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Effect of Serotonin Uptake Inhibitors on Serotonin Metabolism in the Hypothalamus of Freely Moving Rats

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Tricyclic antidepressant clomipramine or selective serotonin reuptake inhibitors (SSRIs) have been commonly used for the treatment of premature ejaculation. In the present study, we analyzed the concentrations of serotonin and 5-hydroxyindoleacetic acid (5-HIAA) in the medial preoptic area (MPOA) of the hypothalamus by awakening animal microdialysis following administration of clomipramine and various SSRIs. We then compared the serotonin metabolism and clinical effects of clomipramine and SSRIs on premature ejaculation. Basal extracellular serotonin level in the MPOA was higher than other brain regions and it was significantly increased by clomipramine and the SSRIs. The rank order of the concentration of serotonin at the MPOA was clomipramine, sertraline, paroxetine and fluoxetine and the concentrations of 5-HIAA was vice versa. The changes in serotonin concentration at the MPOA appeared closely associated with the clinical effects of these drugs on premature ejaculation. These results suggest that the serotonergic neuronal activity in the MPOA may have an selective inhibitory influence on ejaculation, and the effects of clomipramine and SSRIs on erectile function are mainly mediated by MPOA of the hypothalamus.

Key Words: Serotonin, 5-HIAA, Clomipramine, SSRIs, Medial preoptic area, Hypothalamus, Microdialysis

INTRODUCTION

Premature ejaculation is a common male sexual dysfunction due to the absence of voluntary control during sexual activity in which ejaculation occurs before an individual desires (Rodriquez et al, 1984). Animal and clinical studies have implicated various neurotransmitters as being important mediators of the ejaculatory response (Rodriquez et al, 1984; Segraves, 1989). Dopaminergic, serotonergic, and alpha-adrenergic systems all play a role in male sexual function (Bitran & Hull, 1987). The serotonergic system is thought to be a primary means through which ejaculation is controlled and modified (Ahlenius et al, 1971; Mcintosh & Barfield, 1984; Rodriquez et al, 1984). Tricyclic antidepressant clomipramine and selective serotonin reuptake inhibitors (SSRIs) have commonly been used for the treatment of premature ejaculation (Zajecka et al, 1991; Balon, 1996). The mechanism for increased latency of ejaculation probably includes the facilitation of serotonergic neurotransmission by serotonin reuptake inhibition.

It has been reported that sertraline is a more potent serotonin reuptake inhibitor than clomipramine and fluoxetine in vitro (Zajecka et al, 1991), and that paroxetine is more potent serotonin reuptake inhibitor than sertraline in vitro (Grimsley & Jann, 1992). However, it has also been reported in a clinically comparative study that the most efficacious drug useful in increasing ejaculation latency was found to be clomipramine, followed by sertraline and fluoxetine (Kim & Seo, 1998). The total ejaculatory phenomenon is controlled by cerebral factors that can either excite or inhibit the event. However, studies to confirm the discriminating concentration of serotonin

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in the brain are rare in the literature. The exact locations of the cortical ejaculatory centers in man have not yet been elucidated completely. However, the medial preoptic area (MPOA) of hypothalamus appears to play a role as an essential integrating center of sexual function. Serotonergic neurotoxin administered to the MPOA of the hypothalamus promotes ejaculation. These findings suggested that the serotonergic nerves of the MPOA might have an inhibitory influence on ejaculation (Heym & Loe, 1988; Balon, 1996). To investigate the central regulatory role of serotonergic neurons in erectile function, we evaluated the concentrations of serotonin and its metabolite, 5-hydroxyindoleactic acid (5-HIAA) in the MPOA following administration of clomipramine or various SSRIs in this study.

METHODS

Animals

Male Sprague-Dawley rats, weighting 280~330 gm, were assigned to one of five groups: saline, clomipramine, fluoxetine, sertraline, or paroxetine. Each group consisted of eight rats. The rats were allowed free access to food and drinking water.

