Vaginal physiological changes in a model of sexual arousal in anesthetized rats

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Giuliano, François, Julien Allard, Sandrine Compagnie, Laurent Alexandre, Stéphane Droupy, and Jacques Bernabe. Vaginal physiological changes in a model of sexual arousal in anesthetized rats. Am J Physiol Regulatory Integrative Comp Physiol 281: R140–R149, 2001.—The understanding of the pathophysiology of female sexual dysfunction suffers from the lack of a convenient model for the study of female genital sexual response. In this study, systemic arterial blood pressure (BP) as well as partial oxygen tension, temperature, and blood engorgement of the vagina [using laser-Doppler flowmetry in arbitrary units (AU)] were measured in anesthetized, ovariectomized (1 wk before the start of the experiment) female rats. Vaginal sexual arousal was replicated by electrical stimulation of the pelvic nerve (PNS). PNS induced reproducible increases in the different vaginal parameters (from baseline value, respectively: 16 ± 10 to 30 ± 12 mmHg; 34.9 ± 0.6 to 36 ± 0.6°C; 450 ± 196 to 1,500 ± 360 AU; P < 0.05, paired t-test) and BP (90 ± 7 to 123 ± 13 mmHg, P < 0.05, paired t-test). Vaginal vascular resistance was significantly decreased during PNS (from 0.23 ± 0.15 to 0.08 ± 0.02 mmHg/AU). Vaginal wall tension was also measured with a force transducer. PNS induced an increase in vaginal wall tension (1.0 ± 0.2 g), followed by a decrease under the prestimulation value. Intravenous atropine sulfate (1 mg/kg) injection abolished the increase in vaginal wall tension without significantly affecting vaginal vascular resistance. Intravenous vercuronium bromide (2 mg/kg) injection abolished the decrease in vaginal wall tension. Concomitant electrical stimulation of the paravertebral sympathetic chain inhibited vaginal response induced by PNS. Electrical stimulation of the medial preoptic area of the hypothalamus induced a response qualitatively equivalent to PNS with a significant decrease of vaginal vascular resistance. These data support that vaginal contractions involve the autonomic nervous system; female sexual arousal; laser-Doppler flowmetry; medial preoptic area; penile erection.

The increase in vaginal blood flow on vaginal sexual arousal in women was first measured using photoplethysmography (7). It was confirmed using other techniques, namely heat clearance (18), partial oxygen tension (P O2) measurement (36), Xenon washout (38) and laser- and ultrasound Doppler flowmetry (4, 31). Vaginal wall engorgement with blood, combined with enhanced capillary permeability, induces a neurogenic transudate from the vaginal epithelium, which enables lubrication of the inner surface of the vagina. Genital sexual arousal is also accompanied by vaginal luminal diameter and pressure changes. Vaginal luminal diameter increases at the onset of sexual arousal. As sexual arousal increases, there is an increase in vaginal luminal pressure, which culminates with a series of clonic contractions of pelvic and perineal muscles at orgasm (17).

Any impairment of these mechanisms can lead to female sexual dysfunction (FSD), a significant problem that affects the quality of life of 30–50% American women (15). Thanks to the successful launch of Viagra for the treatment of erectile dysfunction, there is now an increasing research effort toward the understanding of the pathophysiology and pharmacological treatment of FSD (11). However, there is a lack of suitable animal models for the study of female genital sexual arousal, and only three studies attempting to fulfill this need have been reported so far (22, 25, 35).

The urethrogenital reflex can be triggered in spinalized female rats (22). The urethrogenital reflex consists of clonic contractions of the perineal muscles and rhythmic vaginal and uterine contractions, and it is assumed to reflect sexual climax (22). Because genital vascular events occurring during the urethrogenital reflex have not been investigated yet, its relevance as a model of vaginal sexual arousal is awaiting further investigation. In the female rabbit, the vaginal and clitoral vasculogenic events occurring with electrical stimulation of the pelvic nerve were investigated using laser-Doppler flowmetry (25). Although this model appeared very promising, a model of female rat genital sexual arousal would also be useful, because a great deal of sexual behavioral as well as pharmacological data have already been gathered in female rats (27).

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line with this idea, the possibility of measuring the increase in vaginal wall blood flow with laser-Doppler flowmetry in the female rat on electrical stimulation of the pelvic plexus nerves has recently been demonstrated (35).

The aim of the present work was to establish a comprehensive female rat model of vaginal sexual arousal by assessing the vaginal vascular events on stimulation of the pelvic nerves with oxymetry and temperature measurements, in addition to the laser-Doppler flowmetry technique. We also intended to investigate the muscular activity occurring within the vagina during sexual arousal, as well as the contribution of major neural pathways to the control of vaginal sexual arousal. As a methodological control, the same microprobes were used to measure the hemodynamic changes occurring within the penile corpus cavernosum on stimulation of the cavernous nerve, a classic model for the study of physiology of penile erection (9).

