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Neural regulation of uterine contractility in the rat: the role of adrenergic and sensory nerves

Ph.D. Thesis

by

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Full papers and abstracts related to the Ph.D. thesis

I. **Klukovits A.**, Mike L., Gáspár R., Falkay Gy.: Az adrenerg denerváció hatása terhes patkány uterusz kontraktilitására *in vitro*. Acta Pharmaceutica Hungarica 2001; 71:181-186.

II. **A. Klukovits**, R. Gáspár, P. Sántha, G. Jancsó, G. Falkay: Functional and histochemical characterization of a uterine adrenergic denervation process in pregnant rats. Biology of Reproduction 2002; 67:1013-1017.

III. **A. Klukovits**, R. Gáspár, P. Sántha, G. Jancsó, G. Falkay: Role of capsaicin-sensitive nerve fibers in uterine contractility in the rat. Biology of Reproduction 2004; 70:184-190.

IV. **Klukovits A.**, Gáspár R., Jancsó G., Falkay Gy: A terhességi adrenerg denerváció funkcionális és morfológiai jellemzése patkány uterusában. A Magyar Élettani Társaság LXVI. Vándorgyűlése, Szeged, 2001. június 6-8.

V. **A. Klukovits**, L. Mike, G. Jancsó, R. Gáspár, G. Falkay: Characterization of pregnancy-induced alterations in uterine contractility in rats: a pharmacological and histochemical analysis. Fundamental and Clinical Pharmacology 2001; 15 (Suppl 1).

VI. Gáspár R., Ducza E., Márki Á., **Klukovits A.**, Falkay Gy.: Non-synapticus adrenerg receptorok jellemzése és farmakológiai reaktivitása terhes patkány uterusban. Magyar Tudomány Napja, Magyar Tudományos Akadémia, Budapest, 2001. november 8.

VII. **Klukovits A.**, Gáspár R., Sántha P., Jancsó G., Falkay Gy: Capsaicin-szenzitív érző rostok funkcionális jelentősége patkány uterusban. Magyar Kísérletes és Klinikai Farmakológiai Társaság V. Kongresszusa, Debrecen, 2002. december 12-14.

VIII. **A. Klukovits**, R. Gáspár, P. Sántha, G. Jancsó, G. Falkay: The modulation of uterine contractile activity by capsaicin-sensitive afferent nerves. 4th Congress of the Federation of European Physiological Societies, 28 June – 3 July, 2003, Nice, France.

IX. **A. Klukovits**, E. Ducza, I. Földesi, G. Falkay: Tocolysis with β_2-adrenoceptor agonists induce oxytocin receptor up-regulation in the late-pregnant rat uterus in vivo and in vitro. Acta Pharmaceutica Turcica 2002; 44 (Suppl).

LIST OF ABBREVIATIONS

Symbols and abbreviations are in accordance with the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature: Nomenclature and symbolism for Amino Acids and Peptides (J Biol Chem 1984; 219: 345-373)

ATP	adenosine triphosphate
CGRP	calcitonin gene-related peptide
GAL	galanin
MLCK	myosin light chain kinase
NGF	nerve growth factor
NKA	neurokinin A
NPY	neuropeptide Y
OT	oxytocin
SP	substance P
SR	sarcoplasmic reticulum
TRPV	vanilloid-receptor-related channel of the transient receptor potential (TRP) superfamily ion channels
VIP	vasoactive intestinal polypeptide
VR	vanilloid receptor

1. INTRODUCTION

1.1. General characteristics of uterine smooth muscle function

The smooth muscle of the uterus, the myometrium belongs to a group of smooth muscles that are spontaneously active. The uterine smooth muscle is characterized by a relative quiescence throughout gestation until the onset of labor. At this time powerful contractions and the dilation of the uterine cervix occur which result in the expulsion of the fetus and the placenta. These tasks, however, are not always performed perfectly, resulting in premature labor or uterine dystocia [Wray, 1993]. Since premature labor is still considered to be the leading cause of neonatal morbidity and mortality [Moore, 2003], a better understanding of the factors affecting uterine contractility would further promote the development of novel strategies in the treatment of this condition.

The control of uterine smooth muscle function is of vital importance during pregnancy and parturition. Mechanisms involving neural, hormonal, metabolic and mechanical factors underlie the control of myometrial activity. In the light of the growing body of experimental data on uterine functions the mechanisms contributing to the modulation of uterine contractile activity are beginning to be understood.

1.1.1. Cellular mechanisms of uterine contractility

The cellular basis of uterine contractility is the interaction between actin and myosin in myometrial smooth muscle cells [Murphy, 1979]. This is driven by calcium through Ca^{2+}-calmodulin-dependent myosin light chain kinase (MLCK) activity [Moore and Bernal, 2001]. The myometrial contractions are primarily dependent on the presence of extracellular Ca^{2+}: the Ca^{2+}-influx via receptor-operated channels and subsequent entry through voltage-gated channels are probably the most important mechanisms of the rise of intracellular Ca^{2+} concentration [Wray et al., 2001]. Uterine activity can be modulated by many G-protein coupled receptors (GPCRs): the agonists binding to uterine catecholamine (α_1) [Ducza et al., 2002; Mhaouty-Kodja et al., 2001] , bradykinin (BK_2) [Figueroa et al., 2001], histamine (H_1) [Gonzalez et al.,1994], oxytocin [Gimpl and Fahrenholz, 2001] or vasopressin (V_1) receptors [Akerlund et al., 1995] elicit contractions by activating the phospholipase C / inositol 1,4,5-

trisphosphate / diacyl glycerol / Ca^{2+} pathway, resulting in the increase in MLCK activity and the phosphorylation of myosin.

A considerable amount of Ca^{2+} is also released from the sarcoplasmic reticulum (SR). The uterine SR has both ryanodine receptors and inositol 1,4,5-trisphosphate receptors. However, the receptorial activation of the SR and the subsequent release of the stored Ca^{2+} probably play little role in the initiation of contractions in either the human or the rat uterus. The release of Ca^{2+} from the SR may lead to the activation of membrane K^+ channels, which tends to decrease calcium entry through L-type Ca^{2+} channels thereby reducing contractions. Thus the SR is playing a role in controlling membrane excitability and hence contractility [Shmigol et al., 1999].

1.2. Neural regulation of uterine contractility

1.2.1. The autonomic innervation of the rat uterus

The rat uterus is densely innervated by autonomic postganglionic nerve fibers. These fibers take their origin from the pelvic plexus and run directly to the genital organs via the hypogastric and the pelvic nerves [Papka et al., 1999; Sato et al.,1989; Baljet and Drukker, 1980]. These nerves have different innervation territories: the uterine horns are mostly innervated by the hypogastric nerve, while the cervix and the vaginal canal are mostly supplied by the pelvic nerve [Berkley et al., 1993a]. Hypogastric and pelvic nerves carry mainly sympathetic and parasympathetic postganglionic axons, respectively. The sympathetic fibers contain noradrenaline as a principal transmitter. The varicose unmyelinated noradrenergic fibers innervate predominantly the blood vessels via axons running in the adventitia, but many of them course freely into the myometrial and the endometrial layers [Berkley et al., 1993b]. They can be demonstrated either by catecholamine fluorescence histochemistry using paraformaldehyde gas and/or glyoxylic acid [Torre and Surgeon, 1976] or by the immunohistochemical localization of tyrosine hydroxylase, the rate-limiting enzyme of catecholamine synthesis [Papka et al., 1985].

Immunohistochemical studies revealed the coexpression of neuropeptide Y (NPY) and of ATP with noradrenaline in the sympathetic nerves of the reproductive tract of the female rat [Fried et al., 1986; Lundberg et al., 1982]. Noradrenaline released from perivascular or myometrial sympathetic fibers may elicit vasoconstriction via α_1 receptors located on vascular smooth muscle cells and may lead to smooth muscle contraction or relaxation depending on

the α_1/β_2 receptor ratio on the myometrial cells. NPY and ATP are both potent smooth muscle contracting agents. NPY exerts its effect via its own receptors on myometrial cells, while the contractile effects of ATP are possibly mediated either directly via P2Y purinergic receptors or indirectly via an increase in prostaglandine synthesis [Aitken et al., 2001].

The influence of parasympathetic fibers on uterine contractility was discussed by some authors, who concluded that parasympathetic nerves probably are of minor importance in the regulation of uterine motor activity in the rat, whereas the excitatory cholinergic innervation represents a major motor component of the rat cervix [Hollingsworth, 1974, Stjernquist et al., 1985; Stjernquist and Owman 1985]. Besides, many uterine cholinergic nerves are associated with blood vessels, and their stimulation results in marked vasodilation via activation of muscarinic cholinergic receptors.

