Long-Term Observations of Uterine Contractions in Nonpregnant Dogs

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ABSTRACT

Uterine contractile activity in nonpregnant conscious dogs was investigated based on 2- to 6-mo-long continuous recording by means of a chronically implanted force transducer. We found that nonpregnant uterine contractile activity could be classified into six major patterns: sporadic contractions, weak and strong tonic contractions, weak and strong phasic contractions, and phasic contraction bursts. The contractile patterns during proestrus and estrus were the most active, with strong phasic and tonic contractions and phasic contraction bursts. The phasic and tonic contractions were inhibited dose-dependently by a β-adrenergic agonist, ritodrine, and reproduced by an α-adrenergic agonist, clonidine. In contrast, the cholinergic inhibitors atropine and hexamethonium did not affect the spontaneous occurrence of these contractions, although bethanechol evoked uterine contractions. Oxytocin and prostaglandin F2α-induced contractions were phasic during estrus, whereas they showed tonic increases with phasic contractions during proestrus, diestrus, and anestrus, and these contractions did not resemble the spontaneous contractions. In conclusion, the nonpregnant uterus and anestrus, and these contractions did not resemble the spontaneous contractions, although bethanechol evoked uterine contractions. Oxytocin and prostaglandin F2α-induced contractions were phasic during estrus, whereas they showed tonic increases with phasic contractions during proestrus, diestrus, and anestrus, and these contractions did not resemble the spontaneous contractions. In conclusion, the nonpregnant uterus contracts continuously in harmony with the estrous cycle phases, and its contractile activity is enhanced by α-adrenergic receptors and inhibited by β-adrenergic receptors.

MATERIALS AND METHODS

Preparation of Experimental Animals

Eight healthy parous but nonpregnant adult female mongrel dogs weighing 9–13 kg were used for the study of contractile activity. The estrous cycle stages were determined by cytological examination of smears of vaginal swab specimens obtained serially and were confirmed by measuring plasma estradiol and progesterone concentrations. General anesthesia was induced by a single i.v. injection of thiopental sodium (Ravonal; Tanabe Pharmaceutical Co., Osaka, Japan; 20 mg/kg BW) and maintained by inhalation of halothane (Fluothane; Takeda Chemical Industries, Osaka, Japan) and oxygen, and the abdominal cavity was opened under aseptic conditions. A miniature force transducer (8.0 × 2.5 mm), constructed in our laboratory as described previously [23], was sutured to the surface of the seromuscular layer of the right uterine horn 3 cm distal to the convergence of the uterine horns for long-term detection of circular muscle contractions. This was the first time we had attempted to measure uterine contractions with a force transducer; therefore another force transducer was sutured to the gastric antrum as a reference to confirm the uterine responses by comparison with the effects of various types of antagonists and agonists on the stomach, as their effects on gastric contractile activity are well known in our laboratory. The lead wires of the transducers were led out through a puncture wound in the lateral abdominal wall, tunneled under the skin, and exteriorized through a skin incision between the scapulae; the ends were secured to the adjacent skin with silk sutures. After the abdominal procedure, a heparinized saline-filled silicone tube (Silascone, Medical tube SH No. 1; Kaneka Medix Co., Osaka, Japan) was introduced into the superior vena cava through a branch vein of the right external jugular vein; the other end of this tube, which was closed with a small plug, was also secured to the adjacent skin with silk sutures. This tube was used as a route for i.v. infusions of test materials and for withdrawing blood specimens. After surgery, a jacket protector was placed on each dog to prevent the dog from scratching the tube and lead wires. The dogs were housed in individual experimental cages, maintained with an i.v. drip-infusion of Solita T3G (Shimizu Pharmaceutical Co., Shimizu, Japan) for 3 days postoperatively, and gradually returned to normal dog food. During the experiment, they were fed once at 1700 h and allowed to drink water ad libitum. Experiments were started after the dogs had recovered completely from the surgery, which usually took about a week.
Recording and Measurement of Uterine and Gastric Contractions

The cable leads from amplifiers (UG-6; Nihon Kohden Kohgyo Co., Tokyo, Japan) were hung from the ceiling just above each experimental cage and connected to the lead wires of the transducers in a small pocket attached to the protector. Signals from the amplifiers were recorded continuously by a pen-writing recorder (ME-175D; Nihon Kohden Kohgyo Co.) at a paper speed of 1 mm/min, day and night, every day except Sunday for 2–6 mo to cover different phases of the estrous cycle and recorded on a tape recorder (RD-110T; TEAC, Tokyo, Japan) when further analysis of data was considered necessary.