MATERIALS AND METHODS

All animal experiments were carried out in accordance with the European Communities Council Directives (86/609/EEC) on the use of laboratory animals. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Animal preparation. Adult male (n = 5) and female (n = 60) Sprague-Dawley rats (Iffa-Credo, L’Arbresle, France) weighing 200–250 g were anesthetized by intraperitoneal injection of urethane (1.2 g/kg; Sigma, Saint-Quentin Fallavier, France). Their temperature was maintained at 37°C using a homeothermic blanket. Rats were tracheotomized to prevent aspiration of saliva and, when required, to perform artificial ventilation. A catheter was inserted into the left carotid artery and the jugular vein for blood pressure (BP) monitoring and drug injection, respectively. In males, the penis was denuded of skin to insert the tip of a catheter fitted with a 25-gauge needle into one penile corpus cavernosum for intracavernous pressure (ICP) monitoring. Arterial and cavernous catheters filled with heparinized saline (100 U/ml) were connected to pressure transducers (EM 750, Elcomatic, Glasgow, UK). Pressure signals (sampling rate 100 Hz) were amplified (Bionic Instruments, Nozay, France) and digitized via an analog-to-digital converter (LabMaster DMA 100, Scientific Solutions). Axotape soft muscles (NXE-100, Phymep, Paris, France) were implanted in the MPOA unilaterally and connected to an electrical stimulator (AMS 2100, Phymep). Stereoatomic coordinates were achieved with the head inclined 1.5°, 0.3 mm lateral to the midline, and 8.6 mm below the skull surface. MPOA stimulation consisted of square-wave pulses (30 Hz, 5 V, 2 ms) for 30 s. At the end of the experiments, the brain was removed and cut serially in the frontal plane on a cryostat in 10-μm-thick sections, and the site of electrode placements was directly determined histologically. Animals in which the stimulation site was outside the MPOA were discarded from the analysis.

Atropine, a muscarinic receptor blocker, was purchased from Sigma. Vercuronium bromide (Norcuron), a neuromuscular blocker, was obtained from Organon Teknika (Fresnes, France).

Measurement of vaginal wall or intracavernous blood flow, PO2, and temperature. Continuous and simultaneous measurements of local PO2 and temperature were obtained from the same microprobe (BF/OT, Oxford Optronics, Oxford, UK) connected to a monitor (OxyLite 2000, Oxford Optronics). The probe consisted of a thermocouple (resolution 0.1°C, accuracy 0.2°C). Blood flow was assessed using laser-Doppler perfusion monitoring (LDPM; stability of reading 1.5% using standard motility solution), using a laser-Doppler probe (Oxford Optronics) connected to another specific monitor (OxyFlo 2000, Oxford Optronics). LDPM is expressed in arbitrary units (AU). The microprobes were placed deep into the penile corpus cavernosum in males or against the inner lateroventral side of the vaginal lumen, 15–17 mm distal from the vulva, in females.

Measurement of vaginal contractile activity. After coeliotomy, a silk thread was superficially attached with one stitch to the surface of the vagina and connected to a force transducer precalibrated with different weights (Bionic Instruments, Nozay, France). Tension was applied so that the silk thread was at right angle with the vaginal surface. The vaginal contractile activity was determined by recording isometric tension of the silk thread. Developed tension was amplified and digitized via an analog-to-digital converter (LabMaster DMA 100, Scientific Solutions). Axotape software (Axon Instruments) was used for tension acquisition at a sampling rate of 100 Hz. Developed tension was expressed in gram equivalent.

Data analysis. BP, ICP, intracavernous or vaginal LDPM, intracavernous or vaginal PO2, vaginal wall tension, and intracavernous or vaginal temperature were the physiologic parameters measured. Recordings were analyzed semi-automatically posteriori using software designed in our laboratory. For each parameter, we determined the baseline value before the stimulation and the maximal value reached with the stimulation. In addition, the latency was determined, which corresponds to the time separating the onset of the electrical stimulation from the parameter to rise over the mean ± 3 SDs of the value before the stimulation. Duration of the variation for each parameter after electrical stimulation was also measured. The different parameters were av-
averaged for each rat for successive electrical stimulations under the same conditions, then averaged per group. The number of rats for each group is designated as *n*. Results are presented as the means ± SD for each group. Vascular resistance in the vagina or penile corpus cavernosum corresponded to the ratio of the mean arterial pressure over LDPM (14). Such a ratio was computed for each stimulation and averaged per rat and then per group.

