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Modulation by Peripheral Serotonin of the Threshold for Sexual Reflexes in Female Rats

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McKENNA, K. E., K. C. KNIGHT AND R. MAYERS. *Modulation by peripheral serotonin of the threshold for sexual reflexes in female rats*. PHARMACOL BIOCHEM BEHAV 40(1) 151–156, 1991.—Using immunohistochemical techniques, a large number of serotonergic paracrine cells were identified in the urethral mucosa of the female rat. The functional significance of these cells was investigated. A model for the study of sexual climax in the anesthetized, acutely spinalized female rat was used. In this model, distension of the urethra with saline elicits a stereotyped coordinated genital response which closely resembles the neuromuscular concomitants of sexual climax. Addition of serotonin to the urethral perfusate (10^{-4}–10^{-7} M) caused a dose-dependent decrease in the mechanical threshold necessary to elicit the climax-like response. This effect was blocked by a specific 5-HT_3 receptor antagonist. The physiological implications of these findings are discussed.

Orgasm Sexual climax Genital Urethra Serotonin 5-HT_3 receptors

THE sensory mechanisms which trigger sexual climax in either sex are unknown. We have recently identified a stereotyped response elicited in anesthetized, acutely spinalized male and female rats, the coitus reflex or urethro-genital (UG) reflex (3,13), which bears many similarities to sexual climax (6,15). This reflex consists, in the male, of penile erection, clonic contractions of the perineal muscles and ejaculation. In the female, it consists of clonic contractions of the perineal muscles and rhythmic vaginal and uterine contractions. Neural recordings indicate that this reflex is essentially the same in the two sexes. The UG reflex is elicited by mechanical stimulation of the urethra, such as probing with a catheter or distension with saline. We have hypothesized that sexual climax may be elicited by urethral sensory stimulation by seminal fluids. In the female, the physiological stimulus is unclear, as the volume of female peraurethral gland secretions seems inadequate to activate the coitus reflex by mechanical stimulation alone. The purpose of this study was to investigate the role of urethral chemosensory mechanisms in eliciting the coitus reflex.

Several researchers have identified paracrine cells in the urethral mucosa of both males and females of several species (4, 5, 7, 8, 12). These cells have been histochemically identified as containing serotonin. The morphology of these cells are similar to chemosensory cells in other organs. However, a functional role for these urethral paracrine cells has not been identified. In the present study, we investigated whether these serotonergic paracrine cells are present in the female rat and what is the physiological effect of serotonin administered into the urethra.

Some of these studies have been previously reported in an abstract (14).

METHOD

Mature, virgin female Sprague-Dawley rats (200–400 g) were used for these experiments. For anatomical experiments, rats were anesthetized by a ketamine and xylazine mixture (86 and 13 mg/kg, IP) and transcardially perfused with phosphate-buffered saline followed by 4% buffered paraformaldehyde (pH 7.4). The urogenital system was removed and 30–50 μm sections were cut. The sections were rinsed, incubated for 1 hour in 3% normal goat serum. Following further rinses, they were incubated overnight in antiserum to serotonin-bovine serum albumen conjugate (dilution 1:1000) raised in rabbit (Incstar). The sections were developed by the ABC method (Vector Labs). Final visualization was carried out with diaminobenzidine (DAB) intensified with nickel ammonium sulfate. The sections were then mounted on gelatinized slides, dehydrated and coverslipped. In some animals, an immunofluorescence technique using whole mount tissue was used. The urethral mucosa was carefully dissected free and pinned out as a whole mount. The whole mount was rinsed, incubated in blocking serum, followed by rinses and the primary antibody overnight. The tissue was rinsed and incubated in fluorescein (FITC) conjugated goat antirabbit secondary antibody (Jackson ImmunoResearch). Following rinsing, the tissue was coverslipped with 60% glycerol in phosphate buffer. The slides were viewed and photographed with a Leitz epifluorescence microscope.

