BIOLOGY OF REPRODUCTION **67,** 1013–1017 (2002)
DOI 10.1095/biolreprod.101.002287

# Functional and Histochemical Characterization of a Uterine Adrenergic Denervation Process in Pregnant Rats[1]

**Anna Klukovits,[3] Róbert Gáspár,[3] Péter Sántha,[4] Gábor Jancsó,[4] and George Falkay[2,3]**

*Department of Pharmacodynamics and Biopharmacy,[3] University of Szeged, 6720 Szeged, Hungary*
*Department of Physiology,[4] University of Szeged, 6720 Szeged, Hungary*



## ABSTRACT

**The time course of pregnancy-induced changes in the contractile responses of isolated uterine rings and sympathetic innervation pattern were studied using electric field stimulation and histofluorescence techniques, respectively, in intact and 6-hydroxydopamine-treated rats. Neurally mediated contractions elicited by field stimulation (0.6 msec, 1–70 Hz, 40 V) were measured in uterine preparations obtained from nonpregnant, 6-hydroxydopamine-treated and 5-, 10-, 15-, 18-, and 22-day (term) pregnant rats. At all frequencies, the amplitudes of contractions were highest in nonpregnant uteri. Stimulation at 1–2.5 Hz evoked contractions in 10-day pregnant uteri but failed to cause contractions on Day 5 and from Day 15 onward. In uterine preparations obtained from term and from 6-hydroxydopamine-treated rats, contractions could not be evoked by stimulation at 1–20 Hz. Fluorescence histochemistry of uterine adrenergic nerves revealed rich perivascular and myometrial innervation in nonpregnant and in pregnant rats through Day 10. Degeneration and loss of adrenergic nerve fibers was apparent by Day 15, and fluorescent myometrial and perivascular nerves were practically absent by Day 22. These findings demonstrate a progressive, frequency-related reduction of nerve-mediated uterine contractions beginning in midterm pregnancy, in parallel with a gradual loss of adrenergic nerve fibers. Pregnancy-induced nerve degeneration may promote the development of nonsynaptic α-adrenergic uterine contractile activity towards term. The reduced responsiveness of uterine smooth muscle to electric field stimulation in early pregnancy appears to be unrelated to alterations in uterine innervation but may be related to changes associated with implantation.**

*catecholamines, female reproductive tract, implantation, pregnancy, uterus*

## INTRODUCTION

The rat uterus is innervated by both autonomic and sensory nerves, including adrenergic [1, 2] and cholinergic [3], as well as by different peptidergic fibers containing vasoactive intestinal polypeptide (VIP), substance P (SP), calcitonin-gene-related peptide, and galanin [4, 5]. During pregnancy, the motor and sensory innervation of the rat uterus undergoes a profound denervation process, although the changes do not affect all types of nerves. Immunocytochemical studies have indicated that myometrial and perivascular VIP-containing fibers disappear at the end of pregnancy [6]; in contrast, SP-containing primary afferent neurons do not degenerate during pregnancy [7]. It is known that, at the end of pregnancy (Days 20–22), the numbers of both myometrial and perivascular adrenergic nerves are decreased in the rat [8, 9], in the guinea pig [10, 11], and in humans [12, 13]. It is important to determine whether these morphologic changes have functional consequences in relation to the pregnancy-induced adrenergic nerve degeneration process that influences myometrial contraction.

The aim of the present study was to investigate the functional consequences of adrenergic denervation on myometrial contractility from the beginning till the end of pregnancy. An in vitro electric field stimulation (EFS) model was used to study the contraction responses of pregnant rat myometrial rings to nerve stimulation. In order to focus on the role of adrenergic control on uterine smooth muscle function, the adrenergic bundles were stimulated electrically and the contractility response was measured. The adrenergic denervation process was also investigated by fluorescence histochemistry.

## MATERIALS AND METHODS

### *Animals*

Animal investigations were carried out with the approval of the Ethical Committee for Animal Research, University of Szeged (registration number 23/1999). Sexually mature female Sprague-Dawley rats (body mass 180–240 g, 60–80 days old) were used in the experiments. Mating with males was carried out in the early morning hours. Copulation was confirmed by the presence of a copulation plug or spermatozoa in the vagina. The day of conception was considered to be the first day of pregnancy. Nonpregnant females were cyclic, virgin rats held in separate cages from males since birth. Animals were housed in temperature- and humidity-controlled and light-regulated (12L:12D) rooms with water and food intake ad libitum.

