Multiple site electromyography recordings of uterine activity following an intraterine bacterial challenge in mares susceptible and resistant to chronic uterine infection

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The electrical myometrial activity of three mares with a documented increased susceptibility to chronic uterine infection (CUI) and three mares considered to be resistant to CUI was investigated. Electrodes were surgically implanted in the myometrium of the mares and electrical activity was monitored by a Grass polygraph. Oestrus was determined by transrectal ultrasonography of the reproductive tract and teasing of the mares with a stallion. Findings were confirmed by blood progesterone concentrations < 0.1 ng ml⁻¹. At the third day of oestrus or when a follicle > 35 mm was detected, the uterus was infused with a genital strain of 5 × 10⁹ Streptococcus zooepidemicus. Myometrial electrical activity was monitored for 1–4 h before the bacterial infusion and continued until a visual stabilization of the activity occurred. No statistically significant differences in electrical myometrial activity were detected between susceptible and resistant mares before the infusion of bacteria into the uterus. A visible increase in myometrial electrical activity was seen in all mares following the bacterial infusion. However, the myometrial response of susceptible and resistant mares was different. Resistant mares demonstrated a greater myometrial activity (P < 0.001) than did susceptible mares. These differences were observed in frequency (P < 0.005) as well as duration (P < 0.001) and intensity (P < 0.001) of the uterine activity. Differences were most marked between 10 and 20 h after the intrauterine inoculation of bacteria. It was concluded from this study that myometrial activity is an important part of the uterine defence mechanism in mares. Mares susceptible to CUI appear to have an impaired electrical myometrial activity in response to an intrauterine bacterial challenge.

Introduction

Chronic uterine infection (CUI) is a common cause of infertility and subfertility in mares. At the time of breeding, the uterus is frequently contaminated with bacteria (Dimmock, 1939; Kenney and Ganjam, 1975). Most young mares can eliminate this infection within 96 h and have been found to be free from infection at the time when the embryo descends into the uterus (Hughes and Loy, 1969). The mechanisms for resistance against a persistent uterine infection is believed to be the combined result of a cellular phagocytic elimination of bacteria (Asbury et al., 1982; Cheung et al., 1985; Liu et al., 1985, 1986; Asbury and Hansen, 1987; Watson et al., 1987a; Troedsson et al., 1990), a local uterine antibody-mediated immunity (Kenney and Khaleel, 1975; Asbury et al., 1980; Mitchell et al., 1982; Williamson et al., 1983; Widders et al., 1984, 1985) and a physical clearance of bacteria and inflammatory products (Evans et al., 1986; Troedsson and Liu, 1991a). In contrast to young fertile mares, older multiparous mares often cannot clear uterine contamination and this can result in a chronic uterine infection (Peterson et al., 1969).

Several workers have investigated at what level compromise in uterine defence occurs in mares susceptible to CUI. It has been shown that the concentration of antibodies within the uterine lumen is the same or even enhanced in susceptible compared with resistant mares (Asbury et al., 1980; Mitchell et al., 1982). It has been suggested that phagocytosis of polymorphonuclear neutrophils (PMN) is dysfunctional in mares that are susceptible to CUI (Cheung et al., 1985; Liu et al., 1985; Watson et al., 1987b). However, in a recent study it was demonstrated that both chemotactic properties of uterine secretion and the ability of uterine PMNs to respond to phagocytosis and chemotaxis were fully functional. An impaired opsonizing function of uterine secretion was, however, found in susceptible mares (Troedsson and Liu, 1991b). It was suggested that this dysfunction is the result of an accumulation of detrimental inflammatory products within the uterus as a result of persistent infections. Older mares and susceptible mares have been found
Materials and Methods

Mares

Six cyclic mares were used in this study. Mares that were considered to be resistant to CUI (n = 3) ranged in age from 3 to 5 years of age. They had no history of reproductive problems and had no significant histopathological findings in uterine biopsies. In addition, resistant mares proved to be capable of completely clearing an induced uterine infection of 5 × 10⁶ Streptococcus zooepidemicus within 4 days of the inoculation. All resistant mares had negative uterine cultures and no inflammatory cells on endometrial cytology at this time. Mares classified as susceptible to CUI (n = 3) ranged from 15 to 20 years of age. They had a history of CUI with associated fertility problems and had severe inflammatory changes and periglandular fibrosis in uterine biopsies (Kenney, 1978). Susceptibility to CUI was confirmed by a bacterial uterine challenge. Only mares that had positive cytology and uterine cultures four days after the inoculation of Streptococcus zooepidemicus were classified as susceptible.

