Reversal of the Effects of 4-Amino-Antipyrine on Myometrial and Cervical Electromyographic Activity by PGF$_{2\alpha}$ in the Ovariectomized Estradiol-Replaced Non-Pregnant Ewe

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Cervical and uterine electromyograms (EMG) have been recorded from ovariectomized non-pregnant ewes. When the animals were infused with saline, the frequency of EMG events lasting less than 180 seconds was not different from those lasting more than 180 seconds. During infusion of estradiol at 100 $\mu$g · 24 hours$^{-1}$ into the maternal jugular, the frequency of events less than 180 seconds increased significantly in the myometrium and in the cervix. Contracture activity (events lasting more than 180 seconds) was not significantly different in the myometrium compared to the cervix before estradiol administration. During estradiol infusion, the contracture activity remained unchanged.

During 4-amino-antipyrine (4AA) administration, the contracture activity decreased significantly in the myometrium, while an insignificant change occurred in the cervix. This state was associated with a decrease in the venous PGFM:6-keto F$_{1\alpha}$ plasma ratio. Infusion of PGF$_{2\alpha}$ (.5 $\mu$g min$^{-1}$ and 1.0 $\mu$g min$^{-1}$ for ten minutes) into the femoral artery resulted in a significant increase in the frequency of events less than 180 seconds in both the myometrium and cervix. For the duration of the ten-minute infusion, the activity was contracture-like.

These findings suggest that the cervix may not only be influenced by mechanical properties (stretch) and local paracrine factors but also by various stimulators and inhibitors irrespective of the myometrium.

INTRODUCTION

Several groups of investigators have described the characteristics of the in vivo electromyogram (EMG) recorded from the whole myometrium and cervix in the sheep and other species in both the pregnant and non-pregnant state [1-10]. The independence of uterine and cervical motility in the ruminant has been investigated [1]. The aim of the present study was to describe the characteristics of EMG activity of the cervix in the ovariectomized non-pregnant ewe, and to investigate the reversal of 4-amino-antipyrine (4AA) effects in the estradiol-replaced ewe by the administration of exogenous PGF$_{2\alpha}$. Cervical and myometrial EMG activity was recorded both before and during the continuous intravenous administration of estradiol, 4AA, and during PGF$_{2\alpha}$ treatment. If differences exist, they may throw light on possible different regulatory mechanisms, enabling us to evaluate possible myogenic (prostacyclin:PGF$_{2\alpha}$ ratio) or neurogenic factors acting on the reproductive tract.

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MATERIALS AND METHODS

Experimental Animals and Surgical Procedures

Four non-pregnant Rambouillet-Columbia ewes were used in the study. Four animals were used because we desired to detect at least a 15 percent change in EMG activity at \((1 - \beta = .80)\), the .05 significance level. Ewes were premedicated with 800–900 mg of ketamine and .5 mg of glycopyrrolate, administered intramuscularly. Under halothane anesthesia, bilateral ovariectomy was carried out in all ewes. At least five days were allowed to elapse after surgery before any infusions were started. Polyvinyl catheters (1.8 mm, outer diameter; 1.02 mm, inner diameter) were inserted into one uterine vein on each side through an entry point near the tip of the uterine horn. The catheters were passed 15 cm in the direction of the inferior vena cava so that the tip of the catheter lay in the main uterine vein. Catheters were also placed in one jugular vein and carotid artery. A fifth vascular catheter was placed in the femoral artery on one side and advanced 30 cm into the dorsal aorta.

Myometrial EMG electrodes were fabricated as described previously [2]. Four pairs of electrodes were placed; two pairs were sewn into the cervix in the region of the external os and one pair into each uterine horn near its junction with the body. In each pair, the electrodes were 2–3 mm apart.