Selective destruction of the adrenergic nerve structures was accomplished by 6-hydroxydopamine (6-OHDA) treatment of cyclic, virgin rats [14]. They received 100 mg/body mass kg of 6-OHDA-hydrochloride (Sigma Aldrich Ltd., Budapest, Hungary) per day for 2 days intravenously, dissolved in physiological saline. The experiment was performed 48 h after the second dose.

### *Uterus Preparation and Electric Field Stimulation Parameters*

The denervation process was studied in nonpregnant and in 5-, 10-, 15-, 18- and 22- day pregnant (term) rats and in 6-OHDA-treated rats. The rats were killed by cervical dislocation at 1000 h, the uteri were removed and trimmed of fat, the feto-placental units were removed, and the decidua was scraped off. The myometrium was immediately placed in an organ bath (de Jongh solution containing [in mM] 137 NaCl, 3 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 12 $NaHCO_3$, 4 $Na_2HPO_4$, 6 glucose; pH 7.4), perfused with 95% oxygen and 5% carbon dioxide. Temperature was maintained at 37°C. Myometrial rings 1 cm long were sliced from the middle part of each horn, including implantation sites in the case of pregnant animals, and mounted vertically between two platinum electrodes in 10 ml of the above mentioned organ bath under the same conditions. After mounting, the uterine rings were equilibrated for 60 min before the experiment. The initial tension was set at 1.5 g. To avoid spontaneous uterine contractions, $10^{-5}$ M verapamil (Chinoin Ltd., Budapest, Hungary) was added to the

[1]This work was supported by Hungarian Research grants OTKA T033126 and T032507.
[2]Correspondence: George Falkay, Department of Pharmacodynamics and Biopharmacy, University of Szeged, Eötvös u. 6, H-6720 Szeged, Hungary. FAX: 36 62 545 567; e-mail: falkay@pharma.szote.u-szeged.hu

Received: 4 December 2001.
First decision: 29 December 2001.
Accepted: 23 April 2002.

ISSN: 0006-3363. http://www.biolreprod.org

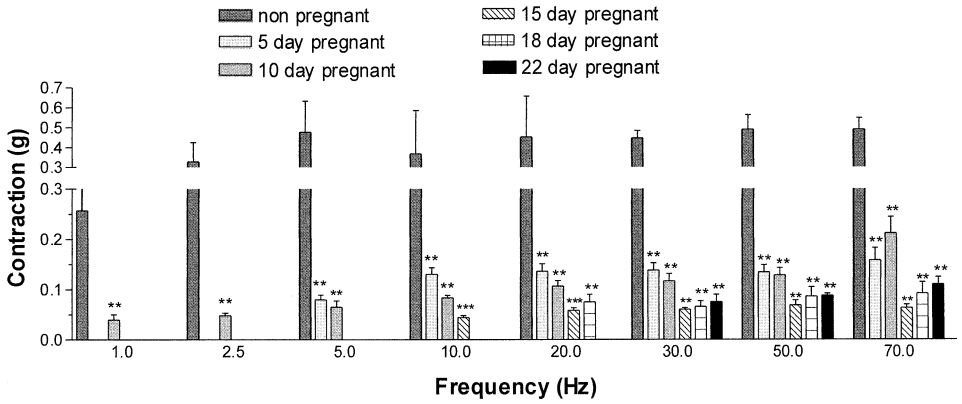


FIG. 1. Myometrial contraction responses versus applied stimulating frequency (in Hertz) in nonpregnant and in 5-, 10-, 15-, 18-, and 22-day (term) pregnant rats. Each bar represents the amplitude of contraction expressed (in grams) as mean ± SEM (n = 6). The contraction data were statistically analyzed at each frequency value independently. The asterisks indicate the level of significant difference from (intact) nonpregnant rat myometria (**$P < 0.01$; ***$P < 0.001$).

