 1974; Kovacs et al., 1976; Pavel et al., 1977; Telegdy and Vermes, 1973; Vermes et al., 1972, 1974; Vernikos-Donellis, 1973, 1980; Westerman et al., 1962).

This study was undertaken to try to clarify the role of serotonin in the pituitary-adrenocortical response to stress. The aims of the present study were to check kinetic parameters of 5-HT turnover as well as steady state concentrations before and after stress and to test whether using relatively sypecific-pharmacologic approaches to stimulate or eliminate central serotonergic system have any consistent effect on the stress-induced activation of HPA system.

MATERIALS AND METHODS

Animals and Chemicals

In all experiments male Sprague-Dawley rats (SNU animl house, Seoul, Korea) weighing 160-250 g 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 1 hr before experimental procedures were started. Experiments were performed between 10 h and 12 h.

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.), pargyline HCl (sigma), parachorophenylalanine (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 stereotaxically into the lateral ventricle and midline raphe nucleus in a volume of 10 μl at a rate of μl/min, according to Pellegrino's rat stereotaxic atlas (Pellegrino et al., 1967). All animals were killed 24 hr after last dose of PCPA and 5, 7-DHT was injected to rats, and the rats were killed 7 days after injection of 5, 7 DHT.

Stress

Stress was induced by exposing the rat for 1 min to an atmosphere saturated in ether vapor at room temperature(20–24°C) or by immobilization of their legs with adhesive tape for 30 min.

Determination of plasma corticosterone levels

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 the decapitation of the preceding animals.

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

Determination of 5-HT and 5-HIAA contents and turnover in brain

In order to conduct the studies on turnover rate of 5-HT in brain, rats were sacrificed at definite time intervals(0, 10, 30 and 60 min) after injection of pargyline(75 mg/kg, i. p.) and the brains were immediately removed and frozen on dry ice and stored at -25°C until used. For each time point, 6 to 8 animals per group were used. The synthesis rate of 5-HT was determined by measuring the accumulation of 5-HT after pargyline hydrochloride (Tozer et al., 1966), and calculated by the regression on the linearity. The elimination rate of 5-HIAA from whole brain tissue was determined based on the measurement of both the steady state level of 5-HIAA and the rate constant of 5-HIAA efflux in rat brain. The rate constant was determined from the slope of the exponential decline of 5-HIAA after pargyline administration.

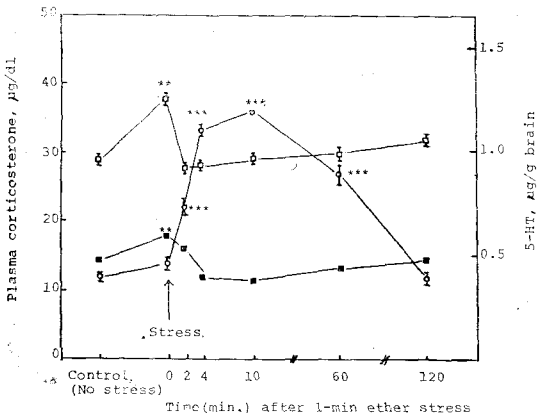

Fig. 1. Time course of the elevation of plasma corticosterone (○) and 5-HT levels in rat hypothalamus (□) and remainder brain areas (■) after 1 min.-ether stress.

Mean values±S.E.M. for 6 to 20 rats per group were shown. S.E.M. were indicated only when they exceed the size of symbol.

Asterisks indicate significant differences from the Nonstressed control group(*p<0.05, ***p<0.01, ***p<0.001).

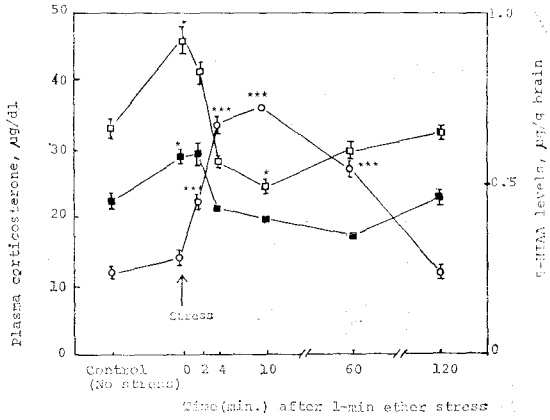

Fig. 2. Time course of the elevation of plasma corticosterone (○) and 5-HIAA levels in rat hypothalamus (□) and remainder brain areas (■) after 1min-ether stress.