Infusions

All vascular catheters were continuously infused with physiological saline (NaCl 0.9 percent W/V) containing 50 U heparin ml\(^{-1}\) at a rate of .5 ml · hour\(^{-1}\). When estradiol was infused, it was administered to the maternal jugular vein in a solution of 2.5 percent ethanol in physiological saline (V/V) at a rate of 100 \(\mu\)g estradiol 24–72 in 39 ml · 24–72. When 4AA (prostaglandin synthetase inhibitor) was infused, it was administered to the maternal femoral vein in a solution of 210 · mg ml\(^{-1}\) of saline. Fifteen mg · min\(^{-1}\) was infused for four hours. PGF\(_{2\alpha}\) was prepared by dissolving .1 ml of PGF\(_{2\alpha}\) (100 \(\mu\)g frozen crystal dissolved in .1 ml of saline) into 4.9 ml of saline. To make up .2 \(\mu\)g, 2 \(\mu\)g, 5 \(\mu\)g, and 10 \(\mu\)g preparations, the 5 ml stock solution was serially diluted. The PGF\(_{2\alpha}\) treatment was repeated sequentially for each concentration. The rates of infusions were .02 \(\mu\)g, .2 \(\mu\)g, .5 \(\mu\)g, and 1.0 \(\mu\)g · min\(^{-1}\) for ten minutes, respectively. It should be noted that PGF\(_{2\alpha}\) was used immediately after preparation because it is unstable in solution. Experimental treatments are presented below. All four ewes were subjected to all four experimental treatments.

Treatment 1: PGF\(_{2\alpha}\) administration without either 4-amino-antipyrine or estradiol

Treatment 2: Continuous estradiol infusion (baseline recording 48–72 hours)

Treatment 3: 4AA administration with continuous estradiol infusion

Treatment 4: PGF\(_{2\alpha}\) and 4AA administration during continuous estradiol infusion

During continuous estradiol infusion, each animal received an administration of the prostaglandin synthetase inhibitor 4AA at an infusion rate of 15 mg · min\(^{-1}\) in the maternal femoral vein for four hours. Resting samples were obtained from the uterine vein catheters. Samples were designated as P\(_4\) (prostaglandin analysis) and S\(_4\) (corticosteroid analysis). Four ml of blood were collected every hour in chilled heparinized tubes (S\(_4\)) and EDTA-aspirin tubes (P\(_4\)), centrifuged for 11 minutes,
frozen with liquid nitrogen, and then stored until analysis. The EDTA-aspirin was used to prevent additional production of prostaglandins in vitro.

As described above, during simultaneous 4AA and estradiol infusion, PGF$_{2\alpha}$ was administered in the femoral arterial catheter for ten minutes at .02 $\mu$g, .2 $\mu$g, .5$\mu$g, and 1.0 $\mu$g • min$^{-1}$. Samples were collected for eight hours and processed as described above. Sampling was staggered in five-minute and 30-minute intervals with, generally, a total of 19 samples per animal.

**Plasma Prostaglandin Measurements**

Blood samples were analyzed from three ewes. PGFM (PGF$_{2\alpha}$ metabolite) and 6-keto F$_{1\alpha}$ (prostacyclin metabolite) titers were determined. The methods of the assay are reported elsewhere [3].

**Recording of EMG Signals and Data Analysis**

EMG signals were recorded and stored on a custom-built data acquisition system as previously described [4]. Individual epochs of activity were analyzed for duration and activity, using a two-hour running mean to set the threshold for distinguishing signal from noise, and an eight-second delimiter to determine when an epoch was completed. The EMG recordings were made continuously and analyzed in two-hour time blocks.

In order to relate the present studies to our findings in the pregnant sheep, we have divided epochs of EMG activity recorded in this study to those of duration less than 180 seconds and epochs of activity greater than 180 seconds' duration [4]. Forty-eight to 72 hours of data were analyzed for each animal without estradiol administration and at least 48 hours of data during estradiol administration. Data throughout are given as means ± SD.