organ bath to block L-type calcium channels after incubation for 55 min. After the incubation period, selective nerve stimulation was elicited by a digital, programmable stimulator (ST-02; Experimetria Ltd., London, UK). Each ring was stimulated by 1-min trains of square pulses with the following frequencies, in increasing sequence: 1, 2.5, 5, 10, 20, 30, 50, 70 Hz. After these 1-min trains of stimulation, the tissue was allowed to recover for 4 min and a higher frequency was then applied. The square pulse duration was set at 0.6 msec and the supramaximal voltage at 40 V. The tension of the myometrial rings was measured with a strain-gauge transducer (SG-02; Experimetria Ltd.) and recorded and analyzed by ISO-SYS Data Acquisition System (Experimetria Ltd.).

### *Histofluorescence Examination of Uterine Adrenergic Innervation*

On Days 5, 10, 15, 18, and 22 of pregnancy, animals were killed by cervical dislocation and were bled. The same was done with the nonpregnant, cyclic virgin rats. After a midline abdominal incision, the uteri were exposed and removed. The tissue samples from the middle part of the uterus, including implantation sites in pregnant animals, were placed immediately on cryostat chucks and frozen onto them with the aid of a small amount of distilled water; longitudinal sections 15 μm in thickness were cut and mounted on glass slides. Specimens were immersed for about 3 sec in a solution of 0.1 M glyoxylic acid containing (in mM) 235 $KH_2PO_4$ and 200 sucrose, pH 7.4 [15]. Air-dried sections were either covered with liquid paraffin and placed in an oven at 95°C for 2.5 min or dried over phosphorus pentoxide overnight and then reacted with paraformaldehyde vapor at 80°C for 60 min. The preparations were examined under a Leica DMLB fluorescence microscope (Wetzlar, Germany) equipped with appropriate filters.

### *Statistical Analyses*

All experiments were carried out on at least six animals and values are given as means ± SEM. Bartlett tests revealed the homogeneity of variances. We investigated the presence of responses and their amplitudes. A one-way ANOVA with a Newman-Keuls test was used to compare the amplitudes of given contractile responses at different gestational ages to each frequency independently. The recorded data were statistically analyzed with the Prism 2.01 (Graph Pad Software Inc., San Diego, CA) computer program.

## RESULTS

### *Electric Field Stimulation Experiments*

Nerve-induced uterine contractions were registered at different times of pregnancy and in nonpregnant rats. Eight distinct frequency values were established, and the presence of a contractility response and its amplitude were detected.

Figure 1 illustrates the neurally evoked contractions of myometrial preparations derived from the uteri of nonpregnant and from 5-, 10-, 15-, 18-, and 22-day pregnant (term) rats. In nonpregnant rats, the uterus responded to all the applied frequencies with contraction. On Day 5 of pregnancy, the uterus did not respond to the lowest frequency stimuli, 1 and 2.5 Hz. On Day 10, contraction responses were detected to all applied frequencies, but contraction amplitudes were lower than those observed in the nonpregnant rats. By Day 15, contraction responses could not be evoked by 1–5 Hz; the lowest frequency that elicited contraction in the myometria of 15-day pregnant rats was 10 Hz. The uteri of the 18-day pregnant rats did not respond to even 10 Hz, but only to values of 20–70 Hz. By term, the uterus responded only to 30–70-Hz stimulation; thus, contractions could not be evoked at 1–20 Hz. The examination of the presence of nerve-induced myometrial contractions therefore revealed a tendency toward a decreasing ability to respond to lower frequency stimulation with increasing gestational age from Day 10 to Day 22 of pregnancy.