1.2.2. The pregnancy-induced sympathetic denervation phenomenon

During pregnancy, the uterus of most mammals undergoes a profound denervation process. Although this neuroplastic change in the innervation pattern of the pregnant uterus is known for a long time, the exact mechanism and time course of this phenomenon are not yet fully understood. Recent investigations revealed an important role of sexual steroid hormones in the regulation of neural noradrenaline level and an effect on the morphology of sympathetic nerves. Further, ovarian steroid hormones have been shown to influence the innervation of the uterus; the presence of estrogen and progesterone receptors on autonomic and sensory nerves supports this notion and point to the possible sites of action [Papka et al., 1997].

At the end of pregnancy, the numbers of both myometrial and perivascular adrenergic nerves are markedly decreased in the rat [Haase et al., 1997], in the guinea pig [Moustafa, 1988; Thorbert et al., 1978], and in humans [Wikland et al., 1984]. At advanced pregnancy the noradrenaline content is significantly reduced in various portions of the rat uterus, both in the vicinity of and distant from the fetuses. Electron microscopy disclosed that degeneration of adrenergic axons underlie these changes in tissue noradrenaline content [Sporrong et al., 1978]. Several factors involving hormonal effects, physical stretching of the myometrium by the developing fetus(es) and changes in uterine nerve growth factor (NGF) content have been suggested to contribute to this degeneration process [Varol et al., 2000]. Despite of the increase in the total content of NGF in the pregnant uterus, it is conceivable that the increase of NGF synthesis lags behind the growth of the uterus, resulting in a relative decrease in tissue NGF concentration. Hence, the sympathetic terminals are possibly not supplied with adequate

amounts of trophic factors or, alternatively, the responsiveness to NGF is reduced. While certain type of neurons remains morphologically and functionally unchanged during pregnancy, an altered sensitivity of sympathetic fibers to NGF should be considered.

1.2.3. Sensory innervation of the uterus: afferent and efferent functions

Uterine sensory nerves may be stimulated by intense mechanical and chemical stimuli which produce pain. The afferent nerves of the uterus are involved in the mediation of nociceptive impulses as well as in reflex actions involving the uterine cervix at labor. These unmyelinated C- and Aδ-type sensory fibers of the uterus and the cervix also travel in the hypogastric and pelvic nerves [Berkley et al., 1988]. The physiological role of sensory nerves is not limited to their "afferent function", i.e. the transmission of noxious information from the periphery to the central nervous system. They also possess an "efferent or local regulatory" function through the release of a variety of neuropeptides involving substance P (SP), calcitonin gene-related peptide (CGRP), neurokinin A (NKA), and galanin (GAL) [Patak et al., 2000; Shew et al., 1992; Shew et al., 1991]. In most visceral organs, the stimulation of sensory nerves – via the release of these neuropeptides - may evoke significant alterations in smooth muscle contractile activity [Anderson and Hedlund, 2002; Giuliani et al., 2001; Baluk, 1997; White, 1997; Lundberg et al., 1992].

In vivo and in vitro studies showed that SP, NKA, and GAL elicited contraction [Crane et al., 2002; Shew et al., 1992; Shew et al., 1991], whereas CGRP [Anouar et al., 1998; Pennefather et al., 1990] caused relaxation of the uterine smooth muscle in nonpregnant rat uteri. Although the effects of the individual neuropeptides are well described, sensory nerve stimulation may result in a masked response due to the co-release of neuropeptides from uterine sensory nerve terminals. The co-release of CGRP with NKA, SP and/or GAL is a common feature occurring in response to the stimulation of sensory nerve fibers and it is an important determinant of the functional response. The relative prevalence of the smooth muscle relaxing and contracting neuropeptides defines the type of response which commences after stimulation of sensory nerves. Assuming that neurotransmitters are released from the same nerve in a relatively constant amount, the final response may depend on the relative amount stored in nerve endings, the affinity of the peptides released and the number of the postsynaptical receptors and also transductional factors [Giuliani et al., 2001]. While these factors show regional differences, the motor responses as a result of sensory nerve stimulation may vary extensively.

The neuropeptides listed above are also believed to play an important role in initiating the cascade of neurogenic inflammation [Jancsó et al., 1980; Jancsó, 1960]. It is generally accepted that SP released from the stimulated sensory nerve endings opens endothelial gaps and causes plasma extravasation via an action on NK1 receptors [McDonald, 1994]. In addition, SP may also acivate mastocytes resulting in the release of mast cell mediators [Holzer, 1992]. The predominant action of CGRP is vasodilation [Brain et al., 1985]. There are several feedback mechanisms in the inflammatory process: for example mast cells liberate histamine, which besides causing smooth muscle contraction, also stimulates sensory nerve endings resulting in further release of SP. Such interactions may be particularly interesting as regards the uterus, where sensory nerve activation can be elicited by several local factors resulting in significant modulation of uterine contractility.

During pregnancy, certain types of sensory nerve fibers in the rat uterus may undergo degeneration [Anouar et al., 1998; Berkley et al., 1993b]. However, there is some controversy as to the nature and extent of these changes. Some immunocytochemical studies have indicated an indiscriminate and practically complete loss of all uterine neuropeptides in full-term pregnant guinea pigs [Alm and Lundberg, 1988], while others provided evidence for the presence of SP- and CGRP-containing sensory nerves in term rats [Traurig et al., 1984]. It has also been suggested that uterine nerves contribute to the implantation process and the maintenance of pregnancy and they may play a role in the increase of uterine contractility and in cervical ripening around term. Recent findings suggested that at late gestation the stimulation of primary afferents in the rat cervix may lead to some tissue rearrangement as a result of the neuropeptide release and action, thus sensory nerves and their transmitters could be part of a neurogenic inflammatory process involved in cervical ripening [Collins et al., 2002; Garfield et al., 1998]

1.2.4. The effect of capsaicin on sensory nerves

A large population of primary sensory neurons are sensitive to the excitatory effect of capsaicin and related compounds referred to as vanilloids. Capsaicin, the active ingredient in hot pepper (Capsicum species) selectively activates neurons expressing the capsaicin/vanilloid VR1/TRPV1 receptor [Szallasi and Blumberg, 1999; Caterina et al., 1997], a nonselective cation channel with a preference for Ca^{2+}. Since the discovery of its selective neurotoxic action, this natural vanilloid has been used routinely to identify capsaicin-sensitive pathways and explore their contributions to physiological and pathological regulatory processes [Jancsó,

1960; Jancsó et al., 1977; Buck and Burks, 1986; Holzer, 1991a]. Capsaicin-sensitive afferent nerves have been demonstrated by immunohistochemistry in the uterus of several species including the rat [Collins et al., 2002; Alm and Lundberg, 1988; Franco-Cereceda et al., 1987]. A significant proportion of these nerves contain SP, CGRP, NKA, and GAL. Although the effects of these sensory neuropeptides on uterine smooth muscle activity are well established, the possible participation of capsaicin-sensitive afferent nerves in the modulation of uterine contractility has not been dealt with in detail, yet.

2. AIMS OF THE STUDY

1. The aim of the present study was to investigate the time course of pregnancy-induced changes in the contractile responses of isolated uterine rings and in the sympathetic innervation pattern, using electric field stimulation and histofluorescence techniques, respectively. The functional consequences of adrenergic denervation on myometrial contractility was studied on days 5, 10, 15, 18, and 22 of pregnancy. The uterine adrenergic nerve function was characterized by measuring uterine contractile responses under in vitro conditions using electric field stimulation.

2. Experiments were also initiated in an attempt to reveal a possible role of capsaicin-sensitive peptidergic sensory nerves in the modulation of uterine contractility in non-pregnant and term pregnant rats. Selective nerve-induced contractions were elicited and the effect of chemodenervation by capsaicin on the contractile responses of uterine preparations was examined. Immunhistochemistry was applied to localize CGRP in intact and capsaicin treated uteri in order to demonstrate the possible depletion of CGRP from capsaicin-sensitve afferent nerves which innervate the uterine blood vessels and the myometrium.

3. MATERIALS AND METHODS

The experiments were carried out with the approval of the Ethical Committee for Animal Research, University of Szeged (Registration number: I-74-8/2002).

3.1. Animals and mating

Sexually mature female Sprague-Dawley rats (body mass: 180-240 g, 60-80 days old) were used in these experiments. Nonpregnant rats were cyclic virgin rats in diestrus phase, held in separate cages from males from the 3rd week after birth. Diestrus phase was detected by taking vaginal smears in the early morning hours; the dominance of leucocytes and mucus in the smear is typical of this phase.

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 and the day of conception was considered to be the first day of pregnancy. Animals were given water and food ad libitum and they were housed in temperature- (20-22 °C) and humidity-controlled (40-50%) rooms with a 12h light/dark cycle.