Awakening animal microdialysis

The rats were anesthetized with chloral hydrate (380 mg/kg, i.p.) and were fixed with a stereotaxic instrument. The skull was burr holed by drill bit and stainless steel screws were inserted into the skull around the hole. An intracerebral guide cannula was inserted into the brain in the right MPOA (0.9 mm posterior, 0.6 mm lateral to the bregma and 8.0 mm from the top of the cortex) according to the atlas of Paxinos and Watson (Paxinos & Watson, 1986). Cranioplastic cement was casted around the intracerebral guide and the stainless steel screws. The scalp was sutured and the rats were returned to the cage. After 24 hours, the intracerebral guide was removed from the guide cannula and a CMA/12 microdialysis probe (CMA/Microdialysis AB, Solna, Sweden), with a membrane length of 2 mm and a molecular weight cutoff of 20,000, was inserted into the MPOA via a guide cannula. Microdialysis probes were perfused with artificial cerebrospinal fluid (NaCl, 125 mM; KCl, 2.5 mM; $MgCl_2$, 1.18 mM; $CaCl_2$, 1.26 mM), at a flow rate of 1.5 μl/min using a CMA/100 microinjection pump (CMA/Microdialysis AB, Solna, Sweden).

Drug administration

Two hours after the insertion of the microdialysis probes, saline, clomipramine (Myungin Ltd., Seoul, Korea), fluoxetine (Lily Korea Ltd., Seoul, Korea), sertraline (Pfizer Korea Ltd., Seoul, Korea) or paroxetine (SmithKlein Beecham Korea Ltd., Seoul, Korea) were administered intraperitoneally at 10 mg/kg. Microdialysates were collected in collecting tubes containing 5 μl of 0.1 M perchloric acid and 1% sodium metabisulfite every 20 min throughout the experiment in awakening animal microdialysis system

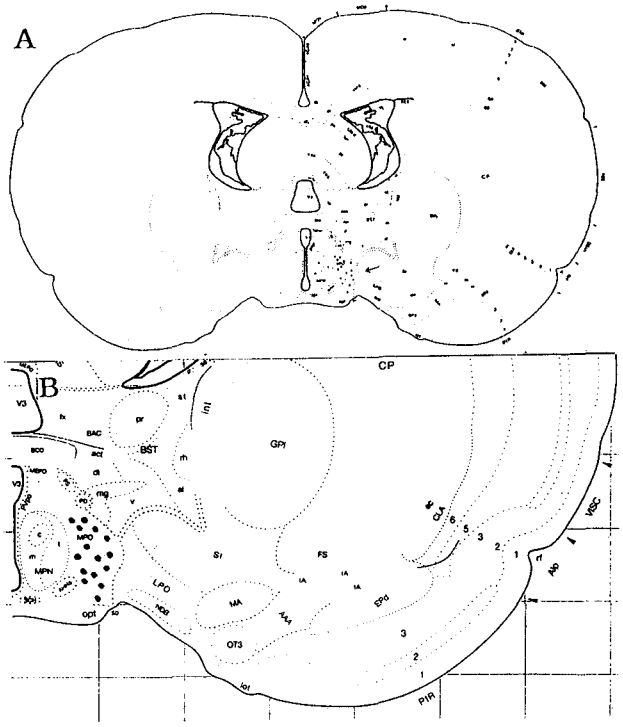

Fig. 1. A: After finishing the microdiaysis procedure, the rat brains were formalin-fixed and paraffin sections in all cases, for localization of probe tips. The arrow indicates the medial preoptic area and the circles mean the location of probe tips. B: Magnification of medial preoptic area was shown. BAC: bed nucleus anterior commisure, BST: bed nucleus stria terminalis, CP: caudoputamen, GPI: globus pallidus lateral segment, LPO: lateral preoptic area, MA: magnocellular preoptic nucleus, MPN: medial paraventricular nucleus, MPO: medial preoptic area, opt: optic chiasm, SCH: suprachiasmatic nucleus, SI: substantia innominata, V3: third ventricle.

and either analyzed immediately or stored at −70°C for later analysis.

In vitro calibration of the probes was carried out by placing the probe in a standard mixture of the compound. The relative recovery was calculated by comparing the concentration found in dialysate with that in the standard solution.