A comparison of the amplitudes of uterine contractions

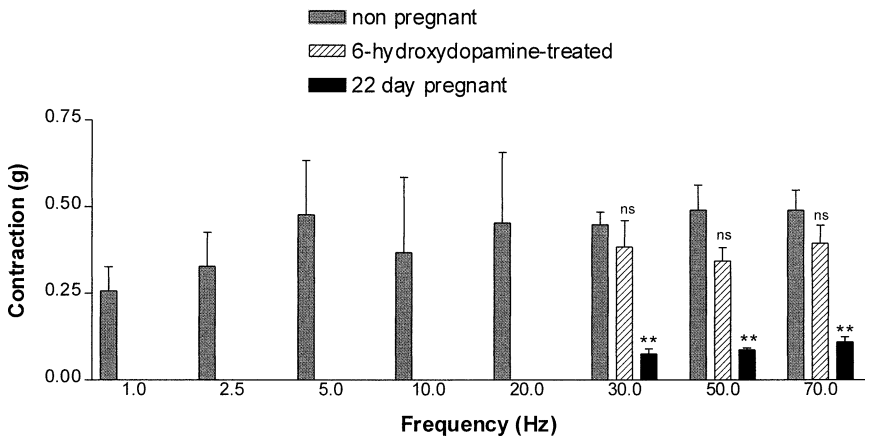


FIG. 2. Myometrial contraction responses versus applied stimulating frequency in nonpregnant, in 22-day (term) pregnant, and in 6-OHDA-treated nonpregnant rats. Each bar represents the amplitude of contraction expressed (in grams) as mean ± SEM (n = 6). The contraction data were statistically analyzed at each frequency value independently. The asterisks indicate the level of significant difference from (intact) nonpregnant rat myometria (** $P < 0.01$; ns, $P > 0.05$, thus no significant difference).

TABLE 1. Summary of the presence of myometrial contraction responses as a function of the applied frequency value of electric field nerve stimulation in nonpregnant, in 6-hydroxydopamine (6-OHDA)-treated nonpregnant, and in 5-, 10-, 15-, 18-, and 22-day pregnant rats according to the data in Figures 1 and 2.

| Frequency (Hz) | Contraction response to electric field stimulation[a] | | | | | | |
|---|---|---|---|---|---|---|---|
| | Non-pregnant rats | Days of pregnancy | | | | | 6-OHDA-treated rats |
| | | 5 | 10 | 15 | 18 | 22 | |
| 1 | + | − | + | − | − | − | − |
| 2.5 | + | − | + | − | − | − | − |
| 5 | + | + | + | − | − | − | − |
| 10 | + | + | + | + | − | − | − |
| 20 | + | + | + | + | + | − | − |
| 30 | + | + | + | + | + | + | + |
| 50 | + | + | + | + | + | + | + |
| 70 | + | + | + | + | + | + | + |

[a] +, Presence of response; −, lack of response.

in different stages of pregnancy to those in nonpregnant rats demonstrated significant differences at all applied frequencies. The amplitudes of the nerve-stimulated myometrial contractions were significantly lower ($P < 0.01$–$0.001$) on each of the examined days of pregnancy compared with nonpregnant animals throughout the whole frequency range of 1–70 Hz. The myometria of the 15-day pregnant rats responded with the lowest amplitude at 10- and 20-Hz frequency, respectively ($P < 0.001$ compared with nonpregnants rats at both frequencies). Although not shown in Figure 1, we note that paired comparisons revealed significantly lower amplitudes on Day 15 at 10 Hz ($P < 0.05$ vs. Day 5 and Day 10) and at 20 Hz ($P < 0.05$ vs. Days 5, 10, and 18).

The uteri of both the 22-day pregnant and 6-OHDA-treated nonpregnant rats did not respond to stimuli of 1–20 Hz (Fig. 2). In the 22-day pregnant rats, significantly lower amplitudes were detected in response to stimuli of 30–70 Hz than in the intact nonpregnant ($P < 0.01$) or in the 6-OHDA-treated nonpregnant rats ($P < 0.01$). In the 6-OHDA-treated nonpregnant rats, the amplitudes of contractions to stimuli of 30–70 Hz were not statistically different ($P > 0.05$) from those in the intact nonpregnant rats.