3.2. Adrenergic innervation

3.2.1. Animals

The uterine adrenergic innervation was studied in nonpregnant and pregnant rats. Tissue samples from pregnant rats were obtained on days 5, 10, 15, 18, and 22 (term) of pregnancy.

3.2.2. Electric field stimulation evoked contractile responses of isolated rat uteri

The rats were killed by cervical dislocation at 10 a.m. and the uteri were removed and trimmed of fat; in uteri obtained from pregnant rats the feto-placental units were removed. The uterus was immediately placed in an organ bath (de Jongh's solution containing, in mM: NaCl 137, KCl 3, $CaCl_2$ 1, $MgCl_2$ 1, $NaHCO_3$ 12, Na_2HPO_4 4 and glucose 6; pH 7.4), perfused with 95% oxygen and 5% carbon dioxide. The temperature of the organ bath was maintained at 37°C. Rings 1 cm in length were cut from the middle part of each horn, including

implantation sites in the case of pregnant uteri, and mounted vertically between two platinum electrodes in the organ bath. After mounting, the uterine rings were equilibrated for 90 min before the start of the stimulation. The initial tension was set at 1.5 g. After the incubation period, selective nerve stimulation was elicited by a digital, programmable stimulator (ST-02, Experimetria Ltd., London, U.K.). The square pulse duration was set at 0.6 ms and the supramaximal voltage at 40 V.

Rings were stimulated by 1-min trains of square pulses with the following frequencies, in increasing order: 1; 2.5; 5; 10; 20; 30; 50; 70 Hz. Each 1-min stimulation was followed by a 4-min recovery period, whereafter a higher frequency was applied. The tension of the myometrial rings was measured with a strain gauge transducer (SG-02, Experimetria Ltd., London, U.K.) and recorded and analyzed by ISOSYS Data Acquisition System (Experimetria Ltd., London, U.K.).

3.2.3. Effect of the α-adrenergic receptor antagonist phentolamine on uterine contractility

In these series of experiments uterine preparations were stimulated as described above, but in the presence of 1 μM phentolamine.

3.2.4. Histofluorescence demonstration of uterine adrenergic innervation

Nonpregnant and pregnant rats were used in these experiments. Pregnant rats were sacrificed on days 5, 10, 15, 18 or 22 of pregnancy. The animals were killed by cervical dislocation and were bled. After a midline abdominal incision, the uteri were exposed and removed. The tissue samples from the medial 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 3s in a solution of 0.1 M glyoxylic acid containing in mM: KH_2PO_4 235 and sucrose 200, pH 7.4. 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 vapour at 80 °C for 60 min. The preparations were examined under a Leica DMLB fluorescence microscope (Wetzlar, Germany) equipped with appropriate filters.

3.3. Capsaicin-sensitive sensory innervation

3.3.1. Animals

The uterine capsaicin-sensitive nerve fibers were studied in intact nonpregnant, intact 22-day pregnant rats, and in capsaicin-treated nonpregnant rats.

3.3.2. Capsaicin treatment

Capsaicin was administered to adult nonpregnant female rats in three subcutaneous injections at doses of 10, 20 and 100 mg/kg on days 1, 2 and 4, respectively, under ether anesthesia. If necessary, terbutaline spray and respiratory assistance was applied. The experiments were taken four days after the last dose.

3.3.3. Uterus preparation and electric field stimulation parameters

Isolated uterine ring preparations were prepared as described above. The preparations were stimulated with square wave pulses of 0.6 ms duration at a supramaximal voltage of 40 V. The rings were stimulated by 30-s trains of square pulses with the following frequencies, in increasing order: 1; 2.5; 5; 10; 20; 30; 50 and 70 Hz. Each 30s stimulation period was followed by a 2-min recovery period, whereafter a higher frequency was applied. The contractions elicited by the first series of stimulations were regarded as the control responses. The tissue samples were washed thoroughly and then incubated for 20 min with capsaicin at final concentrations of 0.1-10 μM. Thereafter, the uterine rings were washed and stimulated again with the same series of frequencies (1-70 Hz). The tension of the myometrial rings was measured with a strain gauge transducer and recorded and analyzed by ISOSYS Data Acquisition System. The changes of the amplitude of the contractions after the exposure to capsaicin were expressed as the percentage of the corresponding control responses.

3.3.4. Effect of a CGRP-antagonist on uterine contractility

In these series of experiments uterine preparations were stimulated as described above except that capsaicin was omitted. The second series of stimulations were performed in the presence of the $CGRP_1$ receptor subtype antagonist, hCGRP(8-37) at final concentrations of 1-10 μM.

3.3.5. Immunohistochemistry

Uterine rings were placed in an organ bath containing de Jongh's solution perfused with 95% oxygen and 5% carbon dioxide, at 37°C. Thirty min later, capsaicin was added at final concentrations of 1-10 μM. Solvent-treated specimens served as controls. After an additional period of 20 min this solution was replaced with fresh physiological solution. Ten min later, tissue specimens were placed in Zamboni's fixative for 2 h at 4°C. The samples were washed in phosphate buffer (0.1 M, pH 7.4) and processed for the demonstration of CGRP-immunoreactivity (-IR) using an indirect immunofluorescence technique. Briefly, cryostat sections 15 μm in thickness were cut and incubated with a rabbit polyclonal CGRP antibody (1:500) overnight. After repeated washing with phosphate buffered saline the sections were incubated with a secondary antibody (goat anti-rabbit IgG conjugated to Cy3) for 1 h at room temperature. After washing in buffer, tissue sections were covered with Cytifluor and examined under a Leica DMLB fluorescence microscope.

3.4. Statistical analysis

All experiments were carried out on at least six animals and values are given as mean ± S.E.M. Bartlett tests revealed the homogenity of variances. One-way ANOVA with Newman-Keuls test was used to compare the amplitudes of contractile responses at each frequency independently. The data were statistically analyzed with the Prism 2.01 (Graph Pad Software Inc., San Diego, CA) computer program.

3.5. Drugs

The drugs used in these experiments were purchased from the following companies: phentolamine, hCGRP(8-37) and glyoxylic acid from Sigma Aldrich Ltd. (Budapest, Hungary), capsaicin from Fluka Chemie AG (Budapest, Hungary), rabbit polyclonal CGRP antibody and the goat anti-rabbit IgG conjugated to Cy3 from Sigma Chemical Co. (St. Louis, MO, USA), Cytifluor from Amersham (Arlington Heights, IL, USA).

4. RESULTS

4.1. Studies on the adrenergic modulation of uterine motor function

4.1.1. Electric field stimulation

Nerve-induced uterine contractions were studied in nonpregnant and pregnant rats. Eight distinct frequency values were established and the presence of a response and its amplitude were detected. **Figure 1** illustrates the neurally-evoked contractions of myometrial preparations obtained from nonpregnant and pregnant rats at gestational ages of 5-, 10-, 15-, 18-, and 22-days. In nonpregnant rats, all the applied frequencies elicited contractions. On day 5 of pregnancy, the uterus did not respond to stimuli at the lowest frequencies of 1 and 2.5 Hz. On day 10, contractions were detected to all applied frequencies, but the amplitudes of the contractions were lower than those observed in the nonpregnant rats. By day 15, contractions could not be evoked by stimulation with 1-5 Hz; the lowest frequency that elicited myometrial contractions in the 15-day pregnant rats was 10 Hz. The uteri of the 18-day pregnant rats did not respond even to stimulation with 10 Hz; contractions were elicited only by stimulations at higher frequencies of 20-70 Hz. By term, the uterus responded only to stimulations at 30-70 Hz, thus contractions could not be evoked at lower frequencies of 1-20 Hz. The examination of the presence of nerve-induced myometrial contractions therefore revealed a tendency of 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 at different gestational ages and that of the nonpregnant uterus 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 as compared to nonpregnant animals throughout the whole frequency range of 1-70 Hz. The uteri of the 15-day pregnant rats responded with the lowest amplitude at 10 and 20 Hz as compared to the nonpregnant uterus at both frequencies. Although we did not indicate it in Fig 1, we note that paired comparisons revealed significantly lower amplitudes on day 15 at 10 Hz ($p<0.05$ vs day 5, and vs day 10), and at 20 Hz ($p<0.05$ vs day 5, 10 and 18).