Comparisons within group (before and after drug treatment) were performed using paired *t*-tests. *t*-Test was used to compare data between groups. Differences were considered statistically significant at *P* < 0.05. All tests are conducted using SigmaStat software, version 2.03 (SPSS, Erkrath, Germany).

RESULTS

Qualitative description of the vaginal response. Electrical stimulation of the pelvic nerve induced reproducible increases in BP, LDPM, PO\textsubscript{2}, vaginal wall tension, and temperature (Fig. 1). As shown in Fig. 2, electrical stimulation of the pelvic nerve induced an immediate and sharp increase in LDPM and tension. The increase in LDPM reached a plateau that lasted until the end of the stimulation. In contrast, there was no plateau phase for the vaginal wall tension response, which started to decrease before the end of the stimulation (Fig. 2). Vaginal wall tension decreased below the basal tension observed before the stimulation and then slowly returned to baseline (Fig. 2). The temperature and PO\textsubscript{2} responses were delayed compared with vaginal wall tension and LDPM. Temperature increased continuously until the end of the electrical stimulation and started to decrease after a short-lasting plateau. Inner vaginal wall PO\textsubscript{2} was the last parameter to increase on pelvic nerve stimulation. The maximal value of PO\textsubscript{2} was reached usually after the end of the electrical stimulation and it returned slowly to baseline (Fig. 2).

On stimulation of the paravaginal nerve in identical experimental conditions in five female rats, reproducible increases in LDPM, PO\textsubscript{2}, vaginal wall tension, and temperature were also observed (Fig. 3). LDPM (Fig. 3B), PO\textsubscript{2} (Fig. 3C), and temperature (Fig. 3E) increases were of smaller amplitude with paravaginal nerve stimulation than with pelvic nerve stimulation. In contrast, the increase in tension was of similar or greater amplitude (Fig. 3D).

We performed the same measurements in males with electrical stimulation of the cavernous nerve (6 V, 10 Hz, 1 ms for 30 s), except for vaginal wall tension, which was replaced by ICP. The aim of the experiments in male rats was not only to compare the dynamics of the responses between males and females, but also to validate the methodology used in this study in a well-characterized model of tissue engorgement with blood. Cavernous nerve stimulation induced reproducible penile intracavernosal responses (Fig. 4). The relative...
time course of the different hemodynamic parameters in males was similar to the one observed in females. Briefly, intracavernosal LDPM increased at the onset of stimulation, reached a plateau, and decreased at the end of the stimulation after a sharp peaklike increase (Fig. 4B). As in female vagina, penile intracavernosal PO₂ increased after a noticeable delay compared with the other parameters, did not display any plateau phase, and reached maximal value after the end of the electrical stimulation (Fig. 4C). ICP increased at the onset of the stimulation and started to decrease at the offset of the stimulation (Fig. 4D). Temperature increased more slowly, so that temperature started to decrease at the end of the stimulation without displaying any plateau phase (Fig. 4E).

**Parameters of the electrical stimulation of the pelvic nerve.** We sought to identify the electrical parameters eliciting the maximal increases in vaginal LDPM. Square-wave pulses of 1 ms, for a total time of 30 s, were used with different values of voltage and frequency (n = 5, 1 trial per rat for each condition). A four-parameter sigmoid best fit was performed \[ y = y_0 + a/1 + \exp -\left(x - x_0\right)/b \], with \( y_0 + a/2 \) and \( x_0 \) giving the value of LDPM and the variable parameter, respectively, at the half-maximal response. The half-maximal response was obtained with 2.1 ± 7.5 V at 10 Hz (Fig. 5A). The vaginal LDPM response was maximal at 6 V and increasing the amplitude of the stimulation to 10 V induced an apparent decrease of the response (Fig. 5A). Regarding the frequency of the square-wave pulses (constant voltage at 6 V), the half-maximal response was obtained with 3.9 ± 0.3 Hz. Maximal response was obtained at 10 Hz and did not change up to 30 Hz (Fig. 5B). According to these results, parameters for electrical stimulation of the pelvic nerve were 6 V, 10 Hz, and 1 ms in the following experiments to induce a maximal response.