Vaginal Cytological Examination

The phases of the estrous cycle were determined according to cytological findings on smears of vaginal swab specimens obtained daily. The proestrous and estrous phases were determined by the observation of serosanguineous discharge, turbidity of vulvae, and early cornification of vaginal epithelial cells evident by morphological alterations of the nuclear and cytoplasmic borders. The estrous state was characterized by maximal cornification and alterations of the nuclear and cytoplasmic borders. The estrous state was characterized by maximal cornification and a lack of white blood cells in vaginal smears [27].

Measurement of Plasma Estrogen and Progesterone Concentrations

Blood samples for measuring estrogen and progesterone were taken once a week in the anestrous and diestrous phases in each of 5 dogs. Collection of blood samples in the proestrous phase was made in only one dog. Five milliliters of blood was collected into a heparinized tube and centrifuged immediately at 3000 rpm for 10 min. The supernatant was stored at −20°C until assay. Progesterone was quantified using a Coat-A-Count Progesterone RIA kit (Diagnostics Products Corp., Los Angeles, CA), which can be used to assay progesterone in canine serum directly. Estradiol was quantified after extracting the samples with diethyl ether and reconstituting each extract in the zero calibrator supplied with the Coat-A-Count Estradiol kit [28].

Experimental Procedures

1. Changes in contractile activity of the uterine horn. Uterine contractions of 3 dogs recorded for 6 mo were adjudged to cover all the contractile activity patterns and were designated recordings A. Contractile recordings of the other 5 dogs could not be taken continuously for mechanical reasons and did not include all 4 estrous phases. These were designated recordings B. First, recordings A were inspected visually to classify their contractile pattern characteristics. The contractile activity of the nonpregnant canine uterus was found to show two major patterns: phasic and tonic (contractions lasting less than or more than 2 min, respectively). A total of 6 patterns were designated (see Fig. 1). Next, these criteria were applied to recordings B, and the contractile amplitudes, frequencies, and durations were analyzed and classified. The pattern designated contraction bursts lasted for over 5 min; the duration of these groups of strong contractions was also measured. With respect to the contraction amplitudes, the absolute contractile force could have been measured in vitro as reported previously [23]; but as the force needed to bend a force transducer is entirely dependent on the amount of uterine muscle actually sutured with silk, comparisons of the absolute forces are meaningless. Therefore, as the force of active phasic contractions is constant and uniform in each dog, no matter how much muscle is sutured to the transducer, the mean amplitude of the strong phasic contractions was taken as the standard amplitude for each dog, and the amplitudes of the other types of contractions were expressed as a percentage of this standard. The contractile activity (motor index) for an equivalent interval of time before and after drug administration was measured quantitatively by determining the area under the contraction curves, using a software program we wrote, after feeding the data into a personal computer (PC-9801 RX computer; NEC, Tokyo, Japan). The mean motor index before administration of the drug was regarded as the control (100%), and the motor index after drug administration was represented as a percentage of the control.

In order to demonstrate the characteristics of each contractile pattern, three typical 24-h recordings of each pattern in 3–5 dogs were analyzed and related to the concomitant vaginal smear findings and plasma estradiol and progesterone levels. Each overall mean value stated in reporting the results is the mean of the mean values obtained for each dog.

2. Cholinergic and adrenergic characterization of nonpregnant uterine contractions. All drugs were given twice to 5 dogs as an i.v. infusion in the diestrous phase. The proestrous and estrous phases were not long enough for testing the effects of all the drugs twice in each dog, but when drugs could be tested, the number of tests is mentioned. Drug administration was done once a day, and the same drug was not given on two successive days.

a. Cholinergic agonists and antagonists. In order to characterize the spontaneously occurring uterine contractions, bethanechol (3, 10, and 30 μg/kg per minute) was given as a continuous i.v. infusion over 10 min. Atropine was given i.v. as a bolus of 0.05 mg/kg followed by a continuous 30-min infusion of 0.05 mg/kg per hour, and hexamethonium was injected i.v. as a bolus of 3 mg/kg followed by 7 mg/kg per hour for 30 min. The contractile activities during the 30 min before and after administration of these drugs were compared.

b. Adrenergic agonists and antagonists. Similarly, the spontaneously occurring uterine contractions were characterized through administration of various adrenergic agonists and antagonists i.v. The following drugs were used: the α1 and α2 adrenergic antagonists prazosin and yohimbine, respectively, phenylephrine (α1) and clonidine (α2) adrenergic agonists; atenolol (β1) and butoxamine (β2) adrenergic antagonists; and dobutamine (β1) and ritodrine (β2) adrenergic agonists. All these drugs, except ritodrine, were given at a dose of 3.0 mg/kg per hour over 30 min, and ritodrine (0.5, 1.0, and 3.0 mg/kg per hour) was given over 30 min.