Anatomical verification of probe tips

Upon completion of the microdiaysis procedure, the animals were anesthetized with chloral hydrate (380 mg/kg, i.p.) and transcardially perfused with 10% formalin (neutral buffered, pH 7.4). The brains were separated and immersed in the fixative overnight and paraffin sections of all cases were examined to determine localization of the probe tips (Fig. 1). If the probe was not located within the MPOA, the collected samples were discarded.

High performance liquid chromatography-Electrochemical detector (HPLC-ECD)

Analysis of serotonin and its metabolites was performed with HPLC-ECD. The instruments used were a LKB/Pharmacia HPLC pump 2248 (Pharmacia LKB Biotechnology AB, Sweden), Rheodyne 7215 injector with 20 μl sample loop, Beckman Ultrasphere ODS column (5 μm, 4.6×200 mm) (Beckman Coulter, CA, USA), LC-4C ECD (Bioanalytical systems, Indiana, USA) and a LKB/Pharmacia REC 2 chart recorder (Pharmacia LKB Biotechnology AB, Sweden). The mobile phase consisted of 0.1 M potassium dihydrogen phosphate, 0.1 mM disodium EDTA, 1 mM sodium octanesulfonate, and 18% methanol (pH 3.1, adjusted with orthophosphoric acid). The flow rate was set at 1.2 ml/min and the detector potential was +0.75 V *vs.* Ag/AgCl. The sensitivity was 1.0 nA full scale.

RESULTS

In vitro relative recoveries were 22.1±1.3% for serotonin and 18.3±1.1% for 5-HIAA. Basal extracellular levels (mean±SEM) in the MPOA were 0.824±0.104 pmol/ml in serotonin and 1.424±0.162 nmol/ml in 5-HIAA. Serotonin levels in the extracellular space were significantly increased following the administration of clomipramine and SSRIs. The time of peak concentration was approximately 120 minutes in the clomipramine and sertraline administered groups, and 240 minutes in the paroxetine and fluoxetine administered groups. The rank order of high serotonin concentration in the MPOA was clomipramine, sertraline, paroxetine and fluoxetine. The concentrations recorded in the clomipramine and sertraline administered groups were similar to each

Table 1. Concentrations of serotonin in the medial preptic area after administration of either clomipramine or selective serotonin reuptake inhibitors

Time (min)	Clomipramine (pmol/ml)	Sertraline (pmol/ml)	Paroxetine (pmol/ml)	Fluoxetine (pmol/ml)
−20	0.816±0.056	0.799±0.082	0.824±0.058	0.841±0.065
0	0.906±0.075	0.865±0.067	0.791±0.066	0.824±0.074
20	0.808±0.083	0.832±0.058	0.832±0.075	0.879±0.083
40	2.637±0.141	1.978±0.124	1.236±0.083*	0.989±0.104*
60	3.625±0.247	3.049±0.219	1.813±0.127†	1.154±0.118$^{\|}$
120	4.284±0.379	3.815±0.329	2.802±0.247†	1.648±0.213†
180	4.120±0.346	3.543±0.247	3.052±0.305†	2.678±0.279¶
240	3.955±0.321	3.461±0.225	3.181±0.288§	2.892±0.324¶
300	3.672±0.224	3.214±0.285	2.966±0.296§	2.472±0.297¶

Data is shown as mean ±SEM, *; comparison of clomopramine or sertraline, $p<0.01$, †; comparison of clomopramine, sertraline or fluoxetine, $p<0.01$, ‡; comparison of clomopramine or paroxetine, $p<0.01$, §; comparison of clomopramine or paroxetine, $p<0.05$, $^{\|}$; comparison of clomopramine, sertraline or paroxetine, $p<0.01$, ¶; comparison of clomopramine, $p<0.01$ comparson of sertraline, $p<0.05$ by Newman-Keuls test

