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Peripheral Effects of Serotonin on the Contractile Responses of Rat Seminal Vesicles and Vasa Deferentia

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ABSTRACT: With the central effects of serotonin (5-HT) on ejaculation having been relatively established, we investigated the peripheral effects of serotonin on the contractile responses of rat seminal vesicles and vasa deferentia. Male Sprague-Dawley rats were grouped on the basis of the agents administered: serotonin, clomipramine, or fluoxetine. The intraluminal pressures of the seminal vesicles and of the vasa deferentia were measured simultaneously. Control responses to hypogastric nerve stimulation (HNS) were recorded in each animal, and HNS was repeated after drug administration. Expression of the mRNAs of the 5-hydroxytryptamine (5-HT) receptors (5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2C}), which have been suggested to be involved in the ejaculation process, were examined by semiquantitative reverse–transcriptase polymerase chain reaction (RT-PCR). Serotonergic agents resulted in the concentration-dependent inhibition of HNS-induced seminal vesicle pressure increases (clomipramine > serotonin > fluoxetine). Vasal pressure responses were effectively inhibited by clomipramine and serotonin, but fluoxetine had no effect. No significant difference was observed in the relative expression levels of 5-HT_{1A} receptor mRNA in seminal vesicles and in the vasa deferentia. However, the expression levels of 5-HT_{1B} and 5-HT_{2C} receptor mRNAs were lower in the vasa deferentia than in the seminal vesicles. These in vivo and in vitro experimental results provide evidence for the peripheral role of 5-HT in the regulation of contractile responses of the seminal tract. Regional differences in the distribution of the 5-HT receptor subtypes of the seminal vesicles and the vasa deferentia might contribute to the different responses to serotonergic agents shown by these organs.

Key words: Ejaculation, seminal tract, 5-hydroxytryptamine.

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Although premature ejaculation is the most common male sexual dysfunction, relatively little attention has been focused on investigating the causes of premature ejaculation or on developing therapeutic strategies. Behavioral studies in animals suggest that the serotonergic system is a primary regulator of the ejaculatory reflex (Ahlenius et al, 1980; McIntosh and Barfied, 1984). Recently, selective serotonin reuptake inhibitors (SSRIs) have emerged as an effective new treatment modality for premature ejaculation (Kim and Seo, 1998; Waldinger et al, 1998b; Kim and Paick, 1999). The clinical efficacy of SSRIs also provides additional evidence that serotonin (5-hydroxytryptamine [5-HT]) plays an important role in ejaculation.

Involvement of the central serotonergic neurotransmission in ejaculation has been investigated in animals, and it has been suggested that the effects of 5-HT are mediated by several 5-HT receptor subtypes (Hillegaart and Ahlenius, 1998; Waldinger et al, 1998a; Ahlenius and Larsson, 1999). However, although the main role of 5-HT in the regulation of ejaculation occurs in the central nervous system, a peripheral role cannot be excluded.

5-HT is present throughout the gastrointestinal tract, which acts as the major reservoir of this substance in the body (Farthing, 1991), and numerous reports are available on the peripheral actions of 5-HT on various physiologic responses (Grahame-Smith, 1988; Farthing, 1991). In addition, some reports indicate that 5-HT has a peripheral role in the regulation of the sexual behavior of male rats (Gonzales et al, 1982; Ahlenius and Larsson, 1998).

To elucidate the possible peripheral role of 5-HT in the ejaculatory process, we investigated the peripheral effects of serotonin on the contractile responses of the seminal vesicles and vasa deferentia.

Materials and Methods

Animals

All studies were approved by the Institutional Animal Care and Use Committee of the Seoul National University College of Medicine. Male Sprague-Dawley rats (300–350 g) were divided equally into 3 groups (n = 10 per group) on the basis of the experimental agents administered: serotonin, clomipramine, or fluoxetine. Rats were anesthetized by an intraperitoneal injection of ketamine (35 mg/kg). Anesthetized rats were secured in the supine position, and a 2-cm midline neck incision was made to access the carotid artery and expose the external jugular vein.