3. Oxytocin and prostaglandin F2α. In order to confirm uterine contractile responses to representative hormonal factors that stimulate uterine contractions, oxytocin (0.3, 0.9, and 2.7 IU/kg per hour) and prostaglandin F2α (PGF2α, 30, 100, and 300 μg/kg per hour) were also given as continuous i.v. infusions over 10 min.

4. Drugs used in the study. The following drugs were purchased: oxytocin, hexamethonium bromide, phenylephrine, prazosin, clonidine, yohimbine, atenolol (Wako Pure Chemical Industries, Osaka, Japan), PGF2α (Ono Chemical Industries, Osaka, Japan), bethanechol, butoxamine (Sigma Chemical Company, St. Louis, MO), atropine (Tanabe Pharmaceutical Co.), dobutamine (Shionogi Pharmaceutical
A: Sporadic contractions

B: Weak tonic contractions

C: Weak phasic contractions

D: Strong phasic contractions

E: Strong tonic contractions

F: Contraction bursts (underlined)

Time intervals, 30 min

FIG. 1. Typical nonpregnant uterine contractile patterns in conscious dogs. A) Sporadic contractions, with contractile forces less than 25% of the mean maximum contractile force, observed during anestrus. B) Weak tonic contractions, with contractile forces less than 65% of the mean maximum contractions. This type of contraction was characterized by its long duration and was observed toward the end of the anestrous phase. C) Weak phasic contractions lasting less than 2 min observed mostly during diestru. D) Strong phasic contractions lasting less than 2 min, the typical contractile pattern during estrus. E) Strong tonic contractions lasting over 2 min, the typical contractile pattern during proestru. F) Strong phasic contraction bursts (underlined). This type of contractile pattern was observed during estrus.

5. Statistical analysis. The results are expressed as means ± SE. Statistical analysis was performed by ANOVA with repeated measures, and comparisons among groups were carried out using Fisher's protected least significant differences. Differences at p values < 0.05 were regarded as significant.

RESULTS

1. Classification of Spontaneous Contractile Patterns

Uterine contractions, including all recorded for all 8 dogs, were classified according to criteria based on amplitudes and durations. Contractions with amplitudes of less than 65% of the mean maximum contractile amplitude were classified as “weak contractions” (Fig. 1, B and C), and those with amplitudes over 65% as “strong contractions” (Fig. 1, D and E). Contractions lasting over 2 min were defined as “tonic contractions” (Fig. 1, B and E), and those lasting less than 2 min as “phasic contractions” (Fig. 1, C and D). In addition, closely grouped strong phasic contractions of a frequency of almost one per minute were defined as “contraction bursts” (Fig. 1F). Contractile patterns other than those mentioned were lumped together and called “sporadic contractions”; these were difficult to characterize, but their amplitudes were less than 25% of the mean maximum value and their shapes were varied, as shown in Figure 1A.

2. Relationship between Each Type of Contraction and the Estrous Cycle

According to the criteria described above, all the recordings were classified into four phases. The first phase was quiescent with sporadic contractions (Table 1), lasted over 4 mo, and, judging from the cytological findings and plasma levels of estrogen and progesterone concentrations, corresponded to the anestrous phase. Plasma levels of estradiol and progesterone during this phase were 8.9 ± 0.69 pg/ml and 0.14 ± 0.03 ng/ml, respectively. These values were obtained for 20 blood samples collected in each of 5 dogs. Toward the end of anestru, weak tonic contractions started to occur at a mean frequency of 1.5 ± 0.20/h (Table 2). The mean duration of these weak tonic contractions was 5.1 ± 0.10 min, the longest in relation to all the types of nonpregnant uterine contractions observed (Fig. 1B). The anestrous phase gradually shifted to the second phase. This phase lasted 9.0 ± 1.7 days, and about 66% of the contractile activity consisted of strong tonic contractions, with the remainder strong phasic contractions. Plasma levels of estradiol and progesterone during this phase were 29.9 pg/ml and 0.97 ng/ml, respectively. These values were obtained from 1 dog. The third phase lasted 11.3 ± 2.4 days,

TABLE 2. Frequency, duration, and amplitude of nonpregnant uterine contractions.