**LDPM.** Semiautomatic quantification of the recording confirmed that LDPM was the first parameter affected by stimulation of the pelvic nerve in female rats (n = 15, 75 trials) and cavernous nerve in male rats (n = 5, 25 trials). The latencies of the increase in
vaginal and penile intracavernosal LDPM on pelvic and cavernous nerve stimulation, respectively, were comparable (2.2 ± 2.7 and 6.2 ± 3.3 s, respectively; Table 1). The mean basal vaginal and cavernosal LDPM were in the same range (450 ± 196 and 325 ± 199 AU, respectively; Table 1). Electrical stimulation led to a significant and similar maximal increase in LDPM in both sexes (230%, \( P < 0.001 \), paired \( t \)-test). Vaginal vascular resistance decreased from 0.29 ± 0.15 to 0.08 ± 0.02 mmHg/AU on pelvic nerve stimulations (\( P = 0.002 \), paired \( t \)-test) and penile corpus cavernosum vascular resistance from 0.38 ± 0.21 to 0.07 ± 0.02 mmHg/AU on cavernous nerve stimulations (\( P = 0.03 \), paired \( t \)-test).

**Vaginal wall tension and ICP.** Analysis of the recordings showed that pelvic nerve stimulations resulted in a biphasic response of the vaginal wall (\( n = 5, 25 \) trials), consisting of an increase in tension followed by a longer decrease in tension (24 ± 6 and 46 ± 16 s duration, respectively; Table 1). In the following, increases in vaginal wall tension are referred to as vaginal contraction and decreases in vaginal wall tension below the basal level as vaginal relaxation. The vaginal contraction was of greater amplitude (1.0 ± 0.2 g equivalent, \( P < 0.001 \), compared with the basal tension, paired \( t \)-test) than the vaginal relaxation (−0.2 ± 0.2 g equivalent compared with the basal tension, \( P = 0.069 \), paired \( t \)-test). In three females, the vaginal lumen was filled with warm saline through the vulva (0.2 ml/min). Simultaneous measurement of liquid pressure in the perfusing catheter was performed to assess luminal pressure variations. An increase in luminal pressure was observed at the onset of the pelvic nerve stimulation, coincident with the increase in tension. The increase in vaginal wall tension in females and ICP in males (\( n = 5, 25 \) trials; latency: 3.2 ± 2.2 and 6.0 ± 1.8 s, respectively; Table 1) were coincident with the increase in LDPM.

Spontaneous contractions of the vagina unrelated to pelvic nerve stimulations, generally of smaller amplitude than the ones induced by electrical stimulation, were often observed during recording sessions. The number of spontaneous contractions was greatly variable among the different rats tested during the 1-h recording session, ranging from 0 to >20. Such contractions were not followed by a relaxation period and were not accompanied by an increase in LDPM (for example, compare Fig. 1, B and D).

**Temperature.** After the onset of stimulation, the latencies of the temperature increase in males (\( n = 5, 25 \) trials) and females (\( n = 15, 75 \) trials) were similar (14 ± 2 and 14 ± 4 s, respectively; Table 1). The basal vaginal and intracavernosal temperatures measured during the experiments were 34.9 ± 0.6 and 29.3 ± 0.8°C, respectively (Table 1). Vaginal temperature was 37.6 ± 0.4°C (\( n = 4 \)) in females that did not undergo coeliotomy. Electrical stimulation induced a mean 1.1°C increase in vaginal temperature in females with coeliotomy (\( P < 0.001 \), paired \( t \)-test) and a mean 2.5°C increase in intracavernosal temperature (\( P = 0.003 \), paired \( t \)-test) compared with the basal temperature (Table 1). Anal temperature (not shown) remained unchanged on pelvic nerve stimulation.

**Oxygen.** \( P_O_2 \) measurement was difficult to obtain, and, because of insufficient baseline stability, it was rejected in 4 of 15 females (55 trials) and 1 of 5 males (20 trials). The basal value of \( P_O_2 \) was slightly greater in males (22 ± 14 mmHg) than in females (16 ± 10 mmHg). \( P_O_2 \) was increased 1.8-fold in females (\( P < 0.001 \), paired \( t \)-test) compared with the basal value using paired \( t \)-test. ICP, intracavernous pressure; BP, blood pressure; LDPM, laser-Doppler perfusion monitoring; AU, arbitrary unit. c, contraction; r, relaxation.