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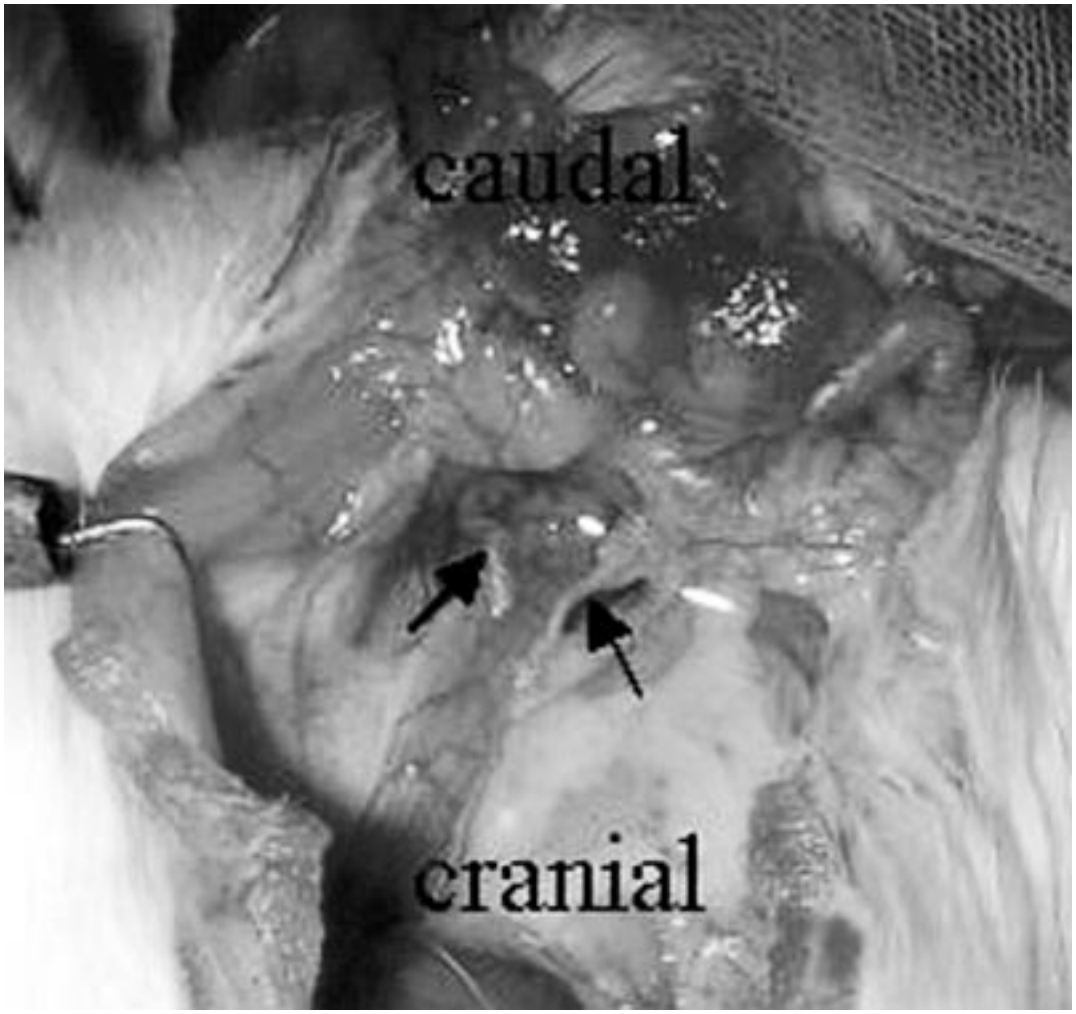


Figure 1. Both hypogastric nerves were transected proximally just below the inferior mesenteric ganglion (arrows indicated).

To continuously monitor systemic blood pressure, a 24-gauge angiocatheter was introduced into the carotid artery. Intravenous drug administrations were accomplished via the external jugular vein.