Mean values−S.E.M. for 6 to 20 rats per group were shown. S.E.M. were indicated only when they exceed the size of symbol.

Asterisks indicate significant differences from the Nonstressed control group(*p<0.05, **p<0.01, ***p<0.001).

Table 1. Estimated rates of 5-HT synthesis of rat brain measured by 5-HT pargyline method before and after stress

Treatment	Steady state level of 5-HT(μg/g)[a]		Synthesis rate of 5-HT(μg/g brain/hr)[b]	
	hypothalamus	remainder	hypothalamus	remainder brain
Control	0.813±0.020	0.327±0.025	0.231±0.032	0.084±0.012
Immobilization stress	0.935±0.048*** (+15.0%)	0.480±0.022*** (+46.7%)	0.763±0.034** (+230.3%)	0.254±0.041** (207.1%)
Ether stress	1.052±0.087*** (+29.4%)	0.552±0.035*** (+68.8%)	1.004±0.059** (+334.6%)	0.233±0.061** (+177.4%)

[a]The values represent mean±S.E.M. of at least 4 to 6 animals of 2 different experiments.
[b]The values represent mean±S.E.M. of 2 different experiments and measured from the accumulation of 5-HT after pargyline hydrochloride(70 mg/kg, i.p.)
( ): % of increase over control.
**: Significantly different from control($p<0.01$)
***: Significantly different from control($p<0.001$)

Table 2. Kinetic parameters of 5-HT turnover in rat whole brain before and after stress

Treatment	Steady state level 5-HIAA (μg/g±S.E.)[a]	Rate constant of 5-HIAA efflux(k) (hr^{-1}±S.E.)[b]	5-HT turnover time(hr±S.E.)[c]	Synthesis rate of 5-HT (μg/g/hr±S.E)[d]
Control	0.672±0.070	0.483±0.071	2.163±0.317	0.325±0.047
Immobilization stress	0.699±0.037 (+4.0%)	0.497+0.032 (+2.9%)	2.029±0.129 (−6.2%)	0.347±0.022 (6.8%)
Ether stress	0.717±0.094 (6.7%)	1.753±0.092** (+262.9%)	0.574±0.030* (−73.5%)	1.257±0.066** (+286.8%)

[a]The values represent mean±S.E.M. of at least 4 to 6 animals of 2 different experiemts.
[b,c,d]The values represent mean±S.E.M. of 2 different experiments and measured from the decline of 5-HIAA after pargyline hydrochloride.
( ): % of increase over control.
* $p<0.05$ Compared to control.
** $p<0.01$ Compared to control

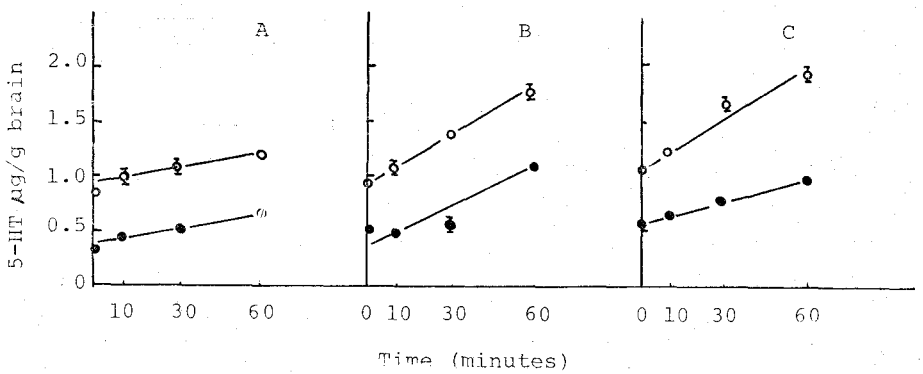

Fig. 3. Accumulation kinetics of 5-HT in rat hypothalamus (○—○) and remainder brain areas (●—●) after pargyline. Slopes were best fitted by least squares linear regression analysis ($r<0.90$).
A: Control; B: immobilization stress(30 min); C: Ether Stress(1 min). Bars represent mean values of at least 4 to 6 animals±S.E. S.E. were indicated only when they exceed the size of symbol.

Statistical analysis was carried out using the student's t-test, one way analysis of variance and least squares linear regression.