Implantation of myometrial electrodes

Six pairs of bipolar electrodes were surgically implanted into the myometrium. The uterus was exposed via a midventral laparotomy performed under general anesthesia induced by 100 mg guaifenesin kg⁻¹ (Rhone-Poulenc, Collegeville, PA) and 2 mg thiamylal kg⁻¹ (Surtal, Parke Davis, Morris Plains, NJ) i.v. and maintained with halothane (Halozein Laboratories, N. Augusta, SC). Four pairs of sterile electrodes (44-gauge multistrand stainless steel Teflon-coated wire) were implanted into the myometrium, spread 3–5 cm apart at the tip of the left horn, the middle of the left horn, the base of the left horn and the uterine body. Similarly, two pairs of electrodes were implanted in the middle and base of the right horn. A loop was made in each wire 5 cm from the distal end of the electrodes. The Teflon was stripped from the electrodes over a 10 mm long segment just distal to the loop. The electrodes were implanted into the myometrium, using a tapered needle, and secured via a suture (prolene 3–0) through the loop. Electrodes within a pair were implanted 1 cm apart. The most distal ends of the electrodes were cut, leaving the stripped area embedded into the myometrium. This technique allowed exposure of the electrodes to both the longitudinal and circular layers of the myometrium. The recorded activity therefore represented the combined activity of the two myometrial layers. Each electrode, covered by a Teflon tube, was passed through the abdominal wall of the left flank, tunnelled subcutaneously, exteriorized near the withers, fixed in place and capped.

EMG recordings

After implantation and recovery from surgery, the mares were allowed to rest for at least one week for the uterus to stabilize. Electrical activity (EMG) was monitored by a Grass polygraph. EMG signals were amplified through Grass DC preamplifiers and the signals were transcribed to a Grass polygraph at a chart speed of 10 mm min⁻¹ and at an amplitude of 50 μV cm⁻¹. Signal filters were set at 10 and 35 Hz (Haluska et al., 1987).

The mares were fed alfalfa hay and kept in stalls during the entire experiment. Oestrous behaviour was detected by daily teasing with a stallion. Transrectal ultrasonography of the reproductive tract was performed when the mares showed signs of oestrus. Oestrus was confirmed by concentrations of progesterone in blood <1.0 ng ml⁻¹ in the presence of a follicle >35 mm.

Collection of blood samples

In conjunction with each recording session, 20 ml of venous blood was withdrawn from the jugular vein into a vacutainer system (Becton Dickinson, Rutherford, NJ) containing sodium heparin as an anticoagulant. The samples were centrifuged at 400 g for 10 min and plasma was removed and stored at −20°C until analysed for progesterone.

Hormone analysis

Plasma progesterone was analysed using a solid-phase microtitre plate enzymeimmunoassay as described by Munro and Stabenfeldt (1984). Progesterone 3-O-carboxymethyloxime—horseradish peroxidase was used as the label and the antisum was raised in rabbits against a progesterone 11α-hemisuccinyl—BSA immunogen. Progesterone 3-O-carboxymethyloxime and 11α-hemisuccinate—BSA were purchased from Steraloids, Inc. (Wilton, NH). Horseradish peroxidase was obtained from Sigma Chemical Co. (St Louis, MO). The mean intra-assay coefficient of variation was 9.97% and the average interassay coefficients of variation for pools of high, medium and low progesterone concentration were 4.9, 6.2 and 10.5%, respectively. The sensitivity of the progesterone assay was <0.1 ng ml⁻¹.