For physiological studies, female rats were anesthetized with urethane (1.2–1.5 g/kg SC). The carotid artery and jugular vein were cannulated for measurement of arterial blood pressure and infusion of drugs, respectively. The trachea was cannulated for

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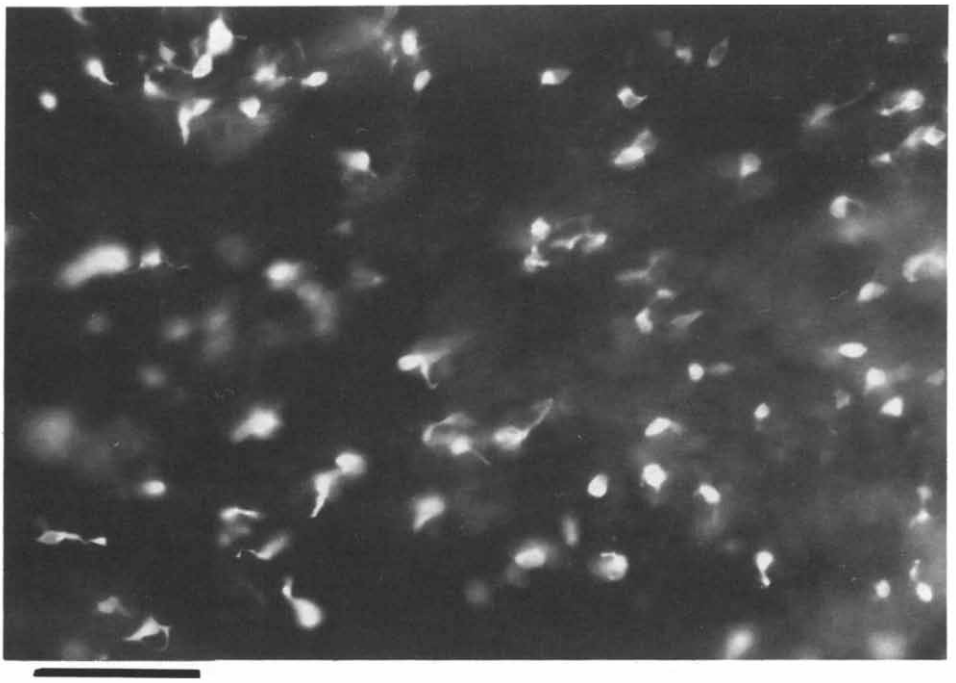

FIG. 1. Photomicrograph of a wholemount of the urethral mucosa stained for serotonin by immunofluorescence. This field shows a region approximately 4 mm from the meatus. Note the high density of the paracrine cells. Scale bar represents 100 μm.

