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Effect of Corticosterone on the Serotonin Receptor Binding Properties in Rat Cerebral Cortex[1]

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= Abstract =A possible role of brain serotonin in the regulation of HPA axis activity including feed-back sensitivity has been studied by many investigators.

The present study was undertaken to check the serotonin binding to rat cerebral cortex membrane before and after corticosterone replacement in adrenalectomized rats.

Both the affinity and the number of serotonin binding sites were significantly increased following corticosterone replacement, compared to those of adrenalectomized control.

In conclusion, the present study strongly suggests that cerebral cortical serotonin receptor sensitivity might be changed by repeated treatment with corticosterone and may support the assumption of serotoninergic regulation in feed-back activity of HPA axis.

Key words: ***Serotonin receptor, Corticosterone, Rat brain, Adrenalectomy***

INTRODUCTION

It has been widely studied that various neurotransmitters in brain may modulate the release of corticotropin-releasing hormone and in-turn regulate synthesis and release of ACTH (Weiner and Ganong 1978; Vernikos-Danellis *et al.* 1977; Jones *et al.* 1978).

There have been several lines of evidence that serotonin plays an important role in the control of hypothalamic-pituitary-adrenal axis (PHA axis) activities, circadian rhythmicity (Scapagnini *et al.* 1971; Krieger and Rizzo 1969; Lee *et al.* 1983; Suh *et al.* 1983), stress responsiveness of ACTH release (Berger *et al.* 1974; Smythe *et al.* 1983; Fuller *et al.* 1976; Steiner and Grahmasmith 1980; Suh *et al.* 1983b), and feed-back sensitivity (Cavagnini *et al.* 1975; Plonk *et al.* 1974; Kawamura *et al.* 1984; Van Loon *et al.* 1981b and 1982). In addition, it has also been demonstrated that adrenal glucocorticoids directly influence serotoninergic neuronal activity via effect on serotonin synthesis (Sze *et al.* 1976; Sze and Neckers 1974; Cho *et al.* 1983; Cho *et al.* 1985) and its turn over rate (Van Loon *et al.* 1981a and 1981b).

Recent advances in radioligand binding assay of serotonin receptor make it possible to analyze the characteristics and location in the brain (Nelson *et al.* 1978; Peroutka and Snyder 1979 and 1983; Bennet and Snyder 1976; Fillion 1983.

Thereafter, *in vitro* measurement of serotonin receptor sensitivity became a useful method to assess *in vivo* serotonin neurotransmission as shown by several studies with several pharmacologic agents affecting serotoninergic acitivity such as imipramin (Segawa *et al.* 1979; Kendall *et al.* 1981), d-fenfluramine and methergolin (Samanin *et al.* 1980).

In the present study, to investigate the role of serotonin in control of hypothalamic-pituitary-adrenal axis activity, particularly in feed back sensitivity, the serotonin receptor sensitivity was studied following corticosterone administration to adrenalectomized rats.

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats (SNU animal house, Seoul, Korea) weighing 160-250 g were used. Animals were housed five in a cage at room temperature of 20-25°C, with 12 hr light cycle

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(lights on 7:00-19:00 hours). They received commercial rat-chow and water ad libitum. Animals were allowed to acclimatize to the condition of a quiet laboratory for 1 hr before experimental procedures.

Adrenalectomy and corticosterone administration

Rats were adrenalectomized bilaterally under Pentothal anesthesia through midline abdominal incision. After adrenalectomy, 0.9% saline was given freely. Corticosterone dissolved in 0.1% ethanol-saline was injected intraperitonealy with different dosages as 1 mg/kg and 5 mg/kg respectively. The first injection was done in 30 min after adrenalectomy and then repeated twice daily. Final injection was given 30 min before sacrifice. Control rats were injected vehicle in same amount.

Determination of plasma corticosterone level

Blood for plasma corticosterone level was obtained from severed neck vessels at the time of decapitation. Plasma levels were measured by spectrophotometric method as described by Zenker and Bernstein (1958).