Infections

Before each experiment, the mares were confirmed to be free from infection as determined by endometrial culture and cytology. At the third day of oestrus or when a 35 mm follicle was detected, the mares were infected with a genital strain of 5 × 10⁶ Streptococcus zooepidemicus. The perineal region was aseptically prepared with extra precaution taken to cleanse...
the clitoral fossa. The bacterial culture suspended in 30 ml phosphate-buffered saline solution (PBS) was infused from an insemination pipette into the uterus. As a negative control, each mare was infused with PBS alone in a subsequent oestrous period.

Uterine activity was monitored for 1–4 h before the infusion of 5 × 10⁶ Streptococcus zooepidemicus or PBS (negative control) into the uterus and was continued until visual stabilization of the activity occurred. At the end of each recording, 20 Iu of oxytocin was given i.v. to serve as a control for myometrial activity.

EMG analyses

All recordings were manually transformed to a digitized form and voltage activity was measured in three ways (Troedsson et al., 1993). (1) Frequency was defined as the number of activity bursts per hour, with each burst consisting of at least ten spikes per minute and separated from other bursts by at least one minute. (2) Duration was defined as the time (min) that elapsed from the start to the end of a burst. (3) Intensity was measured as the number of spikes per minute. Intensity was coded as low (10–15 spikes min⁻¹), intermediate (16–20 spikes min⁻¹), or high (> 20 spikes min⁻¹). Additionally, all activity recorded (regardless of intensity) was classified as total activity and was expressed as the percentage of time any activity was recorded.

In addition, the presence or absence of synchronous activity was expressed as the number of myometrial implantation sites which were active simultaneously and the number which were quiescent simultaneously. Complete synchronous activity occurred when there was simultaneous activity or quiescence at all locations.

Statistical analyses

The mean of the recorded uterine myometrial activity before the infection was tested for significant differences regarding frequency, duration, intensity and total uterine activity between susceptible and resistant mares using a Student's t test. Analysis of variance and covariance with repeated measures were used to compare uterine activity following infection over time between susceptible and resistant mares. Differences in frequency, duration, intensity and synchronization were compared separately. Because of technical problems with the two pairs of electrodes implanted in the right horn of two mares, data from the right horn was excluded from all mares and analyses were made only from the four pairs of electrodes in the left horn and uterine body. Comparisons were not made for each time point owing to the small sample size, but inferences were made from the regularity of the pattern of recordings obtained.

The results were expressed as means ± SEM. Significance was set at P < 0.05.

Results

An increase in myometrial electrical activity was seen in all mares after they were infected with Streptococcus zooepidemicus. Infusion of PBS into the uterus did not affect uterine activity, with the exception of an immediate increase of frequency and synchrony that lasted for only 10–15 min.

Fig. 1. The number of activity bursts per hour following an induced intrauterine challenge in mares (■) susceptible versus (□) resistant to chronic uterine infection. Both groups show an initial increase in activity. Activity bursts subsequently decline to below resting values in the susceptible mares up to 16 h after infection.

A vaginal discharge was observed in all mares, starting approximately 5 h after infection. In the resistant mares, the discharge disappeared after 20 h, whereas in the susceptible mares a purulent vaginal discharge was still visible at the end of the experiment.

Frequency

No differences in the frequency of uterine electrical activity were detected between susceptible (4.42 ± 0.61 bursts of activity h⁻¹) and resistant mares (4.50 ± 0.55 bursts of activity h⁻¹) in the absence of a uterine infection (P > 0.05). However, in both resistant and susceptible mares the frequency of uterine activity for the first 5 h of infection was greater than before infection (Fig. 1). From 5 to 17 h after the infection, significant differences between susceptible and resistant mares were observed (P < 0.001). The increased frequency was sustained during this time in the resistant group until 18 h after infection, when a slow decline to preinfection values occurred. In contrast, the susceptible group showed a marked decline in frequency between 7 and 17 h after infection to values below that seen before infection. After 17 h of infection, the frequency increased to preinfection values in susceptible mares.

Duration

No differences in the duration of the activity burst were found between susceptible and resistant mares before infection (3.93 ± 0.63 min versus 4.24 ± 0.56 min; P > 0.05). Differences between the groups of mares were observed between 12 and 16 h after the infection (Fig. 2). The activity bursts were longer in the resistant mares than in the susceptible mares during this period (P < 0.001).