**Table 1. Measurement of genital response**

<table>
<thead>
<tr>
<th></th>
<th>Baseline Value</th>
<th>Maximal Value</th>
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<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>BP, mmHg</td>
<td>84 ± 5</td>
<td>83 ± 15</td>
</tr>
<tr>
<td>LDPM, AU</td>
<td>325 ± 199</td>
<td>450 ± 196</td>
</tr>
<tr>
<td>Vascular resistance</td>
<td>0.38 ± 0.21</td>
<td>0.23 ± 0.15</td>
</tr>
<tr>
<td>Vaginal wall tension, g</td>
<td>0</td>
<td>†1.0 ± 0.2‡</td>
</tr>
<tr>
<td>ICP, mmHg</td>
<td>12 ± 3</td>
<td>61 ± 8†</td>
</tr>
<tr>
<td>( P_O_2 ), mmHg</td>
<td>22 ± 14</td>
<td>16 ± 10</td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>29.3 ± 0.8</td>
<td>34.9 ± 0.6</td>
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</table>

Values are presented as means ± SD. Hemodynamic changes in the vaginal wall and penile corpus cavernosum upon electrical stimulation of the pelvic and cavernous nerve respectively. \( Top \) corresponds to the value of the different hemodynamic parameters elicited by electrical stimulation of the pelvic nerve in female rats and cavernous nerve in male rats. Recordings identical to the one presented in Fig. 1 from 15 females and from 5 males (Fig. 4) were quantified (vaginal wall tension was only quantified for 5 female rats). Values for each rat corresponded to the average before and quantified (vaginal wall tension was only quantified for 5 female rats). Values for each rat corresponded to the average before and determined (for example, compare Fig. 1, B and D).

**Fig. 5.** Relationship between LDPM and parameters of the electrical stimulation of the pelvic nerve. A: different values of voltage were assessed during electrical stimulation of the pelvic nerve. The electrical stimulation consisted in square-wave pulses of 1 ms, 10 Hz, for a total duration of 30 s, with the voltage varying from 0 to 10 V. B: frequency of the square pulse was the variable parameter (from 0 to 30 Hz). Each point represents the mean and SD of data collected in 5 female rats. The curve represents the best 4-parameter sigmoid fit of the variable parameter to LDPM.
0.001, paired *t*-test) and 1.4-fold in males (*P* = 0.027, paired *t*-test) on electrical stimulation. In females and males, the increase in PO2 was the last change to occur (latency: 26 ± 11 and 21 ± 5 s, respectively; Table 1).

**Effect of additional sympathetic chain stimulation.** Additional stimulation of the paravertebral sympathetic chain (6 V, 10 Hz, 1 ms) was performed for 30 s during a 90-s pelvic nerve stimulation in female rats (*n* = 4, 3 trials per rat), 30 s after the onset of the pelvic nerve stimulation. In this condition, stimulation of the sympathetic chain repeatedly induced a decrease in vaginal LDPM and PO2 (Fig. 6B and C). Temperature was decreased or stopped increasing during sympathetic chain stimulation (Fig. 6E). Vaginal LDPM, PO2, and temperature increased at the offset of sympathetic chain stimulation during continuous pelvic nerve stimulation (Fig. 6B, C, and E).

In these experiments, tension was on its decreasing phase at the time of the sympathetic chain stimulation (Fig. 6D). Sympathetic chain stimulation stopped this decrease and kept the vaginal wall tension at a constant level, usually above the prestimulation baseline value. At the offset of the sympathetic chain stimulation, vaginal tension further decreased and then returned to baseline after the end of pelvic nerve stimulation.

**Effect of atropine sulfate and vecuronium bromide.** Injection of 1 mg/kg iv atropine sulfate suppressed the vaginal contraction elicited by pelvic nerve stimulation, which was replaced by a relaxation (Fig. 7E). The mean values of the vaginal relaxation on pelvic nerve stimulation before and after atropine treatment were not significantly different (0.10 ± 0.06 and 0.16 ± 0.11 g, respectively, *n* = 5 female rat, 3 trials after drug injection per rat, 1 trial before drug injection, *P* = 0.088, paired *t*-test). Vecuronium bromide 2 mg/kg iv injections were performed in two rats. In contrast with atropine sulfate treatment, vecuronium bromide abolished the vaginal relaxation induced by electrical stimulation of the pelvic nerve, but not the vaginal contraction (Fig. 7F). LDPM was nonsignificantly reduced after atropine treatment (maximal LDPM response changed from 1,768 ± 42 to 1,317 ± 519 AU, i.e. −25%, *P* = 0.18, paired *t*-test; Fig. 7C). Vaginal vascular resistance during pelvic nerve stimulation changed from 0.06 ± 0.01 during control stimulation to 0.10 ± 0.04 mmHg/AU after atropine injection ( *P* = 0.22, paired *t*-test). The increase in temperature on electrical stimulation was also attenuated by atropine injection.
(from 0.9 ± 0.3 before the injection to 0.6 ± 0.1°C after the atropine injection, *P* = 0.091, paired *t*-test).

The maximal increase in LDPM and temperature were also reduced by vercuronium bromide injection (Fig. 7D). Injection of the corresponding volume of vehicle did not modify any of the considered parameters (not shown).