<table>
<thead>
<tr>
<th>Contractions</th>
<th>Frequency (contractions/h)</th>
<th>Duration (min)</th>
<th>Amplitude (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weak tonic</td>
<td>1.5 ± 0.20*</td>
<td>5.05 ± 0.10</td>
<td>53.9 ± 0.67</td>
</tr>
<tr>
<td>Weak phasic</td>
<td>10.4 ± 0.73*</td>
<td>1.24 ± 0.01</td>
<td>63.5 ± 4.3</td>
</tr>
<tr>
<td>Strong phasic</td>
<td>12.5 ± 2.36**</td>
<td>1.3 ± 0.04</td>
<td>106.0 ± 0.01</td>
</tr>
<tr>
<td>Strong tonic</td>
<td>6.7 ± 0.34*</td>
<td>2.8 ± 0.17</td>
<td>83.2 ± 18.17</td>
</tr>
<tr>
<td>Burst</td>
<td>15.6 ± 1.48**</td>
<td>0.8 ± 0.06</td>
<td>111.3 ± 19.92</td>
</tr>
</tbody>
</table>

* Determination of amplitude is explained in Experimental Procedures.
*+, **, *: Means ± SE of the mean values obtained for each of 8, 4, and 3 dogs, respectively.

TABLE 1. Distribution of contractile patterns during the four phases of the estrous cycle.

<table>
<thead>
<tr>
<th>Patterns</th>
<th>Anestrus</th>
<th>Proestrus</th>
<th>Estrus</th>
<th>Diestru</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quiescent</td>
<td>51.4 ± 4.54*</td>
<td>0</td>
<td>0</td>
<td>10.3 ± 6.3*</td>
</tr>
<tr>
<td>Sporadic</td>
<td>39.4 ± 4.5</td>
<td>0</td>
<td>0</td>
<td>18.0 ± 1.12</td>
</tr>
<tr>
<td>Weak tonic</td>
<td>9.2 ± 0.85</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Weak phasic</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>71.6 ± 7.39</td>
</tr>
<tr>
<td>Strong phasic</td>
<td>0</td>
<td>33.9 ± 0.56**</td>
<td>85.8 ± 3.70*</td>
<td>0</td>
</tr>
<tr>
<td>Strong tonic</td>
<td>0</td>
<td>66.1 ± 0.56</td>
<td>11.4 ± 2.17</td>
<td>0</td>
</tr>
<tr>
<td>Burst</td>
<td>0</td>
<td>0</td>
<td>2.8 ± 1.53</td>
<td>0</td>
</tr>
</tbody>
</table>

*+, **, *: Values represent the percentage of each phase; mean ± SE of the mean values obtained for each of 8, 3, and 4 dogs, respectively.
The results for these two phases are presented, this is stated in the text.

The strong tonic contractions occurred mainly during the proestrus phase (6.7 ± 0.34 times/h) and lasted 2.8 ± 0.17 min (Table 2). The frequency and duration of the strong phasic contractions were 12.5 ± 2.36 times/h and 1.3 ± 0.04 min, respectively. This type of contractile activity prevailed during the estrous phase, and contraction bursts lasting 8.2 ± 1.00 min always occurred during this phase of strong phasic contractions. Such bursts occurred intermittently 5–10 times a day, the mean being 7.8 ± 1.6 times/24 h; the frequency of the contractions in the bursts was 55.6 ± 1.48 times/h, unequivocally greater than that for any other type of nonpregnant uterine contraction. This active phase of phasic contractions gradually progressed in about 2 wk to the fourth phase, which consisted mainly (72%) of weak phasic contractions and lasted about 60 days. This phase corresponded to the diestrous phase, according to the cytological findings and the plasma levels of estradiol and progesterone. Plasma levels of estradiol and progesterone during this phase were 11.9 ± 2.1 pg/ml and 6.2 ± 1.83 ng/ml, respectively. These values were obtained via 12 collections in each of the 5 dogs. The duration and frequency of the weak phasic contractions were 1.2 ± 0.01 min and 10.4 ± 0.73 times/h, respectively (Table 2). The activity during the remainder of the diestrous phase comprised quiescence and sporadic contractions. The detailed relationships between the contractile patterns and estrous cycle phases are summarized in Table 1.