RESULTS

1) Time course of the elevation of plasma certicosterone and 5-HT and 5-HIAA levels in rat brain after 1 min-ether stress

Steady state brain 5-HT and 5-HIAA concentrations in the hypothalamus and in the remainder of brain after 1 min-ether stress were immediately and significantly elevated without significant rise in the levels of plasma corticosterone, which highly increased 2 minutes after stress (Fig. 1 and Fig. 2).

2) Kinetic parameters of 5-HT turnover in the rat brain before and after stress(30 min-immobilization and 1 min-ether stress)

Figure 3 shows the time-course of the accumulation of brain 5-HT stores in rats after the administration of monoamine oxidase inhibitor, pargyline(75 mg/kg). The slopes of 5-HT accumulation during 30 min immobilization or 1 min-ether stress were shown to be steeper compared to that in non-stressed group(Fig. 3). The rates of synthesis in the hypothalamus during stress were three to four times greater than that of control group(Table 1). The turnover rates in the remainder of the brain were two to three times greater than that of control group as shown in table 1. As shown in table 2, steady state levels and rate constants of 5-HIAA efflux, and 5-HT turnover rates in stressed groups were significantly increased.

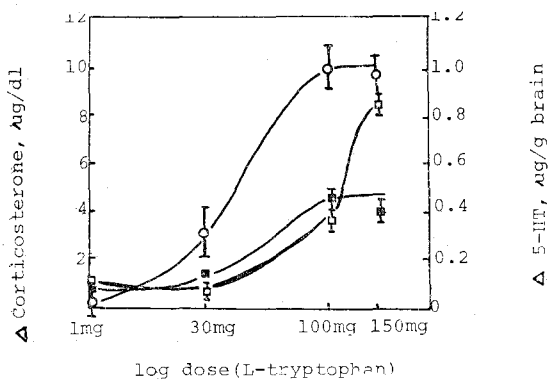

Fig. 4. Log dose response curve for ether induced rise in plasma corticosterone (○—○) and 5-HT levels in hypothalamus (□—□) or remainder brain areas (■—■) after i.p. L-tryptophan.

Bars represent mean±S.E. Groups of 4 to 6 rats were injected i.p. 1 hr before exposure to 1 min ether stress and sacrificed 4 min after stress.

3) Effect of 5-HT precursor, L-tryptophan

L-tryptophan(1mg-150 mg/kg) caused a dose-related elevation of plasma corticosterone and brain serotonin levels in ether-stressed group (Fig. 4).

4) Effect of 5-HT agonist, 5-methoxy-N, N-dimethyltryptamine(5-MeODMT)

As shown in Fig. 5, the ether-stress-induced corticosterone levels were more incresased by serotonin agonist, 5-MeODMT in a dose-related fashion.

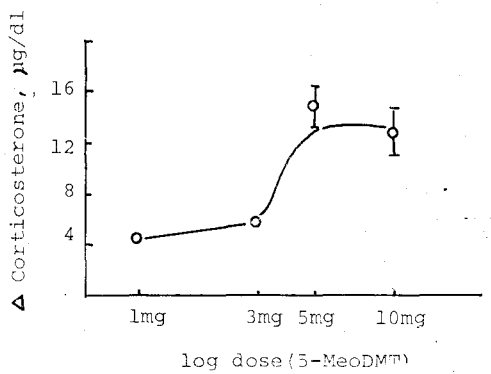

Fig. 5. Log dose response curve for ether-induced rise in plasma corticosterone levels after i.p. 5-methoxy N,N-dimethyl tryptamine (5-MeODMT). Vertical bars are the mean ±S.E.M. of at least 4 to 6 rats.