Preparation of crude synaptosomal membrane

Animals were killed by decapitation and their brains were removed immediately in ice and cerebral cortices were dissected. Crude synaptosomal membrane was prepared as previously described by Peroutka and Snyder (1979) with some modification. The cerebral cortices were homogenized in 20 volume of Tris-HCl buffer (pH 7.4 at 25°C) with Polytron homogenizer (Brinkman Co.) and then centrifuged at 900 g and filtered throught three layers of gauze. The supernatant was carefully decanted and centrifuged at 35000 g to obtain pellet rich in mitochondrial fraction. This pellet was suspended in Tris-HCl buffer and incubated at 37°C so as to facilitate the destruction of endogenous serotonin. Final pellet obtained by washing and centrifugation was resuspended in TCAP buffer containing 50 mM Tris-HCl, 4 mM $CaCl_2$, 0.1% l-ascorbic acid, and 10 μM pargyline. Final tissue preparation of cerebral cortex provided for binding assay corresponded to 40 mg brain tissue/ml.

Receptor binding assay

Binding assay was performed as described by Bennet and Snyder (1976). Briefly, it consists of incubation of aliquots with various concentration of ^{3}H-serotonin (specific activity: 26.2 Ci/mmol New England Nuclear Co.) diluted in TCAP buffer and separaion of bound and free radioactivity with filtration throught GF/B filter (pore size 1.0 μ Whatmann Co.). The radioactivity of filter was counted with liquid scintilation spectrometry in 8 ml scintilation cocktail (Triton X-100, toluene, PPO, POPOP) after 18 hr extraction at 4°C with efficiency of 20-30%. Bound radioactivity was measured in duplicate in single experiment. Specific binding was defined as excess over blank values obtained in the presence of 10 μM unlabeled serotonin. The dissociation constant (Kd) and maximum number of binding sites (B_{max}) were determined from Scatchard analysis (1949).

Statistical analysis was carried out using one way analysis of variance and Neuman-Keul's multiple range test. When p value was less than 0.05, the difference was considered to be significant.

RESULTS

There were single population of binding sites to ^{3}H-serotonin in unoperated control rats. The mean of Kd and B_{max} from 11 separate experiments were 4.3 nM and 9.28 pmol/gm tissue respectively (Table 1).

Table 2 shows plasma corticosterone levels in various kinds of condition. They were in the range of 30-40 μg/dl in sham control, 2-3 μg/dl after adrenalectomy, 40-60 μg/dl in 1 mg corticosterone treated group and 90-125 μg/dl in 5 mg treated group, respectively.

Effect of corticosterone on Kd of ^{3}H-serotonin binding to rat cerebral cortex membrane was shown in Table 3. The Kd was increased significantly two days after adrenalectomy as compared to sham control. On the other hand, following corticosterone replacement Kd was markedly decreased during the period as compared to sham control as well as adrenalectomized group.

Table 4 shows effect of corticosterone on the B_{max} of serotonin receptor. Following corticosterone replacement B_{max} was significantly increased as compared to both adrenalectomized and sham operated groups. However, only after adrenalectomy, significant change of B_{max} was not observed during the observed period.

DISCUSSION

In the past ten years, although many studies have demonstrated that the brain serotonin plays a role in the regulation of "hypothalamopituitary-adrenal" axis activity, it remains controversial whether the role of serotonin in HPA axis is stimulatory (Fuller *et al.* 1976, 1981; Steiner and Grahame-Smith 1980; Krieger *et al.* 1978) or inhibitory

Table 1. Binding prameters of Pecific ^{3}H-serotonin binding to crude synaptosomal membrane of rat cerebral cortex in unoperated control

	n	Kd(nM)	B_{mx} (pmole/gm. tissue)
Unoperated control	11	4.30 ± 0.63	9.28 ± 0.92

Data are mean ± S.E.M. from 11 separate experiments, each in duplicate. Ligand concetration varied from 0.5 nM to 40 nM and nonspecific binding was determined in the presence of 10 μM unlabled 5-HT. Equilibrium dissociation constant (Kd) and maximum number of binding sites (B_{max}) were determined from Scatchard plot.
n: number of experiments.

addition steady state content does not always reflect neuronal activity.

Recently considerable progress has been made on research into the characteristics of brain serotonin receptor which is located mostly in post-synaptic membrane and widely distributed in various brain regions including cerebral cortex and hypothalamus with high concentration (Fillion 1983; Bennet and Snyder 1976; Peroutka and Snyder 1979 and 1983).