3. Characterization of Cholinergic and Adrenergic Control of Nonpregnant Uterine Contractions

Most of the contractions examined in this study of cholinergic and adrenergic control were weak phasic contractions during the diestrous phase. Strong phasic and tonic contractions were also examined, but the number of examinations was limited (n ≤ 3) because of the short durations of the proestrous and estrous phases. However, when results for these two phases are presented, this is stated in the text.

a. Effects of cholinergic agonist and antagonists. It was found that atropine and hexamethonium did not affect uterine contractile activity in the diestrous and estrous phases, although these two cholinergic inhibitors inhibited gastric contractions strongly (data not shown). Bethanechol, a typical muscarinic agonist, stimulated uterine contractile activity in a dose-dependent manner (Fig. 2) and stimulated gastric contractile activity. Bethanechol-stimulated uterine and gastric contractions were inhibited by 0.05 mg/kg atropine.

b. Effects of α2-adrenergic agonists and antagonists. The effects of α1- and α2-agonists and antagonists are shown in Figure 3. The α1-adrenergic (prazosin) and α2-adrenergic (yohimbine) antagonists at a dose of 3.0 mg/kg per hour had no significant effects on weak or strong (n = 2) phasic contractions, whereas α1 (phenylephrine) and α2 (clonidine) agonists (both 3.0 mg/kg per hour) stimulated weak and strong (n = 2) phasic contractions strongly and increased the motor index strongly. Typical contractile responses of the uterine horn and gastric antrum to clonidine are shown in Figure 4. Initially, clonidine evoked tonic contractile activity, but this gradually changed to phasic contractions that resembled the strong tonic contractions, whereas gastric antral contractile activity was abolished by clonidine.

c. Effects of β-adrenergic agonists and antagonists. The effects of β1 and β2 adrenergic agonists and antagonists are...
contractile activity under normal conditions in nonpregnant

evoked by oxytocin. Each phase of the estrous cycle were quite similar to those hormonal-level evidence. The responses to although we were unable to obtain confirmatory plasma fore, we considered this period to be the estrous phase, during the other phases, as can be seen in Figure 7. There-
creases with superimposed phasic contractions; in contrast, anestrus were very characteristic, consisting of tonic in-
contractions and contraction bursts, the contractile response when oxytocin was given during the period of strong phasic

4. Effects of Oxytocin and PGF2α

Figure 7 shows typical uterine contractile responses to oxytocin during the four phases of the canine estrous cycle. Oxytocin and PGF2α both stimulated contractile activity during diestrus in a dose-dependent manner (Fig. 8). Oxytocin-induced contractions during proestrus, diestrus, and anestrus were very characteristic, consisting of tonic increases with superimposed phasic contractions; in contrast, when oxytocin was given during the period of strong phasic contractions and contraction bursts, the contractile response was phasic with no tonic increase, quite different from that during the other phases, as can be seen in Figure 7. Therefore, we considered this period to be the estrous phase, although we were unable to obtain confirmatory plasma hormonal-level evidence. The responses to PGF2α during each phase of the estrous cycle were quite similar to those evoked by oxytocin.

DISCUSSION

The aim of the present study was to measure uterine contractile activity under normal conditions in nonpregnant

conscious dogs in order to establish whether the nonpregnant uterus truly contracts and, if so, what types of contraction occur during the various phases of the estrous cycle and how these contractions are controlled by the cholinergic and adrenergic nervous systems. In order to avoid artifacts, we used a strain-gauge force transducer to measure uterine contractility. This method [11, 15, 23–26] has been validated previously in our laboratory in experiments designed to measure gastrointestinal contractile activity in conscious dogs. A similar study with ovariectomized ewes was conducted by Garcia-Villar et al. [11, 15].