Hypogastric Nerve Stimulation

A 3-cm lower abdominal midline incision was used to visualize the bladder, prostate, seminal vesicles, and rectum after displacing the intestine rostrally by packing the abdominal cavity with gauze. With the use of a surgical microscope, we identified the hypogastric nerves, which originate from the inferior mesenteric ganglion and which travel toward the ipsilateral major pelvic ganglion and can be identified in the area adjacent to the ipsilateral ureter. A microsurgical technique was used to dissect both hypogastric nerves away from the surrounding connective tissue from their origins at the inferior mesenteric ganglion to the area adjacent to a seminal vesicle. The nerve was then transected proximally just below the inferior mesenteric ganglion (Figure 1).

Each hypogastric nerve was mounted on a bipolar silver electrode (Grass SD9; Grass Instrument, Quincy, Mass) and stimulated by a 10-second train of square waves (6-V pulse amplitude, normal polarity) of 1-millisecond pulse duration 1 hour after proximal hypogastric nerve transection. Stimulation frequencies (submaximal frequencies) of 40 Hz for the vas deferens and 80 Hz for seminal vesicles, which were found to cause submaximal contractile responses of each organ during preliminary experimentation, were used.

Pressure Measurements in Seminal Vesicles and Vasa Deferentia

One testis was passed into the abdominal cavity, and the gubernaculum was transected. With the use of a surgical microscope, a transverse incision was made in the wall of the vas deferens about 2 cm from the epididymis. After exposing the lumen of the vas deferens, a polyethylene tube (PE-10) filled with normal saline was inserted into the lumen toward the prostate gland and secured. After unraveling the tail of the contralateral seminal vesicle, an obliquely cut polyethylene tube (PE-60) filled with normal saline was delicately inserted into the main lumen of the seminal vesicle and secured tightly to prevent seminal fluid leakage. The tubes for monitoring the blood and intraluminal pres-

List of oligonucleotide primers used for the semiquantitative reverse-transcriptase polymerase chain reaction (PCR) for 5-hydroxytryptamine (5-HT) receptor subtypes and for the cyclophilin gene

Primer	Sequence	Size, bp	No. of PCR Cycles
5-HT_{1A}		199	30
Sense	5′-GATGTGTTCAGTTTTGGCCAGG-3′		
Antisense	5′-GGAGCGCTCCAAGGCGATGGCA-3′		
5-HT_{1B}		629	30
Sense	5′-AGTCTCCATGATCCTCCCGTCC-3′		
Anitsense	5′-GAAGGGTGGCAGCGAAATCG-3′		
5-HT_{2C}		351	30
Sense	5′-TATCCCTGTGATTGGACTGAG-3′		
Antisense	5′-GTTGATAGCCTGCATGGTGC-3′		
Cyclophilin		216	24
Sense	5′-TGTTCTTCGACATCACGGC-3′		
Antisense	5′-TTATGGCGTGTGAAGTCACC-3′		

sures of the vas deferens and of the contralateral seminal vesicle were connected to pressure transducers and a polygraph (TA6000; Gould Instrument Systems, Valley View, Ohio). The selection of right or left organs for dissection was random. The intraluminal pressures of the vas deferens, the contralateral seminal vesicle, and the systemic arterial pressure were recorded continuously.

In Vivo Study Protocol—Control responses to hypogastric nerve stimulation (HNS) at submaximal frequencies were determined in each animal. On return to baseline values, animals received intravenous administration of the drug in saline. Serotonergic drugs (serotonin, clomipramine, or fluoxetine) were administered intravenously by cumulative injection to each animal in the order 0.01, 0.1, 1, 5, and 10 mg/kg once, respectively. HNS was repeated sequentially 20 minutes after drug administration, and the intraluminal pressures of the vas deferens and contralateral seminal vesicle were measured separately.