### *Fluorescence Histochemistry*

Uterine adrenergic nerves were demonstrated by using glyoxylic acid- and formaldehyde-induced fluorescence histochemistry (Fig. 3). In adult nonpregnant rats, the uterine adrenergic nerve fibers displayed a characteristic topographic distribution: the uterine arteries were richly innervated by thick, varicose adrenergic axons (Fig. 3a), whereas the uterine smooth musculature was served by a moderate mass of fine varicose fibers (Fig. 3b). A similar pattern of rich perivascular and myometrial adrenergic innervation was demonstrated in pregnant rats on Day 5 (Fig. 3c) and Day 10 (Fig. 3d) of pregnancy. Loss of adrenergic nerves was apparent by Day 15. On Day 15, degeneration of both myometrial and perivascular axons was evident: nerve fibers showed clear-cut fragmentation and swollen varicosities (Fig. 3e). On Day 18, the adrenergic innervation of the myometrium was almost completely diminished; few, if any, perivascular nerves were observed around small arter-

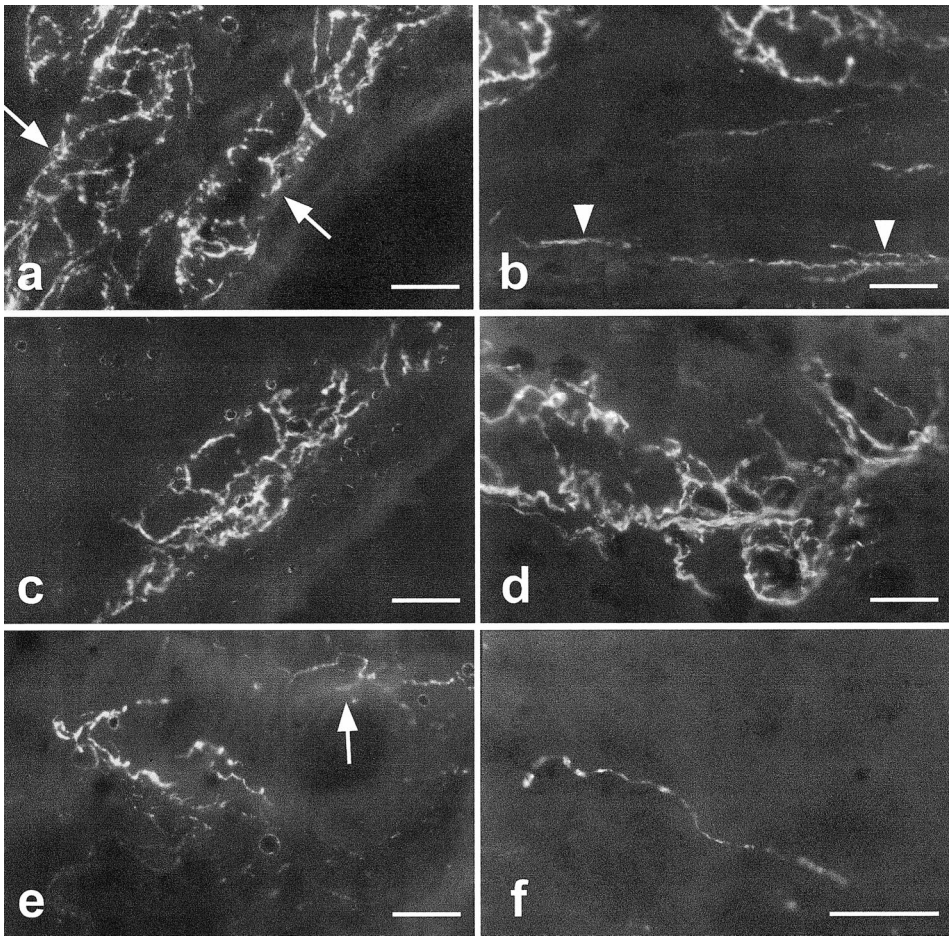


FIG. 3. Fluorescence micrographs illustrating uterine innervation in nonpregnant and pregnant rats. **a**, **b**) Nonpregnant rat. Blood vessels (arrows) and uterine smooth muscle (arrowheads) are richly innervated by adrenergic fibers. **c**, **d**) On Day 5 (**c**) and Day 10 (**d**) of pregnancy, the uterine adrenergic innervation is comparable with the nonpregnant uterus. **e**) On Day 15 of pregnancy, a marked loss of adrenergic nerve fibers is evident: fragmentation of fibers and swollen varicosities are seen. Note the sparse innervation of a blood vessel (arrow). **f**) On Day 22 of pregnancy, irregularly beaded degenerating adrenergic axons can be observed in the uterus. For all micrographs, bars = 10 μm.

ies (data not shown). The fluorescent adrenergic nerves were practically absent on Day 22; only occasional degenerating axons could be seen (Fig. 3f).