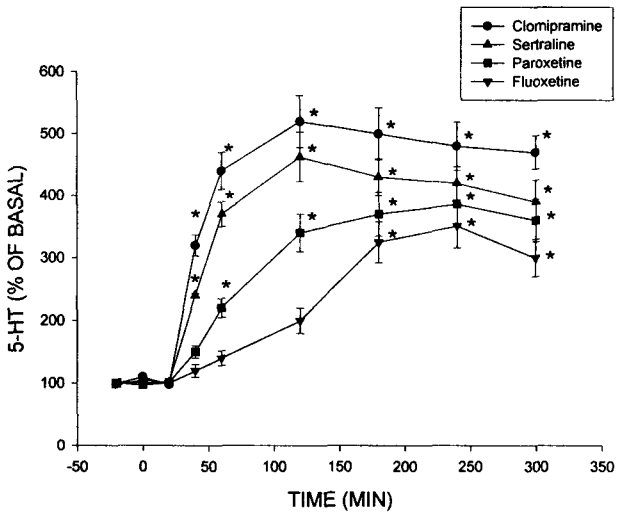

Fig. 2. Dose effect of clomipramine and SSRIs on serotonin levels in rat MPOA dialysates. Values are in percentages of basal levels (mean±SEM); *Significantly different from basal at $P<0.05$ with paired t-test.

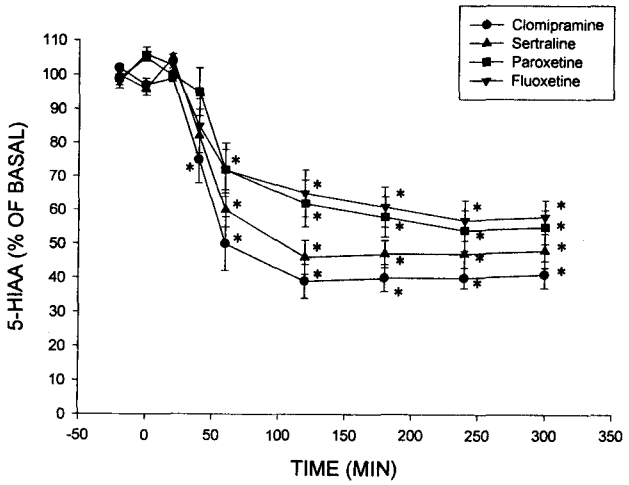

Fig. 3. Dose effect of clomipramine and SSRIs on 5-HIAA levels in rat MPOA dialysates. Values are in percentages of basal levels (mean±SEM); *Significantly different from basal at $P<0.05$ with paired t-test.

other, and those of the paroxetine- and fluoxetine-administered groups were also similar. The difference between the paroxetine- and fluoxetine-administered groups was also not significant except at 60 and 120 min. The significant differences between serotonin concentrations in animals treated with clomipramine and paroxetine, between sertraline and paroxetine, between clomipramine and fluoxetine, sertraline and fluoxetine administered groups were indicated at 40 min, 60 min and 120 min (Table 1, Fig. 2). 5-HIAA levels in extracellular space were significantly decreased after administration of clomipramine and SSRIs. However, the differences of 5-HIAA levels between drug groups were not significant (Fig. 3).

DISCUSSIONS

Premature ejaculation is one of the most common sexual complaints, estimated to affect up to 30% of men (Derogatis, 1980). Cognitive-affective factors such as sexual anxiety, and misperceived levels of sexual arousal are postulated to contribute to premature ejaculation. Behavioral therapies that primarily focus on the treatment of retaining of sexual behavior and on emotional factors are used for the treatment of premature ejaculation (Zajecka et al, 1991; Hawton & Catalan, 1997; Seftel & Althof, 1997). While these techniques are harmless and usually painless, these treatments that have been fairly successful at rates of 60 to 95%. However, most patients who noted improvement in ejaculatory latency with these treatments failed to maintain the gains for the long term (Bancroft & Coles, 1976; De Amicus et al, 1985; Hawton & Catalan, 1986; Seftel & Althof, 1997). Oral pharmacotherapy using clomipramine, tricyclic antidepressants or SSRIs has been commonly used as an alternative to behavioral therapy, particularly in patients for whom psychological treatment has failed, who have rejected psychological treatment, or whose partners were unwilling to cooperate in the treatment (Zajecka et al, 1991; Waldinger et al, 1994; Balon, 1996).