Present studies demonstrated that corticosterone replacement to adrenalectomized rats increased both affinity and number of brain serotonin receptor as compared to those of adrenalectomized rats. Meanwhile, adrenalectomy decreased the affinity.

Table 2. Plasma corticosterone level after various kinds of treatment

Condition \ Days	2		7	
	No. of animal	Mean ± SE	No. of animal	Mean ± SE
Sham operation	8	34.704 ± 3.322	10	31.415 ± 1.617
Adrenalectomy	8	2.323 ± 0.476	8	2.071 ± 0.577
Adrenalectomy with corticosterone 1 mg	7	46.907 ± 3.986	10	56.112 ± 3.942
Adrenalectomy with corticosterone 5 mg	8	92.911 ± 5.612	9	10.821 ± 13.909

(Berger *et al.* 1974; Telegdy and Vermes 1975; Vernikos-Danellis *et al.* 1977).

There have been several studies of serotonin content (Curzon *et al.* 1971; Azmitia and McEwen 1974; Neckers and Sze 1975; Ulrich *et al.* 1975) and turnover rate (Van Loon *et al.* 1981a, 1981b and 1982; Kawamura *et al.* 1984) following adrenalectomy and corticosterone administration. Moreover, our previous studies demonstrated that steady state serotonin content in rat cerebral cortex and hypothalamus were increased after adrenalectomy but were decreased following corticosterone administration (Cho *et al.* 1983; Cho *et al.* 1985).

Above studies support the idea that serotonin can mediate the negative feed-back of glucocorticoid on HPA axis. But interpretation of these results is not simple, because serotonin content was increased (Ulrich *et al.* 1975; Van Loon *et al.* 1981a; Neckers and Sze 1975) or decreased (Curzon *et al.* 1971; Cho *et al.* 1983; Cho *et al.* 1985) after corticosterone treatment in each studies. In Moreover these changes of receptor sensitivity were well correlated with plasma corticosterone levels following adrenalectomy and corticosterone administration.

It is well known that corticosterone can readily penetrate blood brain barrier and has its receptor in rat cerebral cortex (McEwen *et al.* 1970 and 1972). The results of present studies strongly suggest that corticosterone influence the serotoninergic neurotransmission by way of changing the serotonin binding to its receptor.

Of course plasma ACTH level would be changed following adrenalectomy and corticosterone injection. Therefore, the effect of ACTH on brain serotonin may be considered, but it is unlikely that plasma ACTH can penetrate blood brain barrier and increased plasma ACTH level may associate with serotonin turn over rate (Loon *et al.* 1982).

There have been several lines of evidence that postsynaptic serotonin receptor could be regulated by adaptation mechanism, that is, desensitization and supersensitization (Nelson *et al.* 1978; Segawa

Table 3. Effect of corticosterone on the Kd of specific ^{3}H-serotonin binding to rat cerebral cortex membrane

Conditions	Kd(nM)		
	a 2	4	7
A. Sham Adrenalectomy	4.900 ± 0.692 (8)	4.471 ± 0.737 (7)	5.475 ± 0.785 (8)
B. Adrenalectomy	7.200 ± 0.731* (7)	5.775 ± 0.663 (8)	5.257 ± 0.632 (7)
C. Adrenalectomy + C.S. 1 mg	1.914 ± 0.233#* (7)	2.217 ± 0.391#* (6)	2.850 ± 0.251#* (6)
D. Adrenalectomy + C.S. 5 mg	2.538 ± 0.164#* (6)	2.340 ± 0.237#* (5)	3.220 ± 0.465#* (5)

Data are mean ± S.E.M.
a: post operative day in condition A and B, and duration of C.S. administration in condition C and D.
Kd: dissociation equilibrium constant.
C.S.: corticosterone
*: statistical significance at p =0.05 compared with sham adrenalectomy.
#: statistical significance at p = 0.05 compared with adrenalectomy.
( ): number of experiments.