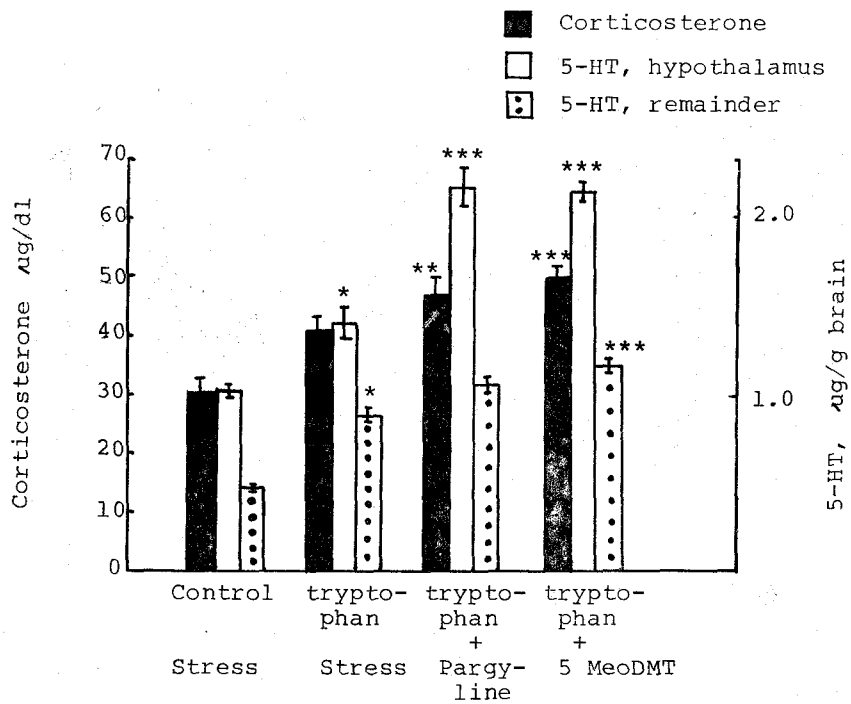

Fig. 6. Brain 5-HT and plasma corticosterone response to ether stress after tryptophan, tryptophan+pargyline, or tryptophan+5 MeoDMT.
* $p<0.05$ Compared to Control.
** $p<0.01$ Compared to Control.
*** $p<0.001$ Compared to Control.

5) Brain 5-HT and plasma corticosterone response to ether stress after tryptophan, tryptophan+pargline, or tryptophan+5-MeODMT

The stress-induced elevation of corticosterone and 5-HT levels in the hypothalamus and the brain were more marked by L-tryptophan (100 mg/kg) and potentiated by monoamine oxidase inhibitor, pargyline (75 mg/kg) or serotonin agonist, 5-MeODMT (5 mg/kg) (Fig, 6).

6) Brain 5-HT and plasma corticosterone response to ether stress after PCPA, 5, 7-DHT or raphe lesion

The stress-induced elevation of corticosterone and 5-HT levels in the hypothalamus and in the remainder of the brain were not significantly decreased by the administration of 5-HT synthesis inhibitor, PCPA and 5-HT neurotoxin, 5, 7-DHT. However, the stress-induced elevation of corticosterone of 5-HT levels were significantly decreased by the destruction of midbrain raphe nuclei (Fig. 7).

7) Correlation between ether-induced plasma corticosterone and 5-HT levels in the hypothalamus and in the remainder of the brain

Figure 8 showed that there was a strong positive correlation between plasma corticosterone and 5-HT concentrations changed by drugs that mainly manipulating 5-HT system in the hypothalamus and in the remainder of the brain.

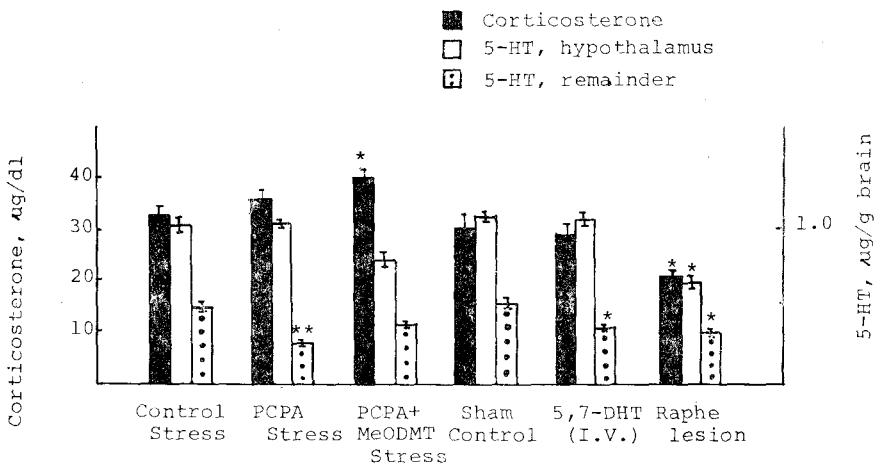

Fig. 7. Brain 5-HT and plasma corticoterone response to Ether Stress(1 min) after PCPA, 5, 7-DHT or raphe lesion. The values were obtained 4 min after 1 min-ether stress.
* $p<0.05$
** $p<0.01$