**Effect of MPOA stimulation.** As shown on Fig. 8, electrical stimulation of the MPOA induced an increase in vaginal LDPM (from 415 ± 145 to 1,152 ± 418 AU, i.e., +177%, *n* = 4 females, 16 trials, *P* < 0.001, paired *t*-test). Vaginal vascular resistance decreased from 0.19 ± 0.06 in the resting state to 0.10 ± 0.02 mmHg/AU during stimulation of the MPOA (*P* = 0.03, paired *t*-test). The LDPM response was rapid, with a mean latency of 2.1 ± 0.8 s. Vaginal tension was recorded in only two rats. The increase in tension was consistent (equivalent to 0.8–1.2 g), but the following decrease in tension was barely detectable. Temperature increases were too weak to be properly distinguished from the ambient noise in rats without coeliotomy, i.e., in rats in which vaginal wall tension was not measured. In rats with coeliotomy, vaginal temperature increases ranged from 0.3 to 0.5°C on stimulation of the MPOA. BP was also remarkably increased on electrical stimulation of the MPOA compared with the basal value (from 77 ± 11 to 121 ± 24 mmHg, i.e., +57%).

**DISCUSSION**

We provided a complete comprehensive physiological evaluation of the rat vaginal response to electrical stimulation of the pelvic nerve. Considering that cyclic sex steroids could influence vaginal sexual arousal (30), our experiments were performed in female rats ovariectomized 1 wk before. As ovariectomy results in a decrease of both neuronal- and endothelial-specific nitric oxide synthase expression after 1 wk (5), it is noteworthy that the genital responses that we observed might differ from the responses observed in intact, cycling female rats. In addition, there might be regional differences in the responses of the vagina, and we here focused on the upper part of the vagina, close to the cervix. Blood flow was assessed with LDPM and vaginal wall mechanical activity with isometric tension recording. Pelvic nerve stimulation did induce a reproducible increase in vaginal LDPM and vaginal wall tension, both events characteristic of the genital sexual response in women (17).

**LDPM.** Measurement of blood flow perfusion is a problematic issue. Taking into account the thickness of the rat female vaginal wall, the most convenient technique to measure blood flow is probably laser-Doppler flowmetry. However, caution must be exercised in the interpretation of the LDPM signal (16). The output signal given by the monitor is correlated to the product of the number of blood cells by their velocity within the volume illuminated by the laser beam. As demonstrated here in males, the synchrony of ICP and LDPM variation elicited by the stimulation of the cavernous nerve shows that LDPM is an index of blood engorgement (as opposed to blood perfusion). Thus the terms “blood flow” and “flux,” which refer to the passage of a quantity of blood through a region of interest, are inappropriate to describe the LDPM output. Moreover, considering the absence of calibration curve between LDPM and blow flow in any tissue (plus the dependence of LDPM output to laser beam incidence on the tissue), it is illusive to try to translate vaginal LDPM measurement into unit of volume of blood/mass of tissue/unit of time at this moment.

An oversimplified but correct definition is that the LDPM signal is an index of the amount of blood present and going through the site of tissue illuminated by the laser beam. In our experiments, LDPM reflected a
“static” component (the blood trapped within the penile corpus cavernosum or the vaginal wall) and a “dynamic” component (the blood effectively perfusing the site of tissue illuminated by the beam). This schematic view was supported by the presence of a peak of the LDPM signal at the end of the electrical stimulation in male rats. This peak likely corresponded to the setting in motion after the relief of venous outflow constriction of the blood cells previously trapped in the sinusoidal space. The relative contribution of blood trapping and blood perfusion to the LDPM signal in the vagina is unknown.

Thus the term “blood engorgement” seems the most suitable to characterize vaginal wall LDPM. Electrical stimulation of the pelvic nerve in females induced a reproducible increase in vaginal blood engorgement (+230% compared with baseline) and a reproducible decrease of the vaginal vascular resistance (~65% compared with baseline). The significant decrease of the vascular resistance demonstrated that the increase in blood engorgement could not be attributed solely to the increased BP. The increase in LDPM was delayed by a few seconds compared with the onset of the electrical stimulation, which contrasts with the observation in female rabbits, where the maximal value was obtained ~40 s after the offset of the stimulation (25). A possible reason is the larger mass of the rabbit vagina, which may require a longer time to be engorged with blood.

Atropine, 1 mg/kg iv, did not significantly affect the vaginal LDPM response to pelvic nerve stimulation despite the fact that cholinergic fibers innervate vascular smooth muscle in the rat vagina (24). Similarly, atropine (0.035 mg/kg iv) did not affect blood flow in women during vaginal sexual arousal despite a rich cholinergic innervation of the vaginal arteries (1, 37). Thus acetylcholine may not be the primary neurotransmitter responsible for the increase in vaginal blood engorgement on sexual arousal.