Intensity

No differences were detected in the intensity of uterine activity before infection between susceptible and resistant

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mares. Low intensity activity was seen for 7.11 ± 0.97 min h⁻¹ in the susceptible mares and 7.8 ± 0.78 min h⁻¹ in the resistant mares (P > 0.05). Intermediate intensity was observed for 4.50 ± 0.80 min h⁻¹ in the susceptible group and 5.73 ± 0.94 min h⁻¹ in the resistant group (P > 0.05). The susceptible mares had a high intensity of uterine activity for 6.54 ± 1.23 min h⁻¹, compared with 9.18 ± 0.91 min h⁻¹ for the resistant mares (P > 0.05).

A statistically significant difference was detected between susceptible and resistant mares for all levels of intensity of uterine electrical activity (P < 0.001) (Fig. 3). Differences in low intensity activity between the groups were observed 13 h after the infection and lasted throughout the remainder of the experiment. At this time, low intensity activity was more frequent among the resistant than the susceptible mares.

Uterine activity of intermediate intensity declined over time in the susceptible group. Intermediate intensity activity occurred more frequently in the resistant mares than in the susceptible mares (P < 0.001). This was particularly obvious at 12 h after infection and beyond.

High intensity uterine activity was observed more often in the resistant than in the susceptible group of mares following infection (P < 0.001). In both susceptible and resistant mares, an increase of high intensity activity was seen during the first 9 h of infection. The increase of high intensity activity was delayed by 2 h in the susceptible mares compared with the resistant group. In susceptible mares, a decline below pre-infection values occurred 9 h after infection. This was not observed in the resistant group.

The total uterine activity following an infection also demonstrated different patterns over time between susceptible and resistant mares (P < 0.001). The activity increased in both groups of mares during the first 6 h after the infection (Fig. 4). However, the onset of activity was delayed and was followed by a sharp decline in activity in the susceptible group. After 11 h and continuing until the end of the experiment (18 h), only a very low uterine activity was present in susceptible mares. In contrast, the resistant mares showed increased uterine activity for a longer period with only temporary decreases in activity occurring at 11, 15 and 19 h after inoculation.

Fig. 2. The duration of uterine electrical activity bursts following an induced intrauterine bacterial challenge in mares (■) susceptible and (□) resistant to chronic uterine infection.

Fig. 3. (a) High, (b) medium and (c) low intensity of uterine electrical activity following an intrauterine bacterial challenge in mares (■) susceptible and (□) resistant to chronic uterine infection. Resistant mares had significantly (P < 0.001) more high intensity uterine activity than did susceptible mares. All levels of intensity progressively declined 6–8 h after infection in susceptible mares.

Fig. 4. Total uterine electrical activity following an intrauterine bacterial challenge in mares (■) susceptible and (□) resistant to chronic uterine infection. Resistant mares showed more total uterine activity than did susceptible mares. After an initial increase, total uterine activity progressively declined in the susceptible mares.
Fig. 5. The percentage of synchronized electrical uterine activity following an intrauterine bacterial challenge in mares susceptible and resistant to chronic uterine infection. The estimated regression lines show that while synchronization does not change in the resistant mares, an increase over time occurs in the susceptible mares.

Synchronization

In the absence of an infection among the susceptible mares, asynchrony of uterine electrical activity between any of the four implantation sites occurred for 38.84 ± 5.1% of the time. This was not statistically different from that of the resistant mares (46.81 ± 4.05%; P > 0.05).

Although synchronization of uterine activity in the resistant group was relatively constant throughout the experiment, synchronization occurred with increasing frequency in the susceptible group for 8–10 h after infection (P < 0.03) (Fig. 5).

Discussion

Electromyographic recordings of myometrial activity have been performed in several species (Zerobin and Spörri, 1972; Naaktgeboren et al., 1973; Taverne et al., 1979a; Krishnamurti et al., 1982; Thorburn et al., 1984; Toutaine et al., 1983; Faltsi and Brikas, 1990). In mares, myometrial activity has been found to change during different stages of the oestrous cycle (Taverne et al., 1979b; Troedsson et al., 1993). Shorter duration of activity bursts is found during oestrus than during dioestrus. In addition, the intensity as well as synchronization between different parts of the uterus are greater during oestrus.