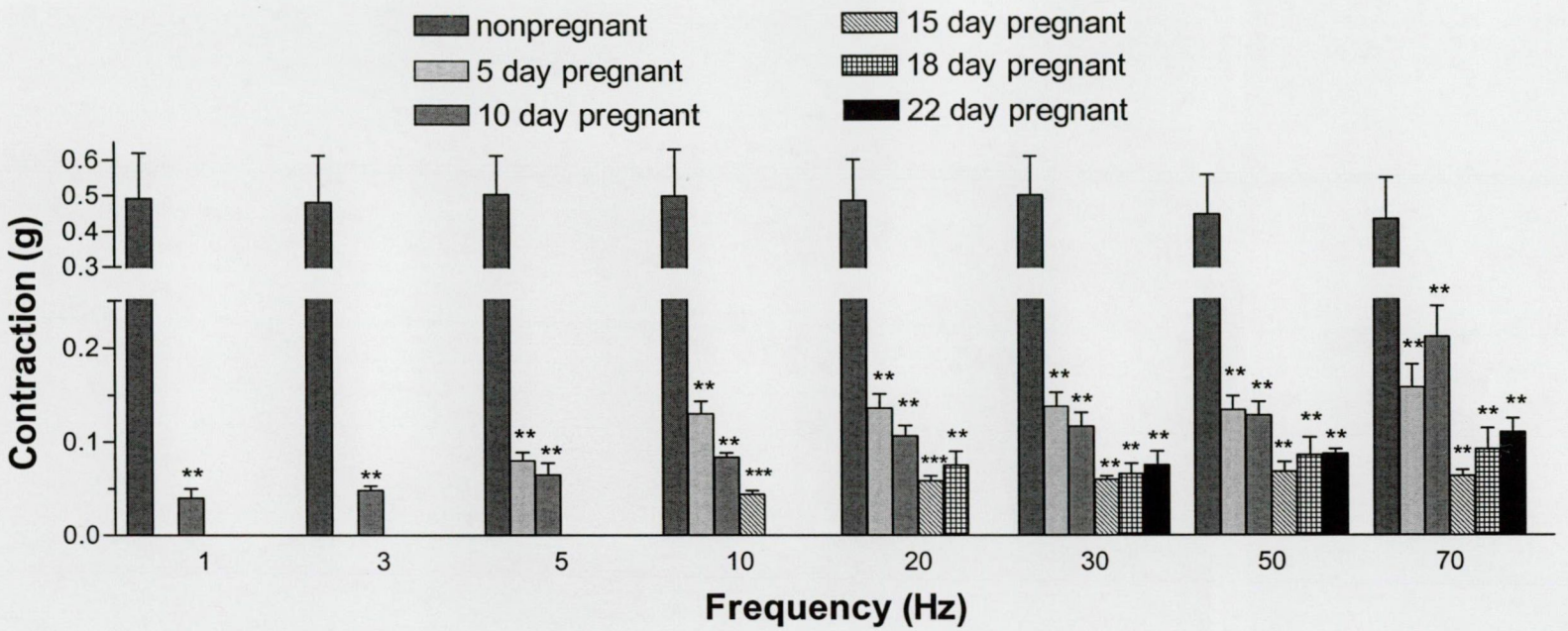

Figure 1. Myometrial contraction responses versus applied stimulating frequency in Herz in nonpregnant and in 5-, 10-, 15-, 18- and 22-day (term) pregnant rats.

Each bar represents the amplitude of contraction expressed in grams, as mean ± S.E.M. (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 (** means $p<0.01$; *** means $p<0.001$).

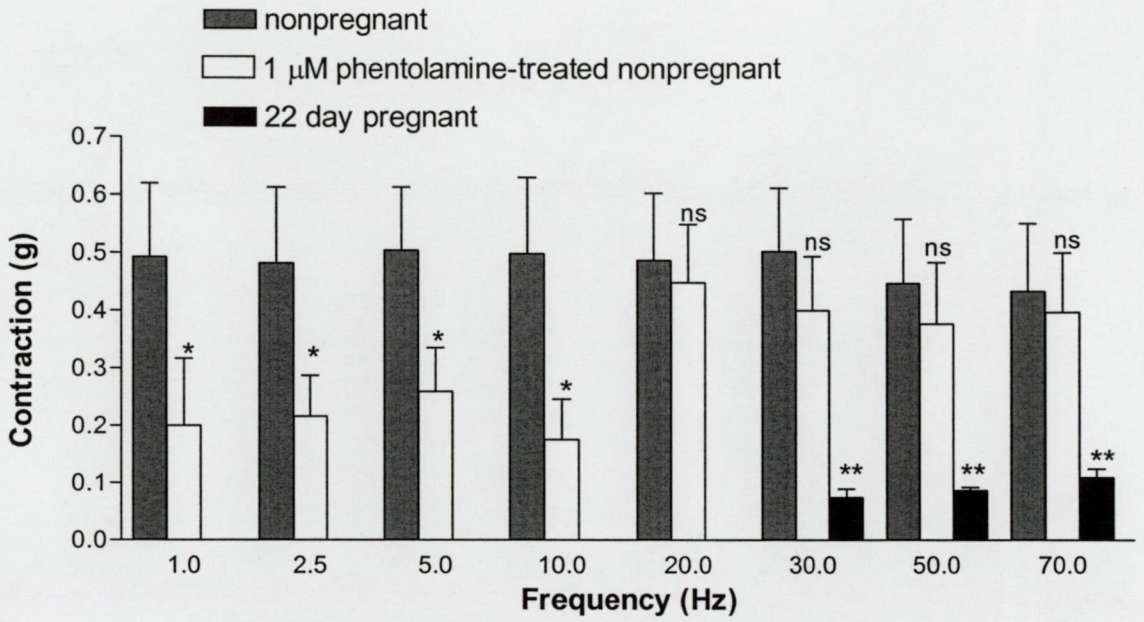

Figure 2. Myometrial contraction responses versus applied stimulating frequency in nonpregnant, in 22-day (term) pregnant and in phentolamine-treated nonpregnant rats.

Each bar represents the amplitude of contraction expressed in grams, as mean ± S.E.M. (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 (** means $p<0.01$ and "ns" means $p>0.05$ thus no significant difference).

The amplitude of contractions evoked by stimulation at 1-10 Hz in nonpregnant rats' uteri in the presence of phentolamine were significantly lower than in intact nonpregnant rats. The uteri of both the 22-day pregnant rats and the nonpregnant rats' uteri treated in vitro with phentolamine did not respond to stimuli lower than 20 Hz (**Figure 2**). In the 22-day pregnant rats, significantly lower amplitudes were detected in response to stimuli of 30-70 Hz than in the intact ($p<0.01$) or in the phentolamine-treated nonpregnant rats ($p<0.01$). In the phentolamine-treated nonpregnant rats' uteri, the amplitudes of contractions to stimuli of 30-70 Hz were not statistically different ($p>0.05$) from those in the intact nonpregnant rats.

4.1.2. Fluorescence histochemistry

Fluorescence histochemistry (**Figure 3**) revealed that in adult nonpregnant rats, the uterine adrenergic nerve fibers display a characteristic topographical distribution; the uterine arteries are richly innervated by varicose adrenergic axons (**Fig 3a**), whereas the uterine smooth musculature was served by a moderate number 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 lost; few if any perivascular nerves could be observed around small arteries. The fluorescent adrenergic nerves were practically absent on day 22; only occasional degenerating axons could be seen (**Fig 3f**).

4.2. Modulation of uterine motor function by capsaicin-sensitive afferent nerves

4.2.1. Electric field stimulation

Figure 4 illustrates the effects of 0.1-10 μM capsaicin administration in vitro on the amplitudes of the neurally-evoked contractions of the isolated nonpregnant rat uteri. At the lowest dose (0.1 μM), the amplitudes increased significantly as compared with the solvent-treated control when the uterine rings were stimulated at 1-50 Hz.