Table 4. Effect of corticosterone on the number of binding sites of serotonin receptor in rat cerebral cortex

Conditions	B_{max} (pmole/gm. tissue)		
	a 2	4	7
A. Sham Adrenalectomy	7.962 ± 0.329 (8)	7.986 ± 0.447 (7)	7.762 ± 0.365 (8)
B. Adrenalectomy	7.314 ± 0.462 (7)	7.637 ± 0.756 (8)	9.271 ± 1.342 (7)
C. Adrenalectomy + C.S. 1 mg	9.271 ± 0.965# (6)	10.683 ± 0.690#* (6)	12.717 ± 1.109#* (6)
D. Adrenalectomy + C.S. 5 mg	9.600 ± 0.511# (6)	9.300 ± 0.598 (5)	10.460 ± 0.988 (5)

Data are mean ± S.E.M.
a: post operative days in condition A and B, and duration of C.S. administration in condition C and D.
B_{max}: maximal number of binding sites.
C.S.: corticosterone
*: statistical significance at p = 0.05 compared with sham adrenalectomy.
#: statistical significance at p = 0.05 compared with adrenalectomy.
( ): number of experiments.

et al. 1979; Kendall *et al.* 1981; Samanin *et al.* 1980). Bennet and Snyder (1976) demonstrated that destruction of midbrain raphe neuclei increased postsynaptic serotonin receptor affinity, indicating sensitization of postsynaptic neuronal membrane following neurotransmitter depletion in synaptic cleft. In addition postsynaptic receptor concentration was increased after treatment with 5,7-DHT known as a selective neurotoxin of presynaptic serotonin neuron (Nelson *et al.* 1978).

These results together with studies of serotonin content (Cho *et al.* 1983; Cho *et al.* 1985) strongly suggest that supersensitization is a possible underlying mechanism to explain the effect of corticosterone on serotoninergic neurotransmission. However appreciable change of receptor number was not observed in adrenalectomized rats. This result appeared to be contradictory to hyposensitization of serotonin receptor after MAO inhibitor treatment as described by Savage *et al.* (1980). But down regulation of serotonin receptor is time consuming process as was shown in other author's studies (Samanin *et al.* 1980; Segawa *et al.* 1979). Long term observation over two weeks might be necessary to observe the down regulation following adrenalectomy.

Besides the adaptation mechanism, direct effect of corticosterone on post-synaptic membrane or receptor protein synthesis may be conceivable but no supportive evidences are available as far as serotonin receptor is concerned.

In conclusion, corticosterone replacement in adrenalectomized rats increased the serotonin receptor sensitivity in cerebral cortex. This study indicated that corticosterone influenced the serotoninergic neutransmission *in vivo* and it strongly suggested a possible role of serotonin in regulation of feed-back sensitivity of hypothalamopituitary-adrenal axis.

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= 국문초록 =

Corticosterone이 흰쥐 대뇌피질 serotonin 수용체 결합성에 미치는 영향에 관한 연구

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시상하부-뇌하수체-부신계의 활성에 대한 신경조절과 corticosterone에 의한 되먹이기 기전이 serotonin성 신경로에 의해 조절될 것이라는 가능성을 추구하고자 부신 적출 및 corticosterone 투여후에 흰쥐의 대뇌피질에 존재하는 serotonin 수용체 결합능의 변화를 관찰하여 다음과 같은 결과를 얻었다.

1. 흰쥐의 대뇌피질에는 ^{3}H-serotonin과 특이적으로 결합하는 고친화성의 단일수용체가 존재하는 것으로 보인다.

2. 부신적출후 2일째, 수용체의 친화성은 대조군에 비해 유의하게 감소하였으나 4일 및 7일째에는 대조군과 유사한 수준을 나타내었으며 관찰기간동안 수용체의 숫자는 유의한 차이를 보이지 않았다.

3. 부신적출후 corticosterone을 투여한 경우에는 수용체의 친화성과 숫자가 부신적출군에 비해 뚜렷이 증가되었다.

4. 부신적출후 corticosterone 1 mg 투여군과 5 mg 투여군 사이에는 수용체 결합능에 있어서 유의한 차이를 나타내지 않았다.

이상의 결과 corticosterone은 흰쥐의 대뇌피질에 존재하는 serotonin 수용체의 ^{3}H-serotonin에 대한 결합성을 변화시키며 이러한 사실은 serotonin성 신경계가 corticosterone에 의한 시상하부-뇌하수체-부신계에 대한 되먹이기 기전을 조절하는데에 관여하리라는 가설을 뒷받침하는 것으로 해석된다.