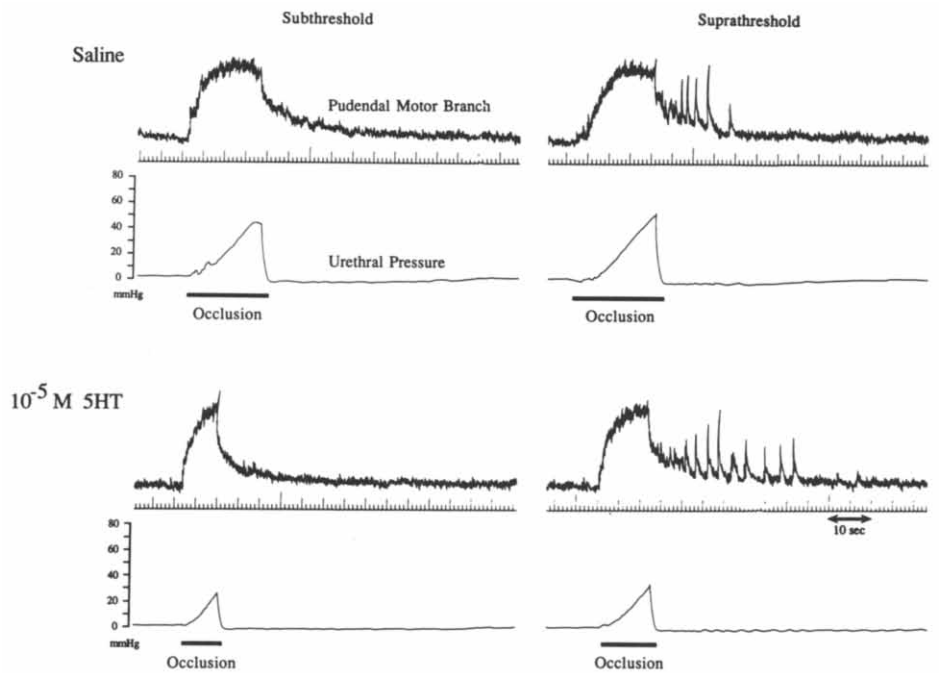

FIG. 2. Polygraph tracings demonstrating the effects of serotonin (10^{-5} M) on the pressure threshold. The top trace in each panel is a recording from the pudendal motor branch, rectified and integrated with a 100 ms time constant. In all cases, the urethra was perfused with fluid at a rate of 0.5 ml/min. The urethral meatus was occluded to raise the urethral pressure to a specified level and then released. If the pressure was subthreshold, no rhythmic firing was observed. If the pressure was suprathreshold, the rhythmic firing of the urethro-genital reflex was elicited. The threshold pressure was defined as the mean of the subthreshold and suprathreshold pressures. In this example, the saline threshold was 45 mmHg. When a solution of 10^{-5} M 5-HT in saline was used, the threshold was decreased to 30 mmHg, a decrease of 33%.

TABLE 1

THE EFFECTS OF SEROTONIN ON THE THRESHOLDS FOR ELICITING THE UG REFLEX

5-HT Dose	Thresholds: Saline Before		5-HT		Saline After
10^{-7}	53.1 ± 15.9 (12)		42.7 ± 11.9 (12)		55.0 ± 12.9 (12)
		$p=0.020$ (12)		$p=0.0004$ (12)	
10^{-6}	53.9 ± 17.0 (14)		42.9 ± 19.2 (14)		59.0 ± 29.4 (10)
		$p=0.006$ (14)		$p=0.034$ (10)	
10^{-5}	70.0 ± 26.0 (7)		44.4 ± 19.6 (7)		68.4 ± 19.5 (7)
		$p=0.005$ (7)		$p=0.002$ (7)	
10^{-4}	77.8 ± 15.7 (4)		46.0 ± 29.4 (4)		70.3 ± 14.6 (4)
		$p=0.059$ (4)		$p=0.010$ (3)	

Thresholds were measured as described in the text. The values shown are the mean ± standard deviation. The number of runs is shown in parentheses beneath the standard deviation. Each trial consisted of a control perfusion run with saline (Before Saline), followed by a specified concentration of serotonin (5-HT) and then another control run with saline (Saline After). Comparisons between runs were made with a paired *t*-test. The *p* value and the number of pairs is shown between the compared saline and serotonin runs. There were no significant differences between any of the Saline Before values and its corresponding Saline After value. This table represents data from 15 rats.

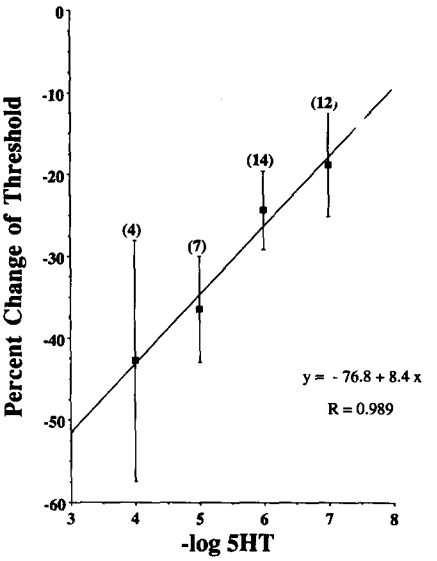

FIG. 3. The effect of serotonin on the threshold. For each trial, a baseline threshold was calculated as the mean between the control saline threshold before and after serotonin. The percent change from this baseline caused by serotonin was then calculated for that trial. The points shown represent the mean of all trials at that dose, the error bars are the standard deviation. A linear regression line was calculated by a least squares fit.

artificial respiration and to prevent aspiration of saliva. The pelvic organs were exposed by a single midline incision and the urethra was cannulated with a PE 50 (0.965 mm o.d.) catheter. The tip was positioned in the pelvic urethra via a bladder incision and secured by a tie at the bladder neck. The urethral catheter was connected to a syringe pump, attached by a T-tube to a pressure transducer. The urethra could be distended by perfusing it with saline and occluding the urethral meatus. The UG reflex is under tonic descending inhibition from the brainstem (16). Thus the spinal cord must be transected in order to elicit the UG reflex in the anesthetized rat. A laminectomy was performed at T6. The spinal cord was sectioned and the wound packed with Gelfoam.