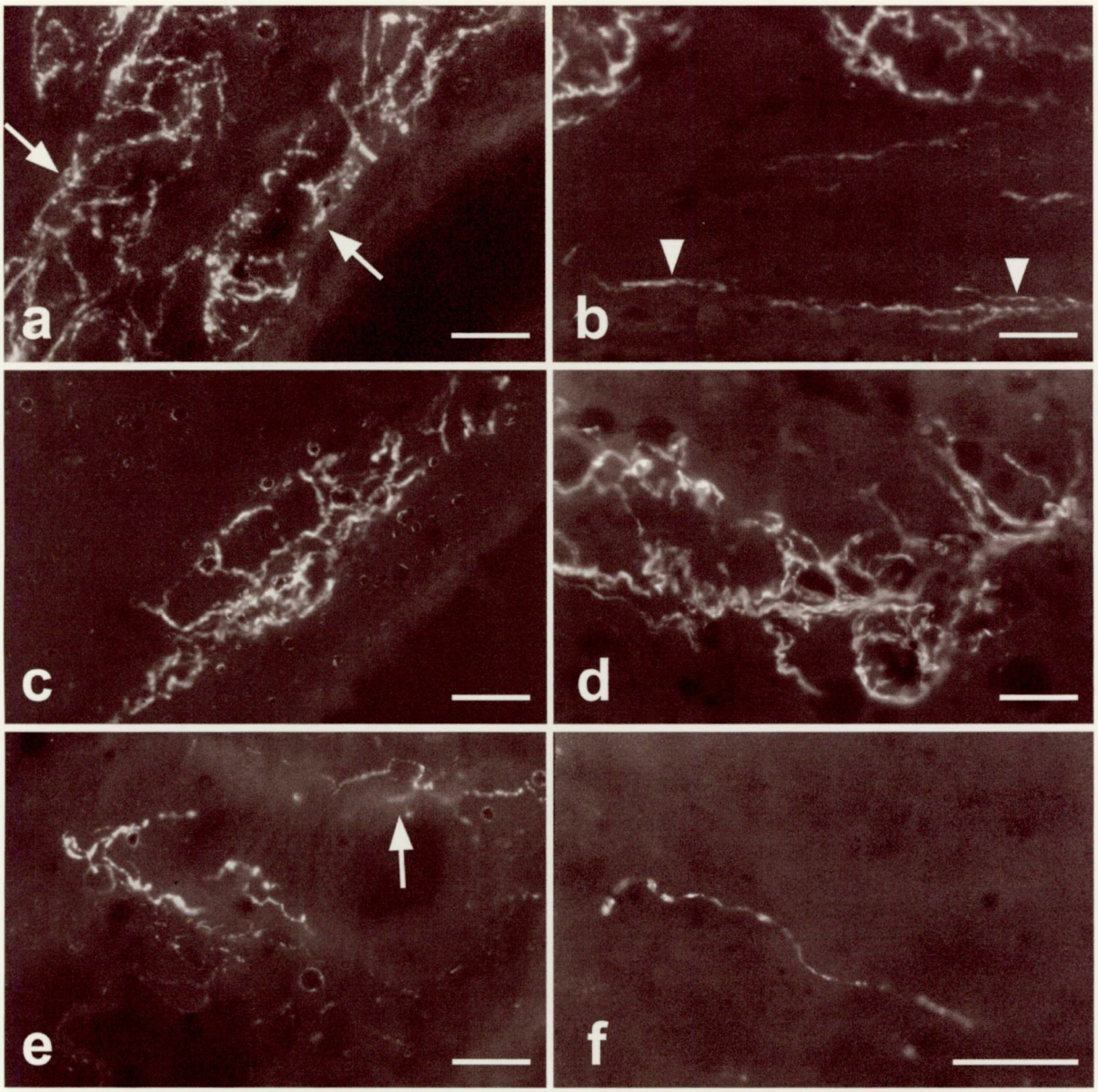

Figure 3. Fluorescence micrographs illustrating uterine innervation in nonpregnant and pregnant rats.

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 10 (**d**) of pregnancy the uterine adrenergic innervation is comparable to 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 scale bars indicate 10 μm.

After incubation with 1 μM capsaicin, the uteri gave significantly greater responses to 1-70 Hz frequency stimulation than those of the samples that were treated with solvent only. Incubation with 10 μM capsaicin did not cause any alteration of contraction responses. The greatest contractions were detected after incubation with 1 μM capsaicin.

In order to exclude the possibility of a direct action of capsaicin on the uterine smooth muscle, a group of nonpregnant rats were pretreated with 130 mg/kg capsaicin and four days after the last day of administration the same experiment was carried out: the effect of 1 μM capsaicin on the uterine contractions in vitro was tested (**Fig 5**). Enhancement of the amplitude of the uterine contractions after incubation with 1 μM capsaicin was not observed when rats were pretreated with capsaicin in vivo. The amplitude of the contractions after incubation with 1 μM capsaicin differed significantly in capsaicin-pretreated nonpregnant rat uteri from that in intact nonpregnant rat uteri.

The effect of the $CGRP_1$-receptor antagonist hCGRP(8-37) was also tested on the neurally evoked uterine contractions (**Fig 6**). After the first series of stimulation, the uterine rings were allowed to rest for 20 min, and then washed, and hCGRP(8-37) was added to the incubation buffer. The second series of stimulation was performed in the presence of 1 or 10 μM hCGRP(8-37). Stimulated at 1-30 Hz, the amplitude of contractions were significantly higher in the presence of hCGRP(8-37) than in the presence of solvent only. The greatest enhancement of the amplitude was observed when the uterine rings were stimulated with 1 Hz in the presence of 10 μM hCGRP(8-37). In that case the alteration in the amplitude of the contraction response was similar to that observed after the uteri were incubated with 1 μM capsaicin. When uteri were stimulated at higher frequencies, the effect of hCGRP(8-37) on the amplitude of contractions was significantly smaller than that of 1 μM capsaicin.

The effect of capsaicin on nerve-induced contractions was also tested in the term pregnant rat uterus (**Fig 7**). Low-frequency stimulation (1-10 Hz) was unable to elicit contractions. The effect of 1 μM capsaicin on the amplitude of contractions was quite similar to that in the nonpregnant uteri: a significant increase of the amplitude was observed at each frequency (20-70 Hz), as compared with the solvent-treated control. On incubation with the lower dose of capsaicin (0.1 μM), the amplitude of the contractions increased significantly in uteri stimulated at 50-70 Hz. When 20-30 Hz was applied, no significant increase of the amplitude was detected. Similarly as in the nonpregnant uteri, incubation with 10 μM capsaicin did not alter the amplitude of the contraction responses.

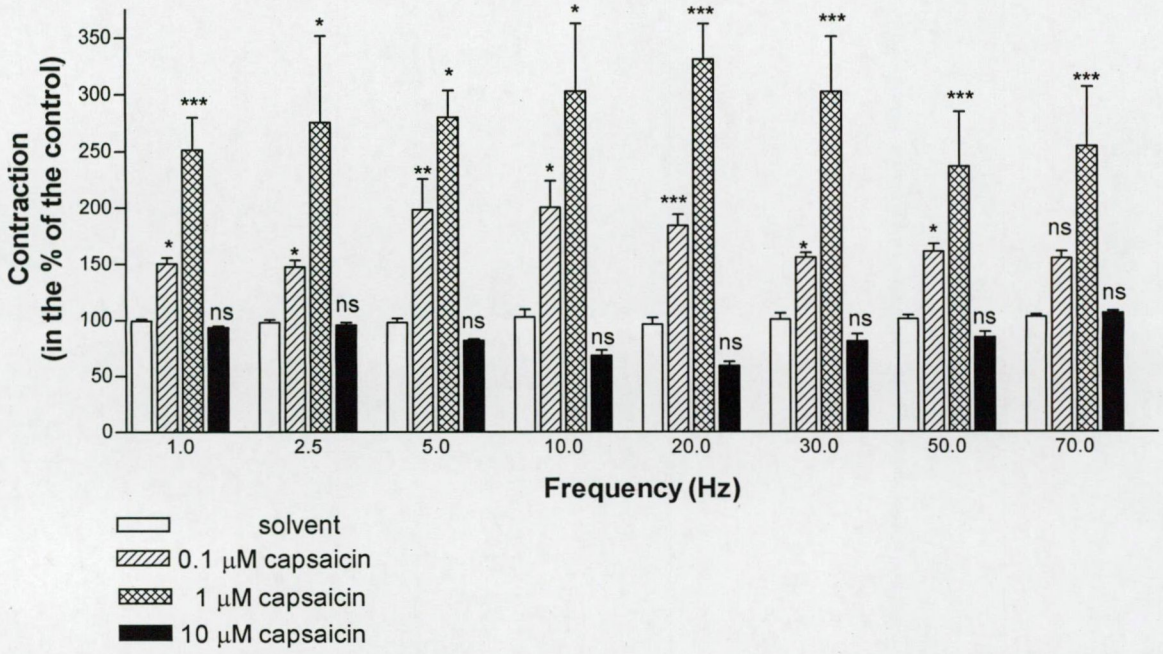

Figure 4. The effect of in vitro capsaicin treatment on the contraction responses evoked by electric field stimulation in isolated uterine rings of nonpregnant rats.

The amplitudes of contractions after a 20-min incubation period with capsaicin are expressed as percentages of the control contractions, elicited at the same frequency. Dose-dependent increase of contractions (0.1-1 μM) is possibly due to partial neuropeptide release, while no change in the amplitude (10 μM) is the consequence of complete destruction of sensory nerve endings by capsaicin. The asterisks indicate the level of significant difference from the solvent treated preparations (* $p<0.05$; ** $p<0.01$; *** $p<0.001$; ns means $p>0.05$, thus no significant difference).