## DISCUSSION

Previous morphological investigations disclosed the innervation patterns of the nonpregnant [16] and the term pregnant and postpartum rat uterus [8, 9]. The time course of these degenerative structural changes in the rat uterus has not been dealt with in detail with special attention to the functional consequences of the impairment of neurogenic contractility during pregnancy. In this study, therefore, we investigated both the functional and the structural changes in the adrenergic innervation in the rat uterus on Days 5, 10, 15, 18, and 22 of pregnancy. In the present experiments, the uterine adrenergic nerve function was studied as concerns the presence or absence of a myometrial contraction response to the eight distinct frequencies applied on different days of pregnancy (Table 1); if present, the amplitudes of contractions were compared.

Field stimulation was considered to be a suitable method to further characterize the role of the adrenergic nerves in the motor responses of the pregnant rat myometrium. The selected frequencies were appropriate to stimulate the adrenergic nerves, as was proven in the experiments on 6-OHDA-treated nonpregnant animals. Two days after the 6-OHDA treatment, there were no detectable myometrial contraction responses to nerve stimulation at 1–20 Hz. Similarly, myometrial contractions could not be evoked by EFS at 1–20 Hz in term pregnant rats (Day 22, i.e., the day of delivery). According to results reported previously, the range 1–20 Hz is considered to be selective for adrenergic nerves if the pulse width is relatively small [17–19]. In the 6-OHDA-treated animals and in the 22-day pregnant rats, stimulation at 30–70 Hz caused uterine contractions, but this frequency range is not selective for adrenergic nerves but rather for others, such as the different peptidergic nerves [20–23]. Hence, the present results support the notion that stimulation with electrical impulses of short duration ($<$5 msec) at frequencies of 1–20 Hz is selective for adrenergic nerves.

The myometrial function was described by the presence of nerve-induced contractions. The lack of a contractile response to stimulation at certain frequencies was considered to be a failure of adrenergic nerve function. The functional alterations could be interpreted in terms of morphologic changes as assessed by fluorescence microscopic demonstration of the adrenergic innervation in the uterus. Our histofluorescence observations indicated that the diminution of the contractility correlated with the progressive loss of uterine adrenergic nerve fibers. Both EFS and fluorescence histochemistry showed the myometrial adrenergic fibers to be present and functional, morphologically not different from those observed in nonpregnant uteri up to Day 10 of pregnancy. The denervation process commenced in midpregnancy and deteriorated toward term. In light of previous reports [24], we interpreted our morphologic data in terms of a degeneration process, but the possibility that disappearance of the staining of adrenergic nerve fibers may also result from a profound depletion of their transmitter content without frank axonal degeneration cannot be excluded.

As concerns the examined days of pregnancy, a parallelism was found between the functional and morphologic changes in the rat myometrium except for the phenomenon that, on Day 5 of pregnancy, no responses were elicited by low-frequency nerve stimulation (1–2.5 Hz), though the histologic picture of a 5-day pregnant myometrium did not show any sign of nerve degeneration. We have not yet found an explanation, but it is worth mentioning that this time period coincides with implantation: in rats, on Day 5 after conception, the hatched blastocysts become attached to the uterine epithelium [25, 26]. Whether the adrenergic nerve or receptor function interferes with this process is not known. Although the uterine adrenergic fibers in the 5-day pregnant myometrium seemed to be similar to those in the nonpregnant myometrium, they may differ in function, e.g., in excitability or in excitation-contraction coupling. The observation of an impaired nerve function despite of intact morphology on Day 5 needs further investigation.