We found that, provided the transducer is small enough to fit across the width of the canine uterine horn, this method is also useful for measuring uterine contractile activity because there are few artifacts attributable to the movements of the animals, and the transducer has a reliable and long life after implantation, with no noticeable influence on the estrous cycle or the organs in the abdominal cavity of the dog. The quality of the information yielded by the transducer method is also superior to that obtained with electromyogram and intrauterine pressure measurement systems: the contractile wave recordings generated by a force transducer provide more details of the characteristics of contractions than an electromyogram.
First, the present study provided us with a huge amount of data on the contractile activity of the nonpregnant uterus, which showed a great variety of contraction waves. Therefore, we had to establish our own criteria to classify the contraction waves after repeated visual inspection of all the canine uterine contraction recordings. We found that the uterine contractions could be classified into six major contractile components, shown in Figure 1, by analyzing them on the basis of the amplitudes and durations of contraction. In addition to analyzing the contractile activity, we analyzed the stage of the estrous cycle with the aid of cytological vaginal smear findings and the plasma levels of estradiol and progesterone; we reached the conclusion that nonpregnant uterine contractile activity in the dog is controlled in association with the estrous cycle. In particular, a period of maximal contractile activity that lasted 20.3 ± 4.10 days between the anestrous and diestrous phases was considered to represent the proestrus and estrous phases. The proestrus phase was characterized by strong tonic contractions, and the estrous phase was characterized by strong phasic contractions accompanied by intermittent strong phasic contraction bursts. The uterine responses to oxytocin and PGF2α were different in the proestrus and estrous phases. Oxytocin- and PGF2α-induced contractions in proestrus were strong tonic contractions accompanied by phasic contractions, whereas during estrus, the contractile response was phasic with no increased tonicity. Quite similar findings were described by Wheaton et al. [18] in 1988.

We observed that the uterus contracted spontaneously and very vigorously during the proestrus and estrous phases. In particular, it should be noted that during estrus, contraction bursts occurred intermittently during the period when strong phasic contractions prevailed, whereas this type of burst activity never occurred during diestrus, proestrus, or anestrus. A review of the literature revealed that nonpregnant canine uterine contraction bursts have not been reported before, although Ruckebusch and Bueno [12] reported bursts of myometrial activity in ewes. Milenov et al. [9] employed the term "burst" in their publication, but they used it to represent "spike potential bursts," not contraction bursts, and therefore their bursts appear to correspond to the phasic or tonic contractions in our study. As shown in our present investigation and other studies [17, 18], the nonpregnant canine uterus is by no means quiescent but, contracts actively in harmony with the phases of the estrous cycle. The physiological role of each type of contraction, however, is unknown, although the uterus may contract to discharge serosanguineous fluid during proestrus, and the characteristic contractile activity observed during estrus may play an important role in the transport of sperm and ova [11, 18]. The physiological significance of uterine contractile activity during diestrus, however, is much unclear, but the continuous phasic contractions may clean the uterine interior by removing desquamated cells and endometrial excretions.

With regard to the involvement of estrogen and progesterone in spontaneous uterine contractions, it is generally considered that estrogen increases uterine contractility [11, 22] whereas progesterone decreases it [2, 5, 6, 11, 16, 29]. In the present study, however, uterine contractile activity occurred during diestrus, even when the plasma progesterone concentration was elevated markedly; and when the plasma estradiol and progesterone concentrations were low, the uterus was quiescent. In contrast, Verma and Chibuzo [16] reported that spontaneous uterine contractions increased after the simultaneous administration of estrogen and progesterone to dogs. In view of these conflicting findings, it cannot be concluded that estrogen simply increases and progesterone inhibits uterine contractility. These findings suggest that uterine smooth muscle contractile activity is controlled by an exquisite balance between estrogen and progesterone and many other neurohumoral factors.

The various responses of uterine smooth muscle to oxytocin and PGF2α during the four phases of the estrous cycle are of interest. In fact, the motor indices for these contractions during proestrus, diestrus, and anestrus differed significantly. In contrast, oxytocin- and PGF2α-induced contractions during estrus were strong and phasic, were not accompanied by tonic increases, and were quite different from those during the other three phases. Similar findings were reported by Wheaton et al. [18]; but we noticed great differences between the durations of each type of contractile activity in their study and ours. Hawk and Conley [26] described strong phasic contractile responses to PGF2α; another study indicated that the uterus has separate independent receptors for oxytocin and PGF2α, and that estrogen increased the affinity and number of uterine oxytocin receptors [30]. Therefore, high plasma estrogen concentrations may increase the responsiveness of the uterus to oxytocin and PGF2α, and appear to be responsible for the strong phasic contractions without tonic increases in response to these hormones.