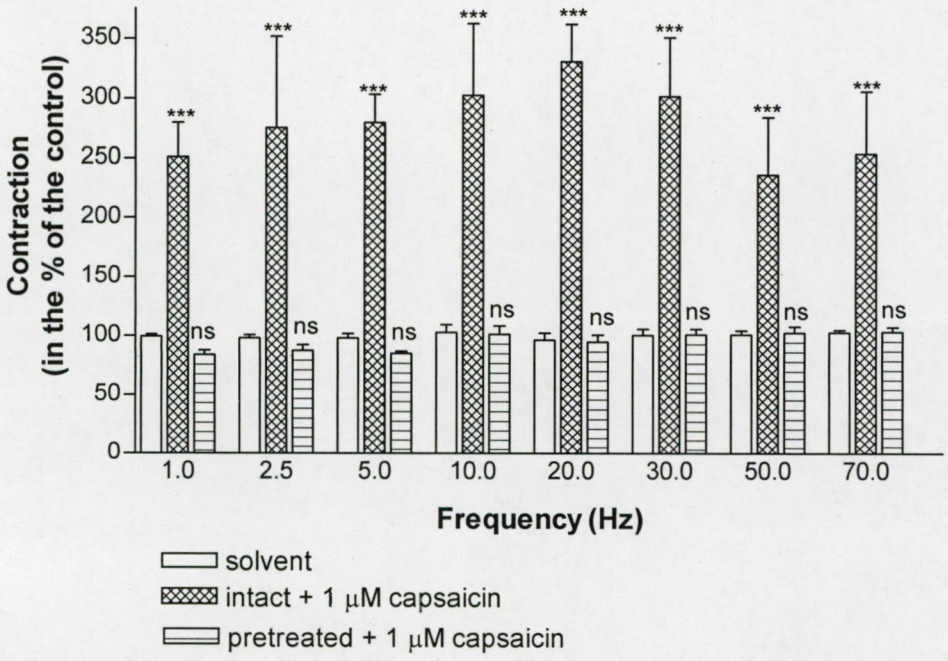

Figure 5. The effects of in vitro 1 μM capsaicin treatment on the contraction responses evoked by electric field stimulation in isolated uterine rings of intact and in vivo capsaicin pretreated (130 mg/kg, subcutaneously) nonpregnant rats.

Prior systemic treatment of the rats with capsaicin abolishes the effect of in vitro capsaicin administration on the amplitude of neurally evoked contractions, suggesting that the effect of capsaicin on uterine contractility is of neural origin. The asterisks indicate the level of significant difference from the solvent treated preparations (*** $p<0.001$; ns means $p>0.05$, thus no significant difference).

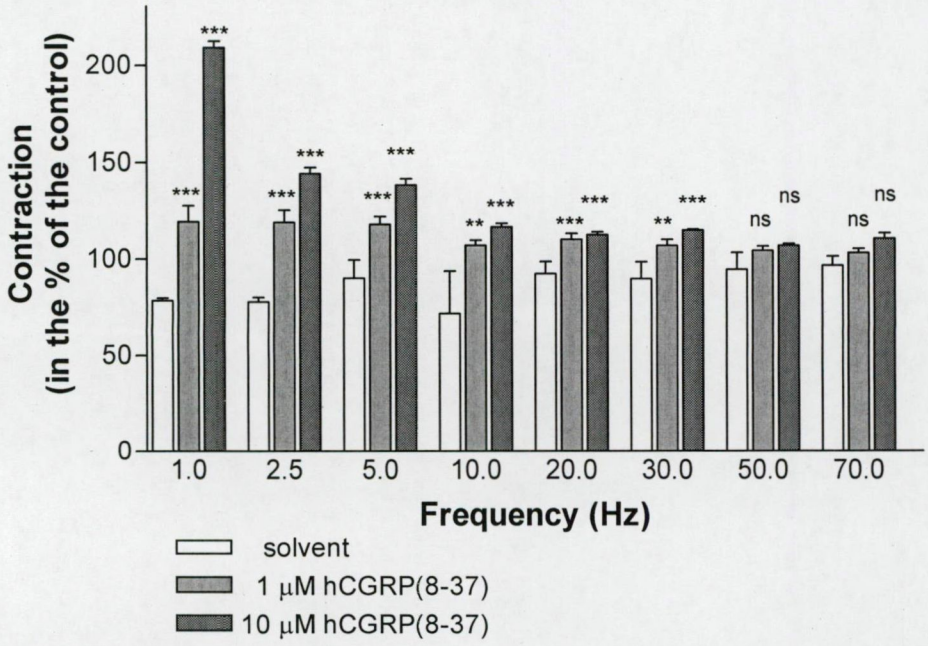

Figure 6. The effect of the CGRP receptor antagonist hCGRP(8-37) on the contraction responses evoked by electric field stimulation in isolated uterine rings of nonpregnant rats.

After a series of control contractions and a 20-min resting period, contraction were evoked by electric field stimulation in the presence of hCGRP(8-37). The amplitudes of contractions in the presence of hCGRP(8-37) are expressed as percentages of the control contractions, elicited at the same frequency. The amplitudes of contractions elicited at 1-30 Hz in the presence of hCGRP(8-37) are significantly higher than in the control, resulting in an augmentation of amplitudes similar to the effects of the in vitro capsaicin treatment. The asterisks indicate the level of significant difference from the solvent treated preparations (** $p<0.01$; *** $p<0.001$; ns means $p>0.05$, thus no significant difference).

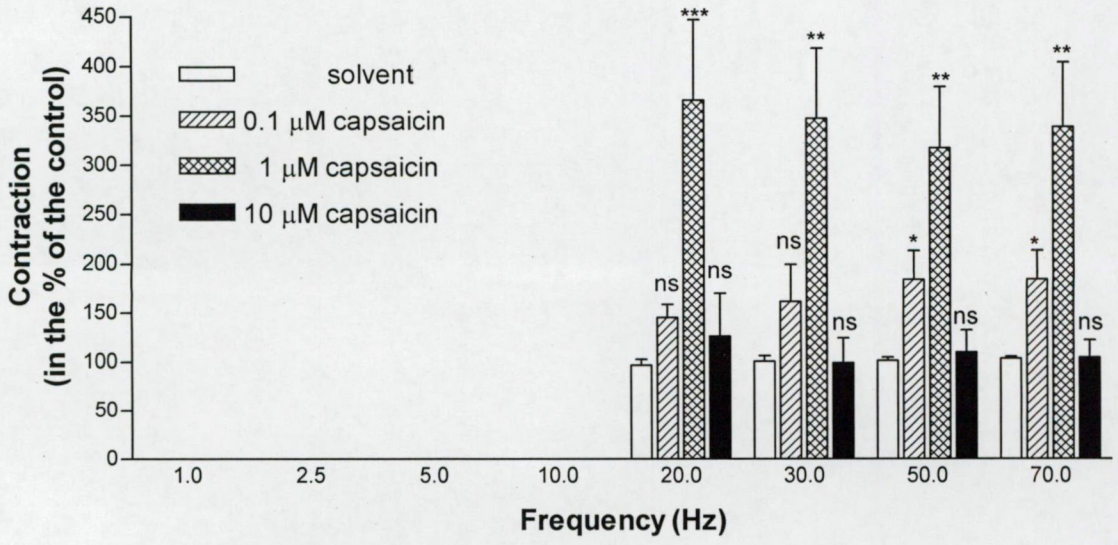

Figure 7. The effect of in vitro capsaicin treatment on the contraction responses evoked by electric field stimulation in isolated uterine rings of 22-day (term) pregnant rats.

Missing bars at 1-10 Hz refer to missing contraction responses to electric stimulation. Stimulation at 20-70 Hz revealed that certain populations of capsaicin-sensitive sensory nerves do not degenerate with pregnancy. The asterisks indicate the level of significant difference from the solvent treated preparations (* $p<0.05$; ** $p<0.01$; *** $p<0.001$; ns means $p>0.05$, thus no significant difference).

4.2.2. Immunohistochemistry

In agreement with previous findings, immunohistochemistry revealed a dense innervation of uterine blood vessels and smooth muscle in control (solvent-treated) specimens (**Fig 8a**). Exposure to capsaicin at concentrations of 0.1-1 μM produced a marked but incomplete depletion of CGRP both from perivascular and myometrial nerve fibers (**Fig 8b**). In contrast, capsaicin administered at a concentration of 10 μM produced an almost complete disappearance of all CGRP-IR nerves from the tissue (**Fig 8c**). Exposure to capsaicin produced similar effects in the term pregnant rat uterus, although the density of CGRP-IR nerve fibres was markedly reduced as compared to nonpregnant control rats (data not shown).