Recordings were made from the pudendal motor nerves with bipolar silver wire hook electrodes spaced approximately 1 mm apart. Vaginal smooth muscle recordings were made by insertion of bared wires into the smooth muscle of the vagina. Intravaginal pressure recordings were made by inserting a pressure transducer (Sensotec. Inc., esophageal pressure transducer). The surgical area was filled with warm mineral oil to prevent dehydration. The rats were maintained at 38°C with a thermostatically controlled heat lamp. Recording signals were fed to a high impedance preamplifier, displayed on a polygraph and oscilloscope, and fed to an audio monitor and digital tape recorder.

The UG reflex was elicited by distension of the urethra with saline or serotonin solutions. The solution was infused into the urethra at a constant rate (0.5 ml/minute). The urethral meatus was occluded to raise the intraurethral pressure up to a specified level. Following release of the occlusion, the UG reflex was elicited. The threshold for eliciting the UG reflex was measured by increasing the intraurethral pressures in 5 mmHg steps until the UG reflex was elicited. The threshold was defined as the average of the lowest pressure at which the UG reflex was elicited and the highest pressure at which it was not elicited. Trials were conducted 2 minutes apart. After a threshold was determined with saline as a perfusate, the urethra was infused with a solution of serotonin. After a 2-minute equilibration, the intraurethral pressure threshold for eliciting the UG reflex was determined for the serotonin solution. The perfusate was switched back to saline for a 10-minute washout period and the saline threshold redetermined.

For determining the mean saline threshold among all animals, a mean threshold value was computed for each animal from all the trials for that animal. The overall mean of the means of each animal was then computed. For determining the effect of serotonin, a paired *t*-test was performed comparing the serotonin thresholds with the saline thresholds before and after the serotonin. A dose-response curve was constructed of the percent reduction of threshold of all the trials at each dose. The average of the saline threshold before and after the serotonin was defined as the baseline threshold. The percent change from this value caused by the serotonin was then computed. A log-linear regression was fit to the data using a least square means. All values are presented as mean ± standard deviation.

Drugs used and their source were serotonin hydrochloride and the 5-HT_3 receptor antagonist, ICS 205,930 methiodide (Research Biochemicals, Inc.). The drugs were dissolved in saline immediately prior to use.