The results of the EFS and histofluorescence studies suggest that maternal adrenergic neuronal factors possibly do not play a prominent role in controlling the myometrial motor activity from midpregnancy toward parturition. However, adrenergic denervation in the pregnant rat uterus does not mean a loss of the ability to respond to adrenergic stimuli. The adrenergic mechanisms in the late pregnant uterus are presumably mediated via circulating catecholamines and the nonsynaptic adrenergic receptors in the uterus that have lost innervation [27]. It is suggested that there is a sensitive balance between the synaptic and the nonsynaptic adrenergic control of the uterine function in rats during pregnancy. The relative lack of sympathetic nervous control of the uterus motor function may promote the importance of nonsynaptic receptorial control toward term and possibly near implantation.

Some previous studies have discussed the possible factors mediating pregnancy-induced uterine denervation, and it seems likely that the fetuses themselves (as physical stretch) [9, 10] and high progesterone levels [28] both may play considerable yet not unique roles in the process. The physiologic significance of adrenergic nerve degeneration is still enigmatic, but it is conceivable that diminution of the adrenergic innervation reduces the contractile responses of the pregnant myometrium elicited by different neurogenic stimuli, such as reflexes evoked by mechanical or chemical stimulation (e.g., infection) of the urogenital organs and mediated, in part, by sympathetic efferent pathways [29–31]. The suppression of nerve-induced contractions as a result of sympathetic nerve degeneration in late pregnancy may contribute to the maintenance of pregnancy by sheltering the fetus in the uterus from possible noxious stimuli. Our findings increase understanding of the temporal constraints of the alterations in adrenergic nervous and receptorial control on uterine contractile activity in the course of pregnancy.

## REFERENCES

1. Sjöberg NO. New considerations on the adrenergic innervation of the cervix and uterus. Acta Obstet Gynecol Scand 1969; 48:28–32.
2. Hollingsworth M. The innervation of the rat cervix and its pharmacology in vitro and in vivo. Br J Pharmacol 1974; 52:539–547.
3. Stjernquist M, Owman C. Cholinergic and adrenergic neural control of smooth muscle function in the non-pregnant rat uterine cervix. Acta Physiol Scand 1985; 124:429–436.
4. Papka RE, Cotton JP, Traurig HH. The comparative distribution of neuropeptide tyrosine-, vasoactive intestinal polypeptide-, substance P-immunoreactive, acetylcholinesterase-positive and noradrenergic nerves in the reproductive tract of the female rat. Cell Tissue Res 1985; 242:475–490.
5. Shew RL, Papka RE, McNeill DL. Galanin and calcitonin gene-related peptide immunoreactivity in nerves of the rat uterus: localization, colocalization, and effects on uterine contractility. Peptides 1992; 13: 273–279.