**Statistical Analysis Methods**

Data were analyzed on the basis of number of events in two-hour time intervals. Each sheep had recordings available from at least six continuous two-hour time intervals. The maximum was twenty-three. The data for individual two-hour time intervals for each sheep were averaged to give data points for the full period of study on that animal on estradiol, 4AA, and PGF$_{2\alpha}$ treatment with or without estradiol. EMG activity in the cervix and myometrium was compared by the two-independent student t-test or the Wilcoxon sign rank test when variances differed greatly. Correlations between plasma prostaglandin concentrations (PGFM:6-keto F$_{1\alpha}$ ratio) and PGF$_{2\alpha}$ dosages were evaluated using the Pearson's correlation coefficient.

**RESULTS**

**Short-Duration Epochs**

In the absence of estradiol administration there was no significant difference in the frequency of short epochs between the cervix 76 ± 18 and the myometrium 60 ± 47 ($p = .20$). During estradiol infusion, the frequency of short epochs in the cervix increased from 76 ± 18 to 89 ± 22 ($p < .05$), while it was more significantly increased in the myometrium from 60 ± 47 to 201 ± 62 ($p < .0001$); also, the frequency of short epochs was significantly greater in the myometrium—201 ± 62 compared with the cervix—89 ± 22 ($p < .0001$). During the administration of 4AA (15 mg • min$^{-1}$ for four hours), the frequency of short epochs was increased insignificantly in both
myometrium—from 66 ± 19 to 74 ± 17 (p = .37)—and the cervix from 81 ± 28 without 4AA to 84 ± 22 (p = .54) with 4AA (Tables 1 and 2).

**Contracture Activity**

In the absence of estradiol, the difference between the frequency of contractures (epochs > 180 seconds) in the cervix (2.0 ± 1.4) and in the myometrium (3.0 ± 1.4) was insignificant (p = .32). During estradiol administration, contracture frequency did not change significantly in either the myometrium—3.0 ± 1.4 to 3.3 ± 1.5 (p = .80), or in the cervix—2.0 ± 1.4 to 2.6 ± .65 (p = .53) (Table 1). During 4AA administration (Table 2), however, contracture frequency decreased significantly in the myometrium—2.8 ± 1.5 to 1.0 ± .8 (p < .02) but was not changed significantly in the cervix—1.5 ± 1.6 to 1.1 ± .9 (p = .49) (Table 2).

**Activity Profile and the Administration of PGF<sub>2α</sub>**

The administration of PGF<sub>2α</sub> immediately elicited contracture-like activity in both the cervix and myometrium (Table 3). The cervix responded well to the .5 μg · min<sup>-1</sup> while in the myometrium, contracture-like activity was elicited at .2 μg · min<sup>-1</sup>. After the withdrawal of the PGF<sub>2α</sub>, the electromyographic activity reappeared progressively both in the cervical and myometrical EMG recording within five to eight minutes. It was similar to that described during the control period (Fig. 1).

When the effect of PGF<sub>2α</sub> stimulation was maximal, i.e., .5 μg · min<sup>-1</sup> or 1.0 μg · min<sup>-1</sup> as suggested by the highest level of electromyographic activity recorded, the cervix and horns displayed continuous activity for the duration of the PGF<sub>2α</sub> infusions. In addition, the response of the cervix and myometrium appeared to be in phase. By 12 hours after the cessation of the 4AA infusion, the uterine horns and the cervix displayed regular electromyographic activity similar to those described during the control period (Fig. 3).

### Table 1

|                | Cervix (<180 Seconds) | Cervix (>180 Seconds) | Myometrium (<180 Seconds) | Myometrium (>180 Seconds) |
|----------------|-----------------------|-----------------------|---------------------------|---------------------------|
| Without E2    | 76 ± 18               | 2.0 ± 1.4             | 60 ± 47                   | 3.0 ± 1.4                 |
| With E2       | 89 ± 22               | 2.6 ± 0.7             | 201 ± 62                  | 3.3 ± 1.5                 |