The functional importance of a more pronounced activity of the myometrium during oestrus has not been established. Cervical–uterine–oviductal activity may be necessary for the transport of spermatozoa. In addition, uterocervical myometrial activity may protect the uterus from invading microorganisms by continually moving endometrial gland secretions through an open cervix. Neither the directions of contractions, nor the direction of the movement of intraluminal fluids were investigated in this study. However, the observed vaginal discharge in all mares and the previously noted ability of the uterus to transport microspheres through the cervix following an infection (Troedsson and Liu, 1991a) indicates that the uterine activity measured in the present study corresponded to uterocervical removal of inflammatory products from the uterus. Monitoring of intrauterine pressure in combination with EMG may better describe movement of intrauterine fluids. However, the presence of instruments in the uterine lumen has the disadvantage that contact with the endometrium is likely to induce mechanical irritation, which may trigger both contractile and inflammatory responses.

Physical uterine defense mechanisms, including the ability to clear inflammatory products following uterine contamination, are believed to be essential for resistance to persistent uterine infection (Evans et al., 1987; Boyd and Allen, 1987; Troedsson and Liu, 1991a). The present study confirms the importance of uterine activity as a component of physical uterine defenses. All mares responded to an intrauterine bacterial challenge with a marked increase in myometrial activity. The enhanced uterine activity lasted for up to 20 h. This corresponded well to a previous observation that radioactively labelled non-antigenic microspheres were almost completely eliminated from the uterus within 24 h after infection (Troedsson and Liu, 1991a).

The mechanism that produces increased myometrial activity during uterine infection is not known. A rapid migration of activated PMNs and high concentrations of prostaglandins (PGF 2α and PGE) have been demonstrated in conjunction with acute uterine infection (Watson et al., 1987a; Pycock and Allen, 1988). Receptors for prostaglandins have been identified in the myometrium and prostaglandin release is believed to increase myometrial activity via an activation of the phosphoinositol cycle and an increase in intracellular Ca 2+ (Carsten and Miller, 1981, 1990). It seems likely, therefore, that prostaglandins, released from activated PMNs, may be responsible for increased myometrial activity during infection.

In addition to identifying enhanced myometrial activity as a response to intrauterine infection, the study reported here shows that there are marked differences in myometrial activity following an infection between mares susceptible to and resistant to chronic uterine infection. A previous study showed that the physical capacity of the uterus to clear non-inflammatory markers was reduced in mares susceptible to compared with mares resistant to CUI (Troedsson and Liu, 1991a). The present study shows that the mechanism behind this may be a dysfunctional myometrial activity during infection. In the absence of inflammation, uterine electrical activity did not differ between susceptible and resistant mares under the conditions of this experiment. However, major differences were demonstrated in association with an intra-uterine bacterial challenge. All mares showed an initial increase in myometrial activity; however, the increase in uterine activity (as measured by number of activity bursts per hour), the duration of the activity bursts, the total activity and the intensity declined rapidly in the susceptible group. Susceptible mares also had poorer synchronization of activity as well as less high intensity activity during the early stages of inflammation compared with resistant mares. The majority of the observed differences between susceptible and resistant mares occurred 12 h or more after the mares were infected. The decline in activity cannot be explained by a decline in inflammatory stimulation of the myometrium because, by definition, all mares susceptible to CUI had a persistent uterine infection 96 h after infection. In addition, a purulent vaginal discharge was observed in all susceptible mares at the end of the experiment. It is more likely that a shorter and weaker myometrical response is, at least in part, responsible for the delayed...
physical clearance of inflammatory products from the uterus. As no differences were detected in myometrial activity in the presence of an infection, further studies of the ultrastructure and biochemistry of the myometrium following an infection are needed to determine the cause of this dysfunction.

In conclusion, this study shows that myometrial activity is an important part of the physical uterine defence mechanism in mares. Furthermore, mares that are susceptible to chronic uterine infection appear to have an impaired electrical myometrial activity in response to an intrauterine bacterial challenge.

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