In Vitro Experiments—This study comprised 10 male Sprague-Dawley rats not subjected to in vivo experiments. Rats were anesthetized, and brain cortex, bilateral vasa deferentia, and seminal vesicles were excised and stored at −80°C. Expression of the mRNAs of the 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2C} receptors, which have been suggested as being involved in the ejaculatory response (Hillegaart and Ahlenius, 1998; Waldinger et al, 1998a; Ahlenius and Larsson, 1999), was then examined by reverse-transcriptase polymerase chain reaction (RT-PCR).

Total RNA was extracted from all 3 organs with the use of TRIzol reagent (Gibco BRL, Gaithersberg, Md). Reverse transcription was carried out with a commercially available kit (Promega, Madison, Wisc). Briefly, 1.0 μg of total RNA was primed with 0.5 μg of oligo$(dT)_{15}$ and incubated for 30 minutes at 42°C. Reactions were carried out in 1× RT buffer (10 mM Tris-HCl, pH 8.8, 50 mM KCl, 0.1% Triton X-100), 1.0 mM dNTPs, 5 mM $MgCl_2$, and 300 units of Moloney murine leukemia virus (M-MLV) RT in a volume of 20 μL.

To amplify the 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2C} receptor cDNA RT product, 3 pairs of primers were prepared (Table), cyclophilin mRNA was amplified as an internal standard (Table). PCR was performed in a final volume of 20 μL containing 4 μL of cDNA product, 2.5 mM $MgCl_2$, 1× PCR buffer (20 mM Tris-HCl, 50 mM KCl, pH 8.4, Triton X-100), 2.5 units of *Taq* DNA polymerase, 0.2 mM dNTPs, and 25 pM of each primer.

PCR was performed with 30 amplification cycles: denaturation at 94°C for 1 minute, annealing at 59°C for 30 seconds (5-HT_{1A}) or 58°C for 30 seconds (5-HT_{1B}) or 54°C for 30 seconds (5-HT_{2C}), and extension at 72°C for 1 minute. Cyclophilin was amplified for 24 cycles (94°C for 1 minute, 54°C for 30 seconds, and 72°C for 1 minute). Preliminary experiments of up to 30 and 24 cycles showed that the PCR amplifications of each 5-HT receptor subtype and of cyclophilin were linear. With the use of a 2% agarose gel, a 10-μL aliquot of each PCR product was subjected to electrophoresis and ethidium bromide staining. The intensities of ethidium bromide fluorescence were determined with a CCD camera (Fuji Film, Tokyo, Japan). The densities of 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2C} were normalized to that of cyclophilin; values are expressed in arbitrary units.

Statistical Analysis

Data are reported as means ± SD. The percent inhibition of seminal vesicle and vasa deferentia intraluminal pressure elevation by each drug at different concentrations was obtained by the baseline and experimental data, and the concentration-response curves of the individual drug were plotted. The significance of each drug in inhibition of intraluminal pressure elevation was determined with a repeated measure 1-way analysis of variance test. Comparisons of 5-HT receptor subtype mRNA expressions were made by the Student's *t* test. Differences were considered to be statistically significant at $P < .05$.

Results

In Vivo Experiments

HNS caused significant increases in intraluminal pressures in seminal vesicles and vasa deferentia. Before drug administration, the peak intraluminal pressures of seminal vesicles and vasa deferentia after HNS were 74 ± 6.8 and 56 ± 5.6 cm H_2O, respectively. Repeated nerve stimulation, each preceded by a 20-minute rest interval, produced