RESULTS

Immunohistochemical staining for serotonin revealed numer-

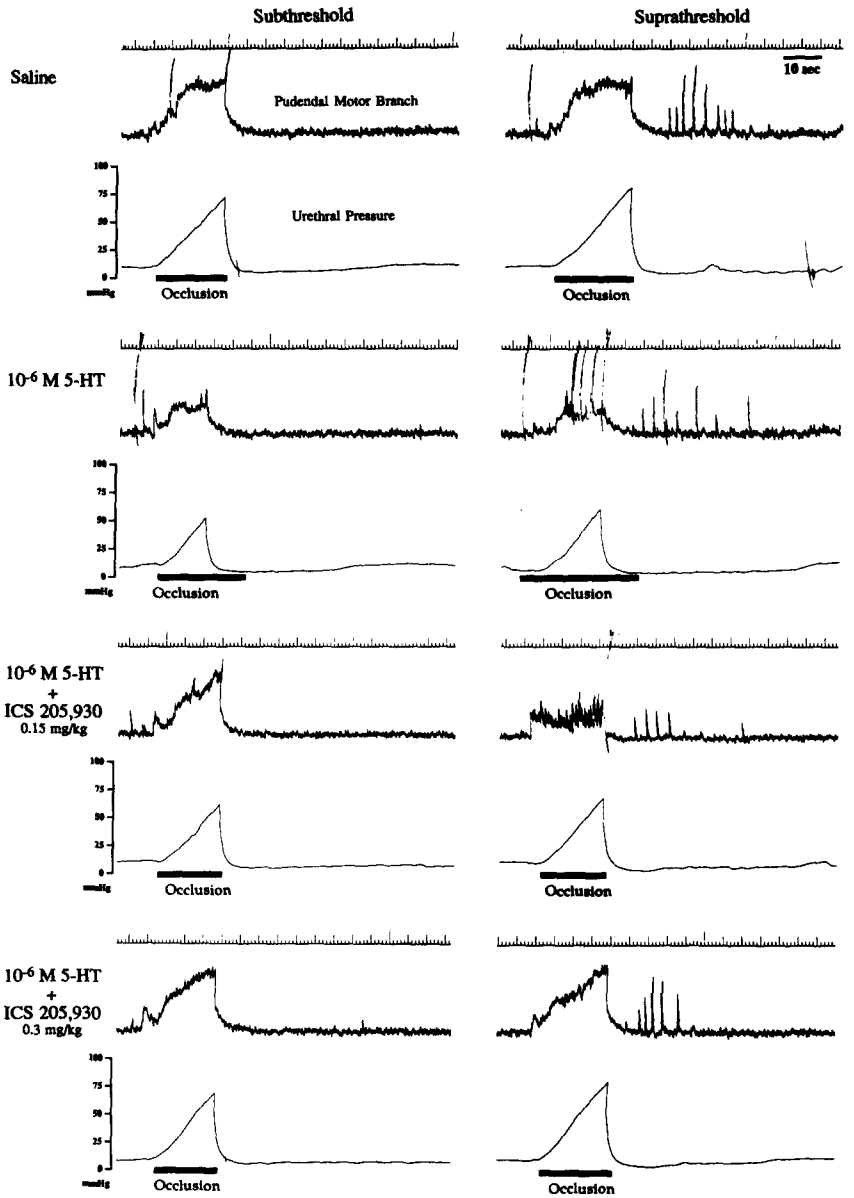

FIG. 4. The effect of the 5-HT_3 receptor antagonist, ICS 205,930 on the serotonin induced decrease in threshold. In the top traces, the saline threshold was 75 mmHg. The addition of 10^{-6} M 5-HT to the perfusate decreased the threshold to 55 mmHg. Administration of ICS 205,930 at a dose of 0.15 mg/kg (IV) 5 minutes prior to a perfusion with 10^{-6} M 5-HT raised the threshold to 65 mmHg. An additional dose of the drug (0.3 mg/kg, IV, cumulative dose) restored the threshold to the control value.

ous intensely labelled cells in the urethra (Fig. 1). Labelled cells were observed from the meatus to the bladder neck. No cells were observed in the bladder. There was a greater density of neurons in the distal urethra compared with more proximal portions, although they were numerous throughout. The paracrine cells were small (10–15 μm in diameter) and usually round or spindle shaped. The cell bodies were usually located in the basal portion of the mucosa. The cells typically had two, sometimes a single, large, short process with prominent club endings which extended laterally and basally. These processes typically ended near the basement membrane of the mucosa. Most of the cells had a single thin, unbranched process which extended to the lumen.

As we reported previously (3,13), urethral stimulation gave rise to a stereotyped pattern of activity that consisted of rhythmic firing (approximately 0.4 bursts per second for 15 seconds) in the cavernous nerve (the autonomic nerve innervating the clitoris) and in the pudendal motor branch leading to clonic contractions of the external anal and urethral sphincters. Vaginal and uterine contractions also occurred. Distension of the urethra with saline was effectively and reliably elicited this reflex. The mean saline threshold was 60.5 ± 16.3 mmHg ($n = 15$ rats, 37 trials).

The addition of serotonin (10^{-4}–10^{-7} M) to the urethral perfusate significantly decreased the pressure threshold necessary to elicit the UG reflex, compared to the saline controls before and after the serotonin (Fig. 2, Table 1). The effect of serotonin was dose dependent (Fig. 3). The facilitatory effect on the threshold by serotonin was partially antagonized by intravenous administration of the specific 5-HT_3 receptor antagonist (2,10), ICS 205,930 methiodide at a dose of 0.15 mg/kg and completely antagonized at a dose of 0.3 mg/kg (Fig. 4).

DISCUSSION

The location and morphology of urethral paracrine cells in the female rat are consistent with previous reports of these cells in the male and female human, dog, cat, rabbit and guinea pig (4, 5, 7, 8, 12). Previous reports have emphasized that the morphology of these cells suggests a chemosensory function. Specifically, the lumen-contacting processes and the concentration of secretory granules containing serotonin in the basal processes suggest that the urethral paracrine cells release serotonin in the urethral mucosa and submucosa in response to some chemical stimulus in the urethral lumen. However, up to now, no functional role for these cells has been proposed.