6. Stjernquist M, Alm P, Ekman R, Owman C, Sjöberg NO, Sundler F. Levels of neural vasoactive intestinal polypeptide in rat uterus are markedly changed in association with pregnancy as shown by immunocytochemistry and radioimmunoassay. Biol Reprod 1985; 33: 157–163.
7. Traurig H, Saria A, Lembeck F. Substance P in primary afferent neurons of the female rat reproductive system. Naunyn Schmiedeberg's Arch Pharmacol 1984; 326:343–346.
8. Moustafa FA. Changes in cholinergic and noradrenergic nerves in the pregnant and postpartum uterus of the albino rat and guinea pig. Acta Anat (Basel) 1988; 132:310–316.
9. Haase EB, Buchman J, Tietz AE, Schramm LP. Pregnancy induced uterine neuronal degeneration in the rat. Cell Tissue Res 1997; 288: 293–306.
10. Thorbert G, Alm P, Owman C, Sjoberg NO, Sporrong B. Regional changes in structural and functional integrity of myometrial adrenergic nerves in pregnant guinea pig, and their relationship to the localization of conceptus. Acta Physiol Scand 1978; 103:120–131.
11. Alm P, Owman C, Sjoberg NO, Thorbert G. Uptake and metabolism of [$^3$H]norepinephrine in the uterine nerves of pregnant guinea pig. Am J Physiol 1979; 236:C277–C285.
12. Wikland M, Lindblom B, Dahlstrom A, Haglid KG. Structural and functional evidence for the denervation of human myometrium during pregnancy. Obstet Gynecol 1984; 64:503–509.
13. Morizaki N, Morizaki J, Hayashi RH, Garfield RE. A functional and structural study of the innervation of the human uterus. Am J Obstet Gynecol 1989; 160:218–228.
14. Kostrzewa RM, Jacobovitz DM. Pharmacological actions of 6-hydroxydopamine. Pharmacol Rev 1974; 26:199–288.
15. Torre JC, Surgeon JW. A methodological approach to rapid and sensitive monoamine histofluorescence using a modified glyoxylic acid technique: the SPG method. Histochemistry 1976; 49:81–93.
16. Garfield RE. Structural studies of innervation on nonpregnant rat uterus. Am J Physiol 1986; 251:41–54.
17. Li Z, Krause DN, Doolen S, Duckles SP. Ovariectomy eliminates sex differences in rat tail artery response to adrenergic nerve stimulation. Am J Physiol 1997; 272:1819–1825.
18. Phillips JK, McLean AJ, Hill CE. Receptors involved in nerve-mediated vasoconstriction in small arteries of the rat hepatic mesentery. Br J Pharmacol 1998; 124:1403–1412.
19. de Haas JR, Terpstra JS, van der Zwaag M, Kockelbergh PG, Roffel AF, Zaagsma J. Facilitatory beta2-adrenoceptors on cholinergic and adrenergic nerve endings of the guinea pig trachea. Am J Physiol 1999; 276:420–425.
20. Manzini S, Perretti F, Tramontana M, Del Bianco E, Santicioli P, Maggi CA, Geppetti P. Neurochemical evidence of calcitonin gene-related peptide-like immunoreactivity (CGRP-LI) release from capsaicin-sensitive nerves in rat mesenteric arteries and veins. Gen Pharmacol 1991; 22:275–278.
21. Maggi CA, Giuliani S, Patacchini R, Santicioli P, Theodorsson E, Barbanti G, Turini D, Giachetti A. Tachykinin antagonists inhibit nerve-mediated contractions in the circular muscle of the human ileum. Involvement of neurokinin-2 receptors. Gastroenterology 1992; 102:88–96.
22. Fernandes LB, Hubbard WC, Undem BJ. Release of inflammatory mediators from guinea pig trachea by electrical field stimulation: lack of neuronal involvement. J Pharmacol Exp Ther 1994; 270:1166–1170.
23. Matsumoto S. The changes in pulmonary C fiber activity and lung mechanics induced by vagal stimulation in rabbits. Life Sci 1997; 61: 2189–2195.
24. Sporrong B, Alm P, Owman C, Sjoberg NO, Thorbert G. Ultrastructural evidence for adrenergic nerve degeneration in the guinea pig uterus during pregnancy. Cell Tissue Res 1978; 195:189–193.
25. Stein BA, Shaw TJ, Turner VF, Murphy CR. Increased lectin binding capacity of trophoblastic cells of late day 5 rat blastocysts. J Anat 1994; 185:669–672.
26. Winkelmann A, Spornitz UM. Alkaline phosphatase distribution in rat endometrial epithelium during early pregnancy: a scanning electronmicroscopic study. Acta Anat (Basel) 1997; 158:237–246.
27. Gáspár R, Márki Á, Zupkó I, Falkay G. Evidence of non-synaptic regulation of postpartum uterine contractility in the rat. Life Sci 1998; 62:1119–1124.
28. Owman C. Pregnancy induces degenerative and regenerative changes in the autonomic innervation of the female reproductive tract. Ciba Found Symp 1981; 83:252–279.
29. Prud'Homme MJ, Rousseau JP. Study of uterine motor responses to vaginal and uterine stimulations in ewe in estrus. Reprod Nutr Dev 1982; 22:597–610.
30. Suzuki H, Okawa T, Endo C, Hoshi K, Sato A. Influence of endotoxin sensitivity to oxytocin in pregnant rat myometrium. Nippon Sanka Fujinka Gakkai Zasshi 1995; 47:377–384.
31. Katsuki Y, Kaga N, Kakinuma C, Takagaki K, Kajikawa S, Shibutani Y. Ability of intrauterine bacterial lipopolysaccharide to cause in situ uterine contractions in pregnant rabbits. Acta Obstet Gynecol Scand 1997; 76:26–32.