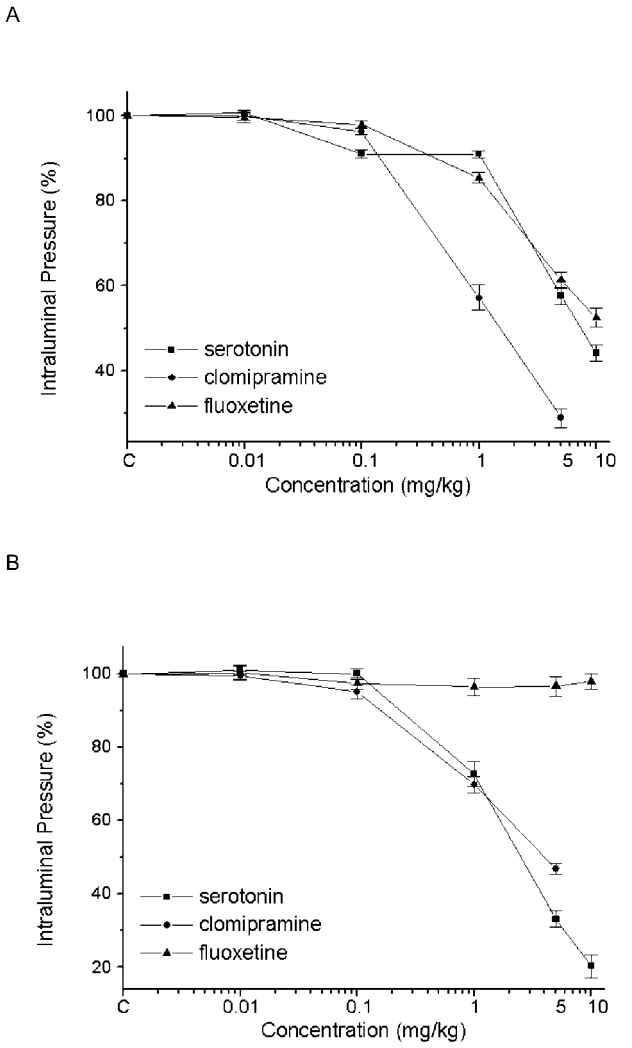


Figure 2. Effects of serotonergic drugs on the intraluminal pressure of seminal vesicles **(A)** and on vasa deferentia **(B).**

reproducible intraluminal pressure recordings in each organ. Also, the baseline values of intraluminal pressure did not vary significantly after intravenous injection of the agents prior to nerve stimulation at the doses administered in this study.

All serotonergic drugs investigated elicited a concentration-dependent reduction in HNS-induced intraluminal pressure elevations in seminal vesicles (Figure 2A). In the vas deferens, 5-HT and clomipramine demonstrated significant concentration-dependent effects, whereas fluoxetine did not (Figure 2B). The administration of 10 mg/kg of clomipramine proved to be fatal, and the corresponding data points were eliminated from the analysis. The administration of serotonergic drugs at the concentrations used in this study did not significantly alter systemic blood pressures. The relative potencies of the drugs in the seminal vesicle and vas deferens were determined by extrapolating the EC_{50}s (concentrations at which 50% inhibition of intraluminal pressure responses). The EC_{50}s for clomipramine, 5-HT, and fluoxetine in the seminal vesicles were 0.16, 7.1, and more than 10 mg/kg, respectively, and the corresponding values in the vas deferens were 5.2, 2.5, and more than 100 mg/kg, respectively.