We previously reported that urethral stimulation in both male and female rats elicits a response, the coitus or urethro-genital (UG) reflex, which closely resembles sexual climax (3, 6, 13, 15). In the male we hypothesize that climax and ejaculation are elicited by the accumulation of seminal fluids in the urethra, leading to sufficient urethral stimulation to elicit the UG reflex. In the female, however, the natural stimulus for eliciting the UG reflex is unclear. It has been hypothesized that secretions from the paraurethral glands in the human female may play a role in eliciting sexual climax (1,9). However, it is unlikely that these glands in the female secrete sufficient volumes to elicit the UG reflex solely by mechanical distension of the urethra. We hypothesize that chemosensory mechanisms may play a role in eliciting the UG reflex. The present results support this contention.

We have shown that serotonin applied intraurethrally decreases the mechanical stimulus necessary to elicit the UG reflex in a dose-dependent manner. During sexual activity, paraurethral gland secretions might stimulate the urethral paracrine cells by a chemosensory stimulus. The release of serotonin from the paracrine cells would cause a similar decrease in threshold. The UG reflex might then be elicited by the paraurethral gland fluid, or more likely, by massage of the urethra by the penis through the vaginal wall.

There is evidence that seminal fluids exert a chemosensory stimulus in the male urethra. In experiments in dogs, Kimura (11) showed that stimulation of the hypogastric nerve elicited seminal emission (secretion of seminal fluids into the urethra). When a threshold pressure was reached (40.1 ± 0.9 mmHg) [n = 13 dogs, 23 trials, our calculation from Table 1 in (11)], rhythmic, ejaculatory contractions were elicited. However, infusion of saline into the urethra at these same pressures was ineffective in eliciting the ejaculatory contractions. This is clear evidence that some component of seminal fluid sensitizes the urethra. Note that the thresholds for ejaculatory responses elicited by seminal fluid in Kimura's experiments are very similar to the thresholds we measured when serotonin was added to the perfusate (Table 1).

The mechanisms by which intraurethral serotonin decreases the pressure threshold for the UG reflex is unknown. One possibility is that the serotonin decreases the compliance of the urethra by contraction of urethral smooth muscle. Depending upon the geometry of the urethral stretch receptors, the smooth muscle contraction could lower the pressures necessary to activate the receptors. This is unlikely to be the case for two reasons. First, addition of serotonin to the urethral perfusate did not cause any increase in the baseline urethral pressure, as would be expected if the smooth muscle were contracted. Second, the decrease in threshold was blocked by ICS 205,930. This drug is believed to be a specific 5-HT_3 receptor antagonist, but additional experiments with other 5-HT_3 receptor antagonists will be necessary to verify that the effects we observed are definitely due to 5-HT_3 receptors. The contraction of smooth muscle by serotonin is usually mediated by 5-HT_2 and 5-HT_1, not 5-HT_3 receptors (2,10). The role of urethral smooth muscle and other serotonin receptor subtypes on the threshold for this reflex are the subject of planned future experiments. The involvement of the 5-HT_3 receptor suggests that the action of serotonin in the urethra may be a direct sensitization of afferent fibers. Serotonin has been shown to produce rapid depolarization in many peripheral afferent fibers, especially visceral afferent fibers. This effect is mediated by 5-HT_3 receptors (10). The decrease in threshold in the present experiments could be mediated by a depolarizing action of serotonin directly on the mechanosensitive urethral afferents.

The present study confirmed the existence of serotonergic paracrine cells in the urethra of the female rat. We determined that the presence of serotonin in the urethra leads to a significant decrease in the threshold for eliciting a sexual climax-like response. This effect was most likely mediated by 5-HT_3 receptors. We hypothesize that the physiological role of the serotonergic paracrine cells may be chemosensory, releasing serotonin and thereby facilitating sexual climax in response to chemical stimulation from paraurethral gland secretions. Thus further research into the role of the urethra and paraurethral gland secretions in sexual function is warranted.

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