Temperature. In males and females, temperature increased at the onset of the neural electrical stimulation and started to decrease at the offset of the stimulation. In our experimental conditions, the increase in temperature is probably a consequence of the difference between the blood flow temperature and the lower vaginal wall or intracavernosal temperature. The basal temperature in the penile corpus cavernosum (29.3 ± 0.8°C) was much lower than the basal temperature of the vaginal wall (34.9 ± 0.6°C) because the denuded penile corpus cavernosum was entirely exposed to the ambient air, whereas the vagina had only its outer surface exposed to the ambient air. The greater increase in intracavernosal temperature (2.5°C) compared with vaginal temperature (1.1°C) reflects the lower resting temperature of the penile corpus cavernosum and the greater filling with blood elicited by neural stimulation.

Increases in vaginal temperature in female rats without coeliotomy was <0.1°C on stimulation of the pelvic nerve. Thus, in contrast with the changes in labial temperature, the basal value of which is lower than core temperature (12, 13), vaginal temperature variation may be minor in women on sexual arousal. Surprisingly, a drop of vaginal temperature has been reported with any kind of sexual arousal using a radiotelemetric device in women, but this result should be confirmed (6).

Vaginal wall tension. A cornerstone of vaginal sexual arousal concerns the vaginal smooth muscle and vagina-connected striated perineal muscle activity. Pelvic nerve stimulations repeatedly elicited an immediate increase in vaginal wall tension, followed by a slight, but consistent, decrease below the basal level. The exact interpretation of these results is at this time rather speculative. The rat vagina is surrounded by a circular layer of smooth muscles, as well as an important longitudinal smooth muscle layer (33). The increase in vaginal wall tension that we observed, concomitant with an increased luminal pressure, is likely due to contractions of both smooth muscle layers, whereas the relaxation phase might involve striated muscles (25, 34). This idea is supported by our pharmacological experiments, as blocking of muscarinic and nicotinic receptors selectively abolished the contraction and relaxation, respectively. The exact nature of the striated muscles involved in the relaxation phase is actually unknown, and accurate anatomic and functional data on vaginal muscle mechanics are required to answer this question (for example, see Ref. 29).

It is noteworthy that vaginal contractions by themselves are not evidence of sexual arousal, as we observed vaginal contractions independent of pelvic nerve stimulation without increase in LDPM. The degree to which the successive contraction/relaxation observed on pelvic nerve stimulation reflects the vaginal wall activity occurring during rat vaginal sexual arousal is actually unknown. In women, an increase in vaginal luminal pressure occurring from arousal to orgasm has been reported, with an increase in inner vaginal diameter at the onset of the sexual arousal (17). This pattern of vaginal wall tension variations may differ in female rats, because of the characteristics of rat copulation, which consists of several brief successive intromissions (300-ms duration) separated by several minutes (28). In our experiments, vaginal wall contractions did decrease the vaginal luminal volume, which would not facilitate intromissions. On the other hand, this would increase sensitive stimulation of the penis, thus facilitating ejaculation. In physiological conditions, vaginal contractions may be more brief and may take place before the intromission just at the onset of the female lordosis response.

There was an apparent contradiction between increased LDPM and vaginal wall contraction. In fact, the peak of tension preceded the peak of LDPM and the plateau of LDPM response occurred when the tension was decreasing. Whether contractions of nonvascular smooth muscles were selectively venoocclusive and thus contributed to the initiation of vaginal blood engorgement or whether contractions limited blood flow in both the arterial and venous bed should be determined.


\[ \text{PO}_2 \]. Our measurements concerning vaginal wall \text{PO}_2 were close to the value reported in women in resting state (9 ± 11 mmHg) and on sexual arousal (39 ± 14 mmHg) (36). As expected, the results that we obtained in males were different with the value obtained with blood sampling and gas blood measurement using polarography in human males (36 ± 6 mmHg in the flaccid state and 84 ± 7 mmHg on erection) (32). This difference supports the idea that \text{PO}_2 in blood sampling from the penis in the flaccid and erect state corresponds to more or less the venous and arterial blood flow, respectively (2), whereas measurement with the ruthenium probe reflected \text{PO}_2 in the tissue i.e., the diffusion of O\textsubscript{2} from the systemic circulation.