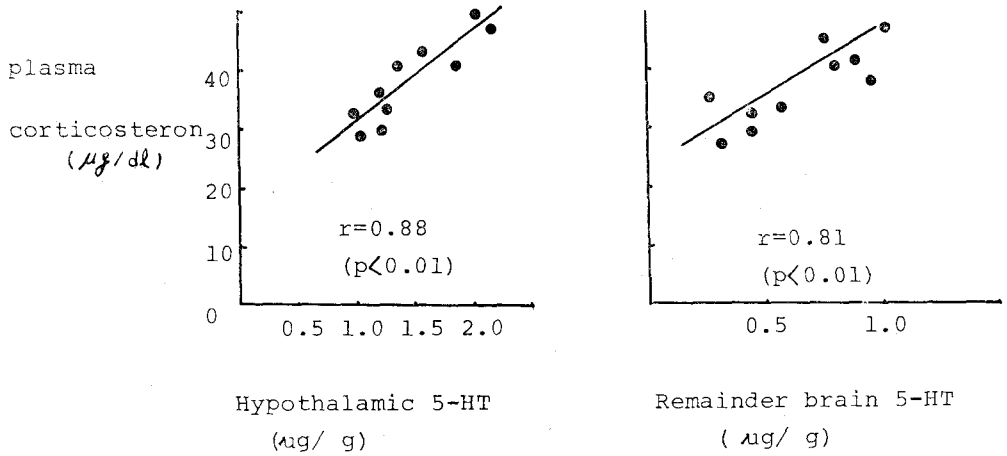

Fig. 8. Correlation between ether-induced plasma corticosterone and brain 5-HT concentration after various treatments affecting 5-HT concentration(control, various doses of tryptophan, tryptophan+ pargyline, PCPA, 5, 7-DHT, raphe lesion).

DISCSSION

Coniderable progress has been made in the past ten years on the elucidation of the role of neurotransmitters in regulating hypothalamopituitary-adrenal(HPA) function. But it is still uncertain which transmitters and which pathways play important regulatory roles. Monoaminergic neural systems involved the hypothalamic control of pituitary function have been widely studied. In the rat hypothalamus *in vitro* secretion of CRF has been shown to be stimulated by acetylcholine and serotonin and inhibited by norepinepinephrine and r-aminobutyic 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 (5-HT is a stimulatory neurotransmitter in the stress responsiveness of Hypothalamo-pituitary-adrenal (HPA) axis (Fuller et al, 1976; Jones et al., 1976, 1979; Knych and Eisenberg, 1979, 1980; Krieger and Krieger, 1970, 1975; Meyer et al., 1978; popova et al., 1972; Steiner and Grahame-Smith, 1980). However, other studies suggested that 5-HT may play an inhibitory role in the stress responsiveness of HPA axis (Berger et al., 1974; Kovacs et al., 1976; Pavel et al., 1977; Telegdy and Vermes, 1983; Vermes et al., 1972, 1974; Vernikos-Danellis, 1973, 1980; Westerman et al., 1962).

Thus, probable reasons for conflicting results are as follows:

1) The availability of selective pharmacologic tools has been limited.

2) Several drugs influence catecholamine neuron as well as serotonin neurons.

3) Steady steate brain amine concentrations do not provide adequate neuronol activity but turnover of a transmitter is a better reflection of neuronal functions.

4) Experiments carriedout at different times of day, using different species.

5) Differential roles for brain serotonin in regulation of circadian rhythmicity and stress responsiveness of HPA have not been undertaken.

The present experiments were undertaken to check 5-HT turnover rates as well as steady state concentrations in the hypothalamus and in the remainder of the brain before and after stress and to test whether using relatively specific pharmacologic approaches to stimulate or eliminate central 5-HT system have any consistent effect on the stress-responsiveness of HPA system.

The present study has demonstrated that steady state concentrations of 5-HT and 5-HIAA in the hypothalamus and in the remainder of the brain after 1 min-ether stress rapidly and significantly rised without significant rise in the levels of plasma corticosterone which increased 2 minutes after stress. Stress-induced increases in the levels of 5-HT and 5-HIAA reflect that there is a stress-induced increase in synthesis and in metabolism of 5-HT with some increase in 5-HT release and functional activity. Thus, the present findings suggest that the increase in the serotonergic neuronal activity precede that in the stress-responsiveness of HPA axis. It has been shown that 5-HT turnover rates and rate constants of 5-HIAA efflux in stressed group were two to three times greater than that in control group. This support above conclusion that stress causes increases in synthesis and turnover of 5-HT and that the functional 5-HT response to stress precede the rise in plasma corticosterone.