In Vitro Experiments

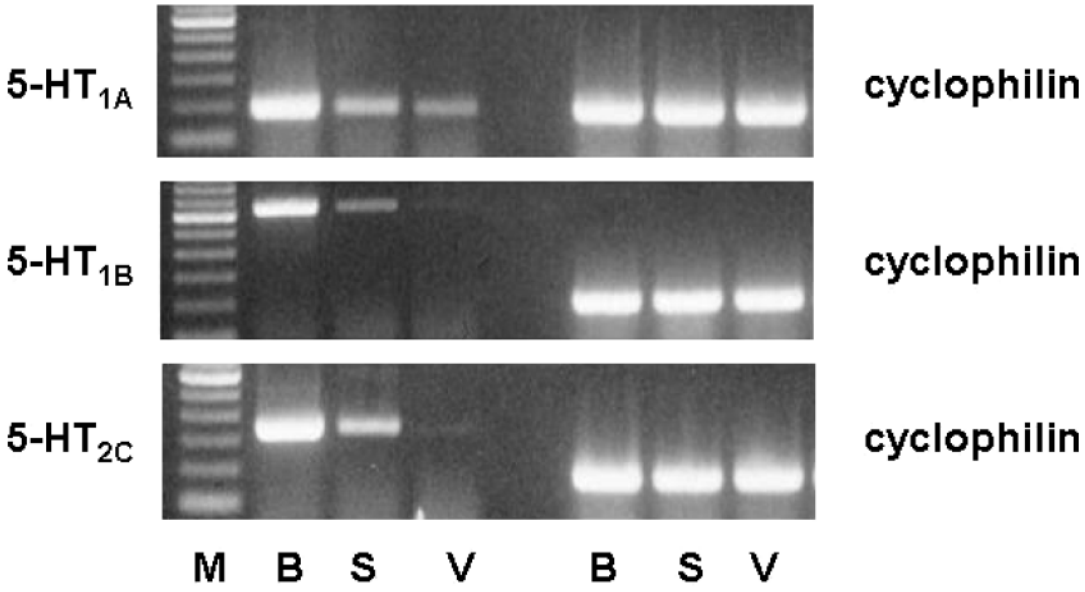
Figure 3A shows the electrophoretic analysis results of the RT-PCR products of 5-HT receptor subtype mRNA. The expected sizes of the PCR products of 5-HT_{1A} (199 bp), 5-HT_{1B} (629 bp), 5-HT_{2C} (351 bp), and cyclophilin (216 bp) were obtained in each organ. Expressions of 5-HT receptor subtype mRNAs were most abundant in brain cortical tissue. The results of the RT-PCR were analyzed further by computing the ratio of each 5-HT receptor to the cyclophilin peak areas for each sample (Figure 3B). No significant difference was observed in the relative expression levels of 5-HT_{1A} receptor mRNA in seminal vesicles or vasa deferentia. However, the expression levels of the 5-HT_{1B} and 5-HT_{2C} receptors mRNAs were lower in vasa deferentia than in seminal vesicles.

Discussion

It is of critical importance that a versatile in vivo model be developed to assess the therapeutic efficacy of the drugs used in the treatment of premature ejaculation. Hsieh et al (1998) proposed a rat model involving electrical stimulation of the lesser splanchnic nerve to induce changes in the intraluminal pressure of seminal vesicles. They measured the inhibition of electrical stimulation–induced seminal vesicle pressure increases induced by several agents (ie, prazosin, 5-HT, clomipramine, fluoxetine, imipramine, or indatraline) and observed concentration-dependent effects for prazosin and all serotonergic agents, except imipramine and indatraline. Of these inhibitory agents, highest efficacy was observed for fluoxetine followed by prazosin, 5-HT, and clomipramine. On the other hand, Kim et al (2000) used a somewhat different model and produced results that contest those of the Hsieh study. They investigated the effects of serotonergic drugs (clomipramine, sertraline, paroxetine, or fluoxetine) on the vasal pressure increase induced by electrical stimulation of the hypogastric nerve. All serotonergic drugs were found to cause a concentration-dependent inhibition of intraluminal pressure elevation in the vas deferens, and clomipramine showed the strongest inhibitory effect followed by sertraline, paroxetine, and fluoxetine.

Aside from the differences between these 2 models in terms of target organ (seminal vesicle vs vas deferens) and stimulated nerve (lesser splanchnic nerve vs hypogastric nerve), there were qualitative differences in the

A



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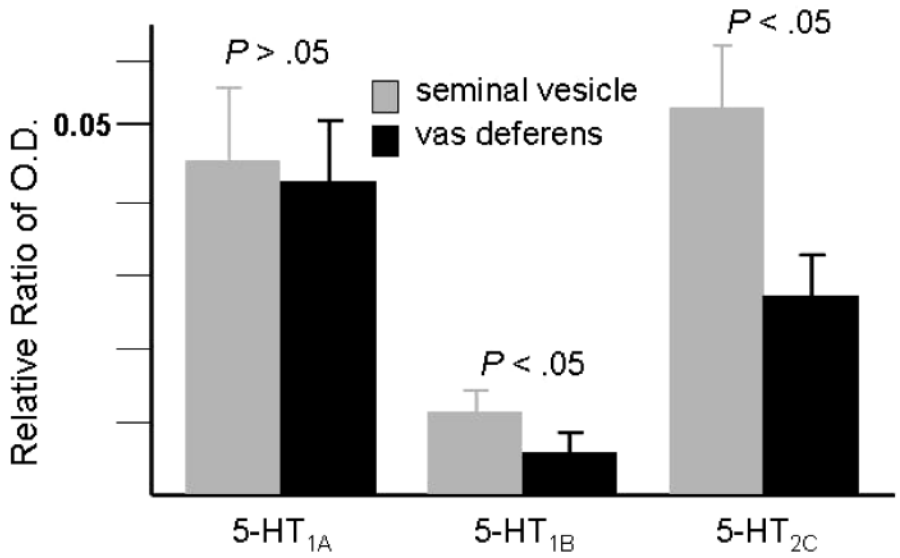