The administration of tricyclic antidepressants or SSRIs caused remarkable increase in ejaculatory latency in patients with premature ejaculation. The mechanism for this increased latency of ejaculation probably includes the facilitation of serotonergic neurotransmission by serotonin reuptake inhibition at the level of the brain. Electrolytic lesions of the midbrain raphe nuclei and the localized administration of a serotonergic neurotoxin both decrease the latency period of ejaculation in the male rat. Similarly, neurotoxic lesions produce a decrease in serotonin uptake in the medial preoptic area of the hypothalamus, a region of the brain where electrical stimulation promotes ejaculation. Lesions of the serotonergic neurons in this area result in an increased

frequency of ejaculation. These findings suggest that the MPOA may have an inhibitory influence on ejaculation (Heym & Loe, 1988; Balon, 1996).

Clomipramine, fluoxetine, sertraline or paroxetine facilitate serotonergic neurotransmission via serotonin reuptake inhibition and increase intravaginal ejaculatory latency effectively in patients exhibiting premature ejaculation (Assalian, 1988; Segraves et al, 1993; Waldinger et al, 1994; Mendels et al, 1995; Kara et al, 1996). It is known that SSRIs have a more favorable adverse effect profile than clomipramine because of their selective inhibition of serotonin reuptake and minimal effect on other monoamines (Waldinger et al, 1994; Mendels et al, 1995; Balon, 1996; Kara et al, 1996). It has been reported that sertraline is more potent inhibitor of serotonin reuptake in vitro than clomipramine and fluoxetine (Leonard, 1993). In anesthetized rats, neurotransmission in the serotoninergic system was enhanced 14 times more by sertraline than fluoxetine (Heym & Loe, 1988; Leonard, 1993). It has been reported that paroxetine is a significantly more potent inhibitor of serotonin reuptake in vitro than sertraline (Grimsley & Jann, 1992). However, it was reported that the most effective drug in terms of prolongation of intravagnal ejaculation latency and improvement of sexual satisfaction in patients and partners was clomipramine, followed by sertraline and fluoxetine (Kim & Seo, 1998). Athough sertraline has been reported to be more potent for serotonin reuptake inhibition than clomipramine, the efficacy of clomipramine in out-patients was better than that of sertraline (Heym & Loe, 1988; Leonard, 1993). Experimental and clinical studies have demonstrated that clomipramine administration induced reuptake inhibition of dopamine and norepinephrine as well as serotonin, which suggests that the mechanism of clomipramine action may be more complicated than the simple inhibition of serotonin reuptake (Heym & Loe, 1988; Leonard, 1993; Balon, 1996; Seftel & Althof, 1997). Serotonin can be released from the serotonergic nerves by the facilitation of both dopamine and norepinephrine. It is increased due to the reuptake inhibition of dopamine and norepinephrine by clomipramine (Done & Sharp, 1994; Rouquier et al, 1994; Yells et al, 1995).

The SSRIs are all very selective inhibitors of serotonin reuptake, but there are a number of pharmacokinetic differences among them that may be of clinical relevance. Paroxetine has the greatest anticholinergic activity. Drug absorption is relatively incomplete with paroxetine and sertraline but high for fluoxetine. Protein binding is highest for sertraline. Peak plasma concentrations following a single dose are achieved within 3 to 7 hours for all of the SSRIs. The plasma half life varies considerably among the SSRIs (Van Harten, 1993). Consideration of these pharmacokinetic differences may be helpful in understanding the difference of concentration of serotonin in brain. Additionally, consideration of the different doses of these drugs may also be helpful. Clinically, clomipramine 50 mg, sertraline 100 mg, fluoxetine 40 mg, paroxetine 40 mg are used. However, we used all drugs at 10 mg/kg, intraperitoneally. Serotonin levels in the extracellular space were significantly increased by clomipramine and the SSRIs probably by inhibiting the neuronal reuptake of serotonin. The rank order of high concentration of serotonin in the MPOA was clomipramine, sertraline, paroxetine and fluoxetine. It was confirmed that the serotonin concentrations were significantly increased in the MPOA.

These findings suggest that the MPOA has an inhibitory influence on ejaculation, and that the effects of drugs on erectile function are mediated by and closely correlated with the increased concentration of serotonin in the MPOA following the administration of drugs.

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