It has been known that exogenous tryptophan increase 5-HT synthesis in the rat brain (Eccleston et al., 1965; Fernstrom and Wurtman, 1971), presumably because tryptophon hydroxylase is not normally saturated by tryptophan (Freedman et al., 1972). Tryptophan, in contrast to L-5 hydroxy-tryptophan, would not lead to serotonin formation at sites that do not form seroton in physiologically.

N, N-Dimethyl-5-methoxytryptamine is indole compounds structurally related to serotonin that is potent serotonin agonist as measured by binding studies in vitro (Fuller and Snoddy, 1979). It was found here that L-tryptophan caused a dose-related elvation of plasma corticosterone and 5-HT levels and that the stress-induced elevation of corticosterone levels were potentiated by prior administration of

strong 5-HT agonist, 5-Meo DMT in a dose-related fastion. Moreover, the stress-induced elevation of corticosterone and 5-HT levels were much more potentiated by prior-combined administration of L-tryptophan+pargylie (MAO inhibitor) or of L-tryptophan+5-Meo DMT. This has been interpreted as indicating that stress-induced activation of HPA axis is largely dependent on the activation of 5-HT system in the brain.

P-chlorophenylalanine(PCPA) inhibits tryptophan hydroxylase irreversibly and lowers serotonin concentration in the brain. Maximum depletion occurs within about 24 hrs and depletion persists for at least 1 week(koe and Weissman, 1968). 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). We previously observed that PCPA(300 mg/Kg/day, 2 days) and 5,7 dihydroxytryptamine(DHT) decreased 5-HT contents in the brain of non stressed rats to about half of control(Suh and Park, 1983). However, the present study demonstrated that PCPA and 5,7-DHT didn't cause decreases in 5-HT and corticosterone levels, probably because the decrease in 5-HT levels may be overcome by the increase in synthesis rate of 5-HT by stress. Raphe destruction caused decreases in corticosterone and 5-HT levels concomitantly. this suggest that decrease in plasma corticosterone may be due to that in 5-HT levels.

The results obtained in the present experiments demonstrated a strong positive correlation between the contrations of plasma corticosterone and 5-HT in the hypothalamus and in the remainder of the brain.

In concluion, these experiments strongy suggest that 5-HT(serotonin) is a main stimulatory neurotransmitter involved in the regulation of stress induced activation of the HPA system.

SUMMARY

A role for brain serotonin(5-HT) in regulation of the HPA axis has been suggested but remains contoversial and poorly defined.

The present experiments were designed to check kinetic parameters of 5-HT turnover in rat hypothalamus and remainder brain areas before and after stress and to test whether using various different pharmacologic approaches to stimulate or eliminate the control serotonergic system have any consistent effect on the stress-induced activation of HPA system.

Steady state brain serotonin and 5-HIAA concentrations during 1 min ether stress were significantly elevated without significant rise in the levels of plasma corticosterone, which highly increased 2 minutes after stress. This suggests that the increase in serotonergic neuron activity precede that in HPA activity.

Furthermore, during 1 min-ether stress or 30 min immobilization stress there is a marked increase in hypothalamic and remainder brain serotonin(5-HT) turnover or synthesis rates assessed by both the pargline/5-HT method and pargyline/5-HIAA method.

The stress-induced corticosterone levels were increased by serotonin precursors and serotonin agonist in a dose-related fashion. The stress-induced corticosterone levels were highly elevated by L-tryptophan(100 mg/kg) and potentiated by monoamine oxidase inhibitor, pargyline or serotonin agonist, 5-MeoDMT.

The stress-induced elevation of corticosterone and 5-HT levels in rat brain were not significantly decreased by the administration of 5-HT

synthesis inhibitor, PCPA and 5-HT neurotoxin, 5,7-DHT. However, the stress-induced elevation of corticosterone and 5-HT levels were decreased by the destruction of midline raphe nuclei. There was a strong positive correlation between plasma corticosterone and 5-HT concentrations changed by drugs which mainly manipulating 5-HT system in the hythothalamus and in the remainder of the brain.

In conclusion, our present data stongly suggest that 5-HT is an important key neurotransmitter involved in the stress-induced activation of the HPA system.

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