Figure 3. **(A)** Expressions of the 5-hydroxytryptamine $(5\text{-HT})_{1A}$, 5-HT_{1B}, and 5-HT_{2C} receptors by semiquantitative reverse-transcriptase polymerase chain reaction in rat seminal vesicles. **(B)** Relative expressions of the 5-HT receptor subtype mRNAs in the seminal vesicle and vas deferens (expressed as relative optical densities). Relative ratio of optical densities (OD) = (OD of 5-HT receptor)/(OD of cyclophilin). M indicates marker (100-bp DNA ladder); B, brain cortex; S, seminal vesicle; and V, vas deferens.

findings of these 2 reports. To settle this matter, we modified these previous models and devised a new in vivo experimental model in which the intraluminal pressure responses of a seminal vesicle and of a vas deferens can be measured in a single animal. We chose to stimulate the hypogastric nerve because we believed it likely to be more specific in terms of both action and distribution than the lesser splanchnic nerve for inducing seminal tract contraction by electrical stimulation because the former is a postganglionic branch from the inferior mesenteric ganglion, whereas the latter is located cranial to the ganglion (Kihara and de Groat, 1997). In humans, electrical stimulation of either the presacral nerve (superior hypogastric ganglion) or of the hypogastric nerves produces contraction of the bladder neck, seminal vesicles, vasa deferentia, and ejaculatory ducts (Learmonth, 1931).

The basic hypothesis of our in vivo study is that the drugs used to treat premature ejaculation produce their effects by inhibiting intraluminal pressure elevations of the seminal tract. All serotonergic agents were found to significantly inhibit the increased seminal vesicle pressure induced by HNS (clomipramine $>$ 5-HT $>$ fluoxetine). On the other hand, vasal pressure responses are not significantly inhibited by fluoxetine, which has been demonstrated to have a therapeutic effect on premature ejaculation (Kim and Seo, 1998; Waldinger et al, 1998b). In this study, 5-HT was found to be the most effective at inhibiting vasal pressure response.

Although doses of various antidepressants for delaying ejaculation in patients with premature ejaculation have not been clearly established, 50 mg of clomipramine and 40 mg of fluoxetine have been commonly used. Therapeutic doses of the test drugs for this experiment can be determined by halving the usual doses for a 60-kg patient. In this sense, those of clomipramine and fluoxetine can be calculated to be 0.42 and 0.33 mg/kg, respectively. These calculated therapeutic doses of the drugs are within the ranges of doses applied in this study.

Another interesting observation made during the course our study is that seminal vesicle and vasal contractile responses to HNS were both significantly inhibited by peripherally administered 5-HT. Because circulating serotonin cannot readily cross the blood-brain barrier (Erspamer, 1966), this observation indicates a peripheral role with respect to the regulation of contractile responses of the seminal tract. A few reports have provided evidence for the peripheral role of 5-HT in the regulation of male rat sexual behavior (Gonzales et al, 1982; Ahlenius and Larsson, 1998). Mount and intromission latencies increased in rats after the peripheral administration of 5-HT (Gonzales et al, 1982), and the administration of the 5-HT precursor 5-hydroxytryptophan (5-HTP), in combination with an inhibitor of peripheral 5-HTP decarboxylase, produced a dose-dependent increase in the ejaculation latencies of male rats (Ahlenius and Larsson, 1998).