**Conclusion about the different parameters measured.** The combined use of LDPM, ruthenium probe, and measurement of vaginal wall tension allowed some unique insights into the dynamic interplays of the different parameters. There was a shift between the increase in vaginal LDPM and increase in \text{PO}_2 in females: the beginning of the increase in \text{PO}_2 coincided with the maximal value of the vaginal LDPM, and the maximal value of the \text{PO}_2 was clearly reached after the return to baseline of LDPM. The filling with arterial blood could lead to an increase of O\textsubscript{2}, resulting in saturation of O\textsubscript{2} within the tissue. The slow return to baseline corresponded to the progressive O\textsubscript{2} consumption and washout from the tissue.

Nevertheless there is a redundancy in these different parameters. The variations of temperature and \text{PO}_2 that we observed are both secondary phenomenon of the increased blood engorgement, which was directly assayed by LDPM. Moreover, LDPM displayed several advantages over temperature and \text{PO}_2 measurement. LDPM response was more sensitive and rapid than temperature, and, in experiments without coeliotomy, temperature measurement was not accurate enough to evaluate vaginal sexual arousal. \text{PO}_2 measurement could not reflect the dynamic of vaginal engorgement and appeared delicate to obtain.

In contrast with \text{PO}_2 and temperature, vaginal wall tension is a parameter independent of vaginal wall blood engorgement. Spontaneous increases in vaginal wall tension of amplitude equal to the one induced by pelvic nerve stimulation were observed without change of LDPM. Moreover, on atropine injection, the increase in LDPM was only accompanied with a decrease of the vaginal wall tension, clearly showing that different neurotransmitters are involved in the regulation of vaginal wall contractions and blood engorgement.

**Neural control of vaginal sexual arousal.** In anesthetized rats, the neural control of male and female sexual responses were comparable at any level considered. The same electrical parameters could elicit genital sexual responses in both sexes on stimulation of the pelvic or cavernous nerve.

Stimulation of the pelvic or paravaginal nerve elicited similar vaginal responses. This result is in agreement with the idea that the parasympathetic outflow to the vagina is conveyed by the pelvic nerve and, after synapsing in the paracervical ganglion, in the different branches of the paravaginal nerve (3). The minor differences observed in the responses with the different nerves may be due to the more specific innervation of the vagina by the paravaginal nerve compared with the pelvic nerve. In addition, it is likely that more efferent and afferent fibers to and from the vagina are recruited on pelvic than paravaginal nerve stimulations.

It is accepted that the sympathetic outflow runs through the hypogastric nerve and the paravertebral sympathetic chain in female rat (23). In males, erection is determined by a balance between antierectile sympathetic and proerectile parasympathetic tone (8). Here, stimulation of the sympathetic chain reduced or inhibited the vaginal response induced by pelvic nerve stimulation. To our knowledge, such a result constitutes the first experimental evidence that vaginal sexual arousal is oppositely regulated by the sympathetic and parasympathetic system in female rats, in the same fashion as in male rats.

We further investigated similarities of the neural control of genital arousal in males and females. The brain is the master organ in sexual function in both males and females (21). MPOA has been identified as a key structure in the supraspinal control of penile erection in male rats. Electrical stimulation of the MPOA facilitates copulatory behavior in freely moving male rats (19) and induces penile erection and urethrogenital reflex in anesthetized animals (10, 20). The implication of MPOA in vaginal sexual arousal has not been explored in female rats, but hypothalamic neurons are critical for expression of sexual behavior (27). Here, electrical stimulation of the MPOA induced a significant increase in vaginal LDPM and decrease in vaginal vascular resistance, suggesting that sexual genital arousal may also be triggered by MPOA activation in female rats.

In conclusion, to our knowledge, this study constitutes the first comprehensive approach to the study of the vaginal physiological changes occurring in female rats on sexual arousal. LDPM appeared as a reliable assessment of vaginal wall engorgement. Variations of the vaginal wall tension compatible with the vaginal relaxations/contractions observed on genital female sexual arousal were measured. We have shown that acetylcholine may play an important role in the contractile phase induced by pelvic nerve stimulation, whereas its participation to the increase in vaginal blood engorgement seemed minor. The first experimental evidence of the negative regulation of vaginal sexual arousal by fibers running in the paravertebral sympathetic chain and of the implication of the MPOA in the generation of vaginal sexual arousal was provided. These data support the idea that there are great similarities in the neural control of the genital sexual responses in males and females rat.

**Perspectives**

This model should allow a better knowledge of the peripheral and central neurophysiology and neuromediation of female genital sexual arousal. It may also
help to understand the pathophysiology of FSD using female rat models of menopause, neuropathies, and/or vasculopathies, as these complications are suspected to cause sexual dysfunction in humans. Such studies are necessary conditions for the development of drugs aimed at treating FSD.

REFERENCES