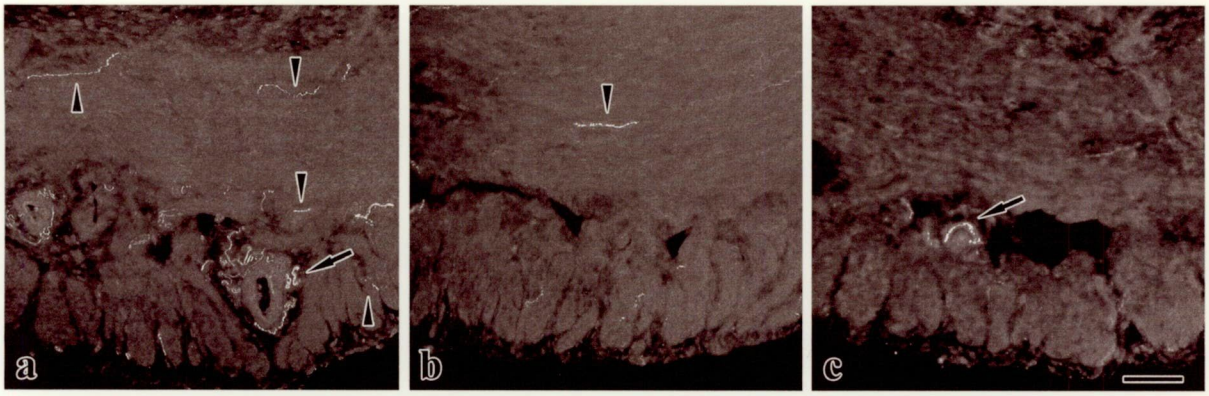

Figure 8. Immunohistochemical demonstration of CGRP-IR perivascular (arrows) and myometrial (arrowheads) nerve fibers in uterine specimens of the rat.

In control samples (**a**) CGRP-IR fibers innervate the blood vessels and the longitudinal and circular smooth muscle layers. Incubation of uterine samples with capsaicin at a concentration of 1 μM resulted in a marked depletion of CGRP-IR nerves (**b**). In samples incubated with capsaicin at a concentration of 10 μM only a few fragmented degenerating fibers can be seen (**c**). For all figures, bar = 10 μm.

5. DISCUSSION

The exact mechanism(s) of the initiation of labor at term are still poorly understood. Several factors have been shown to contribute to trigger this remarkable physiological event indicating that it can be attributed to diverse but strictly coordinated sequel of neural, humoral and biochemical procesess rather than a single mechanism.

These complex changes involve both maternal and fetal pathways mainly consisting of adrenergic and oxytocinergic mechanism and the mediators of the arachidonic acid cascade [Garfield et al., 1998]. Some hormonal (elevated plasma cortisol level) and humoral changes (elevated plasma levels of interleukin-1 and 8 which enhance prostacycline and thromboxane A_2 production) in the developing fetus may also act like triggers [Sadowsky et al., 2003]. Despite of the wealth of clinical and experimental data on putative modulators of uterine contractility, the exact physiological organization of the neural and humoral processes ultimately leading to the initiation of uterine contractions at labor is still elusive.

Previous morphological investigations revealed the innervation patterns of the nonpregnant [Garfield, 1986], the term pregnant and the postpartum rat uterus [Haase et al., 1997; Moustafa, 1988] and disclosed a marked decrease in the number of adrenergic nerves in the pregnant uterus. However, the time course of these degenerative changes in the rat uterus has not been dealt with in detail, with respect to parallel functional changes, in particular uterine neurogenic contractility during pregnancy. In the present study, we studied both the functional and the structural changes in relation to alterations in the adrenergic innervation of the rat uterus on days 5, 10, 15, 18 and 22 of pregnancy. Analysis of contractile responses evoked by electric field stimulation at selected parameters was considered to be an appropriate approach to characterize the role of the adrenergic nerves in the motor responses of the pregnant rat myometrium. Both the experiments on uteri of term pregnant rats and on nonpregnant rats' uteri treated in vitro with phentolamine revealed that myometrial contractions could not be evoked by EFS below 20 Hz. This is in agreement with previous findings showing that adrenergic nerves can be stimulated at low frequencies if the pulse width is relatively small (<0.5 ms) [de Haas et al., 1999; Phillips et al., 1998; Li et al., 1997].

These functional alterations can be interpreted in terms of morphological 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. Fluorescence histochemistry and EFS showed that, similar to nonpregnant uteri, myometrial adrenergic nerve fibers are morphologically intact and functional up to day 10 of pregnancy. The denervation process commenced in mid-pregnancy and progressed towards term (**Table 1**). In line with previous reports [Sporrong et al., 1978] we interpreted our morphological data in terms of a degeneration process but the possibility that disappearance of neuronal catecholamine fluorescence may result from a profound depletion of their transmitter content without frank axonal degeneration cannot be excluded. The results suggest that contractions mediated by adrenergic nerves do not play a significant role in controlling myometrial motor activity from midpregnancy until term.

In the second half of pregnancy the degeneration of adrenergic nerves in the rat uterus may result an altered adrenergic responsiveness of the myometrium. Considering that noradrenaline has a greater affinity to α_1-receptors than to β_2-receptors, and the myometrial contractions are mediated via the stimulation of α_1-receptors, it seems likely that the degeneration of adrenergic nerve fibers in the pregnant rat uterus might have a preventive function: sympathetic stimulation may result in diminished contractile responses or they may be completely absent. This mechanism may contribute to the maintenance of pregnancy by sheltering the fetus from adverse maternal effects.

The relative lack of sympathetic nervous control of the motor function of the uterus may emphasize the importance of nonsynaptic receptorial control at term. By the end of pregnancy the adrenergic receptors - both α_1- and β_2-receptors - are supposed to be nonsynaptic receptors activated by circulating catecholamines secreted by the adrenal medulla [Gáspár et al., 1998]. At the initiation of labor, α_1-receptors are upregulated in the rat uterus [Legrand et al., 1987] while the G-protein coupling of β_2-receptors is lower than in mid-pregnancy [unpublished observation]. Consequently, in the term pregnant rat uterus, the circulating catecholamines are more likely to elicit myometrial contractions via α_1-receptors than relaxation via β_2-receptors. Probably, α_1-receptors play a crucial role in mediating term uterine contractions.

It is suggested that there is a critical balance between the synaptic and nonsynaptic adrenergic control of the uterine function in rats during pregnancy. The adrenergic system has a dual function in controlling uterine contractility: protection of the developing fetus and initiation of labor contractions. On the one hand, the diminution of the adrenergic innervation after midpregnancy reduces the contractile responses of the pregnant myometrium elicited by

Table 1.

Summary of the presence of myometrial contraction responses as a function of the applied frequency value of electric field nerve stimulation in the uteri of intact nonpregnant and 5-, 10-, 15-, 18- and 22-day pregnant rats, and in uteri of nonpregnant rats treated in vitro with phentolamine, according to the data in Figures 2 and 3.

Frequency (Hz)	Contraction response to electric field stimulation						
	Intact non-pregnant rats	Days of pregnancy					Phentolamine-treated non-pregnant rats
		5	10	15	18	22	
1	●	○	●	○	○	○	○
2.5	●	○	●	○	○	○	○
5	●	●	●	○	○	○	○
10	●	●	●	●	○	○	○
20	●	●	●	●	●	○	●
30	●	●	●	●	●	●	●
50	●	●	●	●	●	●	●
70	●	●	●	●	●	●	●

● presence of response

○ lack of response

different neurogenic stimuli, such as reflexes evoked by mechanical and chemical stimulation of the urogenital organs and mediated, in part, by sympathetic efferent pathways [Katsuki et al., 1997; Suzuki et al., 1995; Prud'Homme et al., 1982]. On the other hand, at term the elevated α_1-receptor density is an important requirement for the development of contractions, i.e. initiation of labor. The physiological significance of adrenergic nerve degeneration is still enigmatic; the possible factors mediating this process might involve the fetuses themselves (as physical stretch) [Haase et al., 1997; Thorbert et al., 1978] and high progesterone level [Owman, 1981].

Concerning the time course of the degeneration process of adrenergic nerves, a parallelism was found between the functional and morphological changes of the rat myometrium, except for the 5th day of pregnancy. At this time, low-frequency nerve stimulation (1-2.5 Hz) failed to elicit contractile responses, although the histological picture of the 5-day pregnant myometrium showed an apparently intact adrenergic innervation. We have not yet found an explanation for this phenomenon, but it is worth mentioning that this time period coincides with implantation: the hatched blastocysts become attached to the rat uterine epithelium on day 5 after conception [Winkelmann and Spornitz, 1997; Stein et al., 1994]. 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 seem 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.

Characterization of uterine contractile responses under in vitro conditions using EFS revealed that adrenergic contractions may be elicited at lower frequencies of 1-10 Hz. Indeed, the amplitudes of contractions elicited at lower but not higher frequencies were reduced by phentolamine. It is generally accepted that stimulation at higher frequencies (20-70 Hz) activates mainly peptide containing afferent nerves [Matsumoto, 1994; Fernandes et al., 1994; Maggi et al., 1992; Manzini et al., 1991]. We suggest, therefore, that contractile responses elicited at higher frequencies result from the activation of sensory nerves in the rat uterus and, like in most visceral organs, can be attributed to the release of neuropeptide(s) from sensory nerves.