### Table 2

|                | Cervix (<180 Seconds) | Cervix (>180 Seconds) | Myometrium (<180 Seconds) | Myometrium (>180 Seconds) |
|----------------|-----------------------|-----------------------|---------------------------|---------------------------|
| With E2       | 81 ± 28               | 1.5 ± 1.6             | 66 ± 19                   | 2.8 ± 1.5                 |
| 4AA + E2      | 84 ± 22               | 1.1 ± 0.9             | 74 ± 17                   | 1.0 ± 0.8                 |
TABLE 3
Average Duration of EMG Epochs of >180 Seconds for a Two-Hour Period, Recorded from Four Ovariectomized Non-Pregnant Sheep with 4AA Infusion at 15 mg · min⁻¹ and During PGF₃₀ Infusion .5µg · minute for 10 minutes (Data during PGF₃₀ administration were obtained for ten minutes.)

|                   | Cervix >180 Seconds | Myometrium >180 Seconds |
|-------------------|---------------------|-------------------------|
| PGF₃₀ alone       | 1,060 ± 25          | 960 ± 32                |
| E₂ only           | 680 ± 700           | 720 ± 140               |
| E₂ + 4AA          | 432 ± 70            | 468 ± 44                |
| E₂ + 4AA + PGF₃₀ | 1,020 ± 40          | 1,074 ± 26              |

Plasma Prostaglandin Concentration

Plasma PGFM (PGF₂α metabolite) levels increased from 55 ± 5.5 pg · ml⁻¹ to 180 ± 215.4 pg · ml⁻¹ during estrogen replacement while 6-keto F₁α (prostacyclin metabolite) dropped from 58 ± 33.8 pg · ml⁻¹ to 25 ± 7.2 pg · ml⁻¹.

During the administration of 4AA, the plasma PGFM decreased from 180 ± 215.4

FIG. 1. Electromyogram from a bilaterally ovariectomized non-pregnant ewe infused with saline. A, myometrium left horn  B, cervix  C, myometrium left horn during PGF₃₀ infusion (1.5 µg · min⁻¹)  D, cervix during PGF₃₀ infusion (.5 µg · min⁻¹).
to 47.0 ± 28.4 pg · ml⁻¹, compared to 6-keto F₁₆ — 25 ± 7.2 without 4AA to 34 ± 16.5 with 4AA. None of the above values were found to be statistically significant.

Without estradiol replacement the PGFM:6-keto F₁₆ ratio in the plasma increased proportionately with dose (r = .88, p < .05), while with estradiol replacement the association was even more pronounced (r = .97, p < .01) (Table 4). Samples were taken five minutes after the start of infusion.

**DISCUSSION**

In all four ewes studied, the cervix in the non-pregnant ewe demonstrated regular EMG activity. The cervical EMG activity exhibited both similarities to, and differences from, the EMG recorded simultaneously from the myometrium. In the presence of estradiol, there was no difference in the frequency of contractures, but the myometrium demonstrated significantly more short epochs than the cervix. During 4AA treatment, the contracture activity of the myometrium significantly decreased while the cervical contracture activity remained unchanged. There was an insignificant increase in the frequency of short epochs both in the myometrium and cervix during 4AA administration. We have data, as yet unpublished, to suggest that 100 µg estradiol · day⁻¹ is a supramaxial dose in regard to the myometrial EMG activity.
Although an $n$ number of 4 is too small a sample size on which to base treatment effect, the reader might wonder whether or not we can accept as valid any statistical conclusions drawn on the basis of this particular small sample. The truth of the matter is that, as long as we have met the underlying assumptions for the statistical test, the rejection of $H_0$ at a .05 significance level is no more significant, statistically, when $n$ is large than when $n$ is four. Since we used the .05 significance level and rejected $H_0$, the changes in EMG activity in question are just as rare or unusual no matter how many ewes we test. If anything, we might be more impressed by our rejection of no treatment differences $H_0$ with our small sample size of four.

There are several possible explanations of the mechanisms underlying the difference between the myometrium and cervix in response to