We hypothesized that the different effects of the individual serotonergic drugs on seminal vesicles and on vasal pressure responses might be the result of different distributions of 5-HT receptor subtypes in seminal vesicles and vasa deferentia. To date, no biological study has examined the mRNA expressions of 5-HT receptors in the seminal tract. In this study, we were able to demonstrate the mRNA expressions of the 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2C} receptor subtypes in the seminal vesicle and vas deferens, which have been suggested to be involved in the central regulation of the ejaculation process (Hillegaart and Ahlenius, 1998; Waldinger et al, 1998a; Ahlenius and Larsson, 1999). This finding provides additional evidence that 5-HT plays a role in the contractile responses of the seminal tract in a peripheral manner. We compared the relative expressions of each 5-HT receptor subtype mRNA in the seminal vesicle and vas deferens. We found a significant difference between the relative expression levels of 5-HT_{1A} receptor mRNA in the 2, but the expressions of 5-HT_{1B} and 5-HT_{2C} receptor mRNAs were lower in the vasa deferentia than in seminal vesicles.

The involvement of central serotonergic neurotransmission in the ejaculation process has been examined in animals. The drug stimuli of SSRIs, fluoxetine, and paroxetine were found to resemble 5-HT_{2C} receptor activation most closely (Berendsen and Broekkamp, 1994). On the other hand, selective activation of the 5-HT_{1A} receptors was found to shorten the ejaculatory latency time and to reduce the number of intromissions preceding ejaculation in animals (Ahlenius and Larsson, 1999). On the basis of these animal studies, Waldinger et al (1998a) hypothesized that premature ejaculation was caused by a disruption in the functional balance of 5-HT receptor subtypes (ie, increased sensitivity of the 5-HT_{1A} receptors or a reduced sensitivity of the 5-HT_{2C} receptors or both). Another animal study demonstrated the opposite effects—facilitation and inhibition—on male rat ejaculatory behavior after 5-HT_{1A} or 5-HT_{1B} receptor stimulation, suggesting that the SSRI-induced inhibition of male ejaculatory dysfunction is due to 5-HT_{1B} receptor stimulation (Hillegaart and Ahlenius, 1998).

Although the peripheral role of 5-HT receptor subtypes is not necessarily related to central regulation of the ejaculatory process, one would expect that the different mRNA expression levels of 5-HT receptor subtypes might explain the different responses of the seminal tract to individual serotonergic drugs. As mentioned previously, vasal pressure responses were not significantly inhibited by fluoxetine, and the expression of 5-HT_{2C} receptor mRNAs was significantly lower in the vasa deferentia than in seminal vesicles in this study. Furthermore, it has been reported that fluoxetine displays a high affinity for the 5-

HT_{2C} receptor and interacts directly with the 5-HT_{2C} receptor by radioligand binding assay (Palvimaki et al, 1996).

One of the fundamental shortcomings of this and other animal models in terms of investigating the contractile responses of the seminal tract is that these models reflect electrical stimulation–induced emission rather than true ejaculation. The minimum concentration or pressure range at which a drug demonstrates an effect, whether by causing ejaculatory delay or outright suppression of the ejaculatory reflex, has not been determined. Prior studies thus far (including this study) entailed nerve stimulation shortly (10–30 minutes) after drug administration. Therefore, these studies are limited to observation of the acute effects of these drugs. This is counterintuitive when one considers the well-documented, protracted, multiple-day lag time before ejaculatory delay is observed in premature ejaculation patients treated with SSRIs. For this reason, we are currently in the process of revising this model in rats by feeding serotonergic drugs daily for 1 week or more.

Nevertheless, the results of the in vivo and in vitro experiments described in this study provide evidence for the peripheral effects of 5-HT on the contractile responses of the seminal vesicle and the vas deferens; furthermore, this study presents new evidence on the possible role of 5-HT in terms of regulating the ejaculatory process in rats.

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