Our aim was to study the possible role of capsaicin-sensitive afferent nerves in uterine contractility. The putative contribution of sensory nerves was assessed by the analysis of neurally evoked uterine contractions after incubation with capsaicin. This natural vanilloid

has been used routinely to identify capsaicin-sensitive pathways and to explore their contribution to physiological and pathological regulatory processes [Holzer, 1991b; Jancsó et al., 1977]. Through the administration of capsaicin, the activation of sensory nerves and a consecutive neuropeptide release can be evoked. In the present study the possible role of capsaicin-sensitive sensory nerves in the modulation of neurogenic contractility was studied in nonpregnant and term pregnant rat uteri. Our observations show that capsaicin-sensitive afferents in the nonpregnant rat uterus can be stimulated at a wide range of frequencies: capsaicin pretreatment caused a dose-dependent increase of the amplitude of uterine contractions evoked by stimulation at 1-50 Hz. The specificity of electric field stimulation to activate nerves instead of smooth muscle was ensured by applying short pulse width (<5 ms) [Tomita, 1970].

Although the chance of direct smooth muscle stimulation by the electric field was excluded by the application of stimuli of 0.6 ms in duration, it seemed necessary to study the possibility of a direct effect of capsaicin on myometrial smooth muscle. To make a negative pharmacological animal model, nonpregnant rats were treated with capsaicin in order to produce a depletion of sensory neuropeptides and/or structural damage of sensory nerves. Four days after the last dose of in vivo capsaicin administration, neurogenic contractions could also be elicited at 1-70 Hz in the isolated uterine preparations obtained from these animals. However, the amplitude of the contractions failed to show any change after the in vitro application of capsaicin. The lack of the contractility-increasing effect of in vitro capsaicin was considered to be a consequence of the sensory nerve damage and consecutive neuropeptide depletion [Ferdinandy et al., 1997; Jancsó, 1992]. These findings clearly demonstrate that the contractility-increasing effect of capsaicin is fully dependent on an intact sensory nerve function, and it can be regarded as an indirect action on the myometrium.

While the augmentation of contraction responses was detected after an in vitro incubation with 0.1 or 1 μM capsaicin, we hypothesized that the phenomenon is related to an altered neuropeptide content in the sensory nerves. Capsaicin did not cause any change in the basal uterine tone when it was added to the organ bath for a 20-min incubation period [unpublished observation]. It is proposed that in this experimental design capsaicin influences uterine contractility via a release of different neuropeptides from the sensory nerve endings leading to a change in the amplitude of the contractions after ESF stimulation. The finding of an enhancement of the contraction amplitude after the application of 0.1 or 1 μM capsaicin led us to consider the possibility of a differential depletion of neuropeptides by such doses of

capsaicin resulting in a relatively lower concentration of relaxing than constricting neuropeptides in the local sensory nerve endings. The highest dose of capsaicin (10 μM) did not give rise to a significant change in the amplitude of the contractions, which might be presumably explained by an irreversible damage of sensory nerves [Király et al., 1991].

Since the most abundant relaxing neuropeptide in the sensory nerves of the rat uterus is CGRP, we assumed that the observed enhancement of uterine contraction responses is related to a marked loss of CGRP from the capsaicin-sensitive afferent nerves. CGRP is presumed to be responsible for the maintenance of pregnancy by ensuring a relaxant state of the myometrium. In the present experiments, when the uteri of intact nonpregnant rats were stimulated at low frequency in the presence of a CGRP receptor antagonist CGRP(8-37), an enhancement of the amplitude of the contractions was detected as compared with the contractions evoked in the absence of CGRP(8-37) after stimulation at the same frequencies. These findings support the idea that CGRP-containing nerves participate in the mechanism of uterine contractions induced by low-frequency (<30 Hz) EFS. Although several reports have indicated that CGRP-containing nerves are sensitive to a higher frequency stimulation in the trachea [Matsumoto, 1994; Fernandes et al., 1994], ileum [Maggi et al., 1992] or in mesenteric arteries [Manzini et al., 1991], low frequency stimulation of afferent nerves has also been shown to activate CGRP-containing nerves and to elicit antidromic vasodilation [Magerl et al., 1987], where CGRP is the key mediator. In our experiments the amplitudes of contractions in the presence of CGRP(8-37) were the highest when the uteri were stimulated at 1 Hz. It is interesting, since the amplitude can approach of those detected at the same frequency after the uteri were incubated with 1 μM capsaicin. This supports our suggestion that the increased amplitude of responses after in vitro capsaicin treatment is a consequence of, at least in part, the capsaicin-induced release and depletion of CGRP from the sensory nerve endings.

Our immunohistochemical findings also support the role of CGRP in the mechanism of this phenomenon. Indeed, CGRP-IR nerve fibers were sparse after a prior exposure to capsaicin at the lower concentrations of 0.1 and 1 μM. CGRP-IR nerves were practically absent after pretreatment with capsaicin at a concentration of 10 μM. These observations support our suggestion that changes in the tissue levels of CGRP play a significant role in the mechanism of the capsaicin-induced augmentation of uterine contractile responses.

An important question is whether the observed increase in contractility is detectable in the uteri of term pregnant rats. Studies on the possible effect of pregnancy on uterine sensory

nerves are sparse. In the light of our present findings changes in the functional condition of sensory nerves may bear of some significance as regards the mechanism of preterm labor when enhancement of uterine contractility develops closely before term. Studies on the functional alterations of adrenergic nerves have already disclosed the influence of pregnancy-induced denervation process on neurogenic contractions in the pregnant rat uterus: the lack of contraction responses to ESF at 1-20 Hz appeared in parallel with the progressive loss of uterine adrenergic fibers detected by histofluorescence. Thus, it was not surprising that the uteri of term pregnant rats can not be stimulated at such low frequencies, which is a remarkable difference from the nonpregnant uteri. The application of hCGRP(8-37) showed that CGRP-containing nerves in the nonpregnant uterus are possibly stimulated at 1-10 Hz, thus these nerves are probably affected by pregnancy-induced degeneration. The remaining contraction responses in term pregnant rat uteri also proved to be elevated after incubation with 0.1 or 1 μM capsaicin, similarly as in the nonpregnant rat uteri, which supports the notion that certain populations of sensory nerves in the rat uterus [Traurig et al., 1984] do not degenerate with pregnancy.

In conclusion, our findings suggest that capsaicin-sensitive sensory nerves, by the release of sensory neuropeptides, may significantly modulate uterine contractile activity. The increased amplitude of uterine contractions after an in vitro exposure to capsaicin may be a result of an integrative mechanism which involves an altered neuropeptide content, a relative deficiency of CGRP, in the sensory nerve endings. Pregnancy-induced denervation might likewise affect afferent nerves [Anouar et al., 1998; Berkley et al., 1993b], possibly involving CGRP-containing nerves. The augmentation of the responses upon high frequency stimulation cannot be attributed to an involvement of CGRP. Our preliminary experimental data, showing a relative sparing of galanin- (GAL-) immunoreactive fibers following an in vitro exposure to capsaicin, suggest that increased contractile responses to high frequency stimulation may be related to an effect of GAL. Indeed, GAL is abundant in reproductive tissues of the female rat, it participates in the regulation of the reproductive process and causes contractions of the myometrium [Stjernquist et al., 1988]. However, only a detailed quantitative immunohistochemical analysis could reveal the exact nature of the populations of capsaicin-sensitive nerves involved in the mechanism of enhanced myometrial contractility elicited by capsaicin.

Capsaicin proved to be a suitable pharmacological tool to investigate the contribution of sensory pathways to the uterine motor activity. Through the administration of capsaicin,

the activation of sensory nerves and a consecutive neuropeptide release can be evoked. The mechanism of contractility enhancement due to neuropeptide depletion from stimulated afferent nerves may be particularly interesting, considering that such stimulation may occur in the uterus under certain pathological conditions, in particular tissue inflammation. Local mediators of inflammation e.g. protons (low pH) acting via VR1/ TRPV1 and/or acid-sensing ion channels or local proteases acting via protease-activated receptors [Fiorucci and Distrutti, 2001] were reported to stimulate sensory nerve endings. Taking into account that in approximately 25% of all preterm delivery a genital infection is the most probable trigger factor, the further study of the involvement of sensory nerves in uterine pathology may offer a promising approach to a better understanding of the role of sensory nerves in uterine function under physiological and pathological conditions. Finally, pharmacological targeting of uterine sensory nerve function may offer a novel way of modulation of uterine motor control.

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