With regard to the major concern of this study, i.e., the neural control of spontaneously occurring uterine contractions, acetylcholine and noradrenaline are the major neurotransmitters of the autonomic nervous system, and the uterus is no exception to cholinergic and adrenergic control. The uterine contractile responses to these neurotransmitters have been studied widely, mostly in vitro, in various experimental animal species [3, 10, 14, 19, 25, 26, 31–33] as well as in humans [4, 7, 8, 34]; considerable information about the mechanism or mechanisms by which these neurotransmitters stimulate contraction has been gathered. With respect to the contractile response of the uterus to cholinomimetics, many studies have indicated the existence of abundant muscarinic receptors in the uterine smooth muscle [3, 4, 7, 8, 35–38]. In fact, in the present study, bethanechol strongly stimulated contractions of the uteri of conscious dogs, but it is interesting to note that the spontaneously occurring phasic contractions were not affected by muscarinic or nicotinic receptor antagonists. In fact, Wray [33] stated that the role of cholinergic nerves in uterine contractile activity can only be a small one, such as coordination of activity. Marnet et al. [13] demonstrated that ovine myometrial responses to phenylephrine and clonidine consisted of activation of contraction and assigned α2 receptors a chronotropic function, whereas they suggested that α1 receptors were responsible for inotropic control. In our experiments, however, we did not observe these two different responses and therefore concluded that the spontaneously occurring phasic contractions of the nonpregnant canine uterus are not attributable to cholinergic innervation.

On the other hand, it is well known that noradrenaline, a sympathetic nervous system transmitter, has varying effects in different animal species and during the estrous cycle [32, 33]. In reviewing uterine adrenoreceptors, Digges [32] noted, for example, that noradrenaline relaxes the longitudinal muscle of the rat uterus during the middle period of pregnancy through inhibitory β-receptors, whereas it strengthens uterine contractile activity at delivery through excitatory α receptors. The effects of noradrenaline on cir-
cular muscle contractile activity, however, are the opposite [32, 33]. Of the adrenomimetics, ritodrine [39–42], a β2 adrenergic receptor agonist, significantly inhibited the spontaneous uterine contractions of diestrus in nonpregnant conscious dogs in a dose-dependent manner, but the fact that complete inhibition was not achieved, even at a high dose of 3.0 mg/kg per hour, suggests strongly that other unknown nonadrenergic and noncholinergic factors are involved in the control of spontaneous uterine contractions. With regard to other adrenergic receptors, phenylephrine (α1 agonist) [13, 14, 25], clonidine (α2 agonist) [13], and butoxamine (β2 antagonist) were found to enhance uterine spontaneous contractile activity, whereas prazosin (α1 antagonist) [10], yohimbine (α2 antagonist) [10], dobutamine (β1 agonist), and atenolol (β1 antagonist) did not influence the spontaneous contractions at all.

Uterine smooth muscle has been demonstrated to possess excitatory α receptors and inhibitory β-receptors, and its actual response to catecholamines is controlled by the predominant receptor [32, 33]. The type of receptor that predominates has been reported to depend on the muscle layers [3] and the animal species [31] and on whether the animal is pregnant, nonpregnant, or parturient [32, 33]. In the present study on nonpregnant conscious dogs, we found that α1 and α2 receptors were responsible predominantly for contraction and β2 receptors for inhibition of contractions during the diestrous phase. However, the adrenergic receptor subtypes responsible for contractile activity during proestrus and estrus could not be analyzed fully as we were within the diestrous phase. However, the adrenergic receptor subtypes responsible for contractile activity during proestrus and estrus could not be analyzed fully as we were unable to perform a sufficient number of experiments, including a dose-response study, to cover all receptor subtypes because the duration of proestrus and estrus is very short in the dog.

In conclusion, the uterus of the nonpregnant conscious dog is by no means quiescent but contracts actively throughout the estrous cycle. The characteristics of the contractions vary with the phase of estrus. During estrus and proestrus, the uterus is most active, with vigorous phasic and tonic contractions accompanied by intermittent contraction bursts. Phasic contractions occur even during diestrus, although their frequency is diminished in comparison with those during proestrus and estrus. The contractile activity is controlled by α1 and α2 receptors, which increase it, and by β2 receptors, which inhibit contraction. The participation of cholinergic control in uterine activity can be ruled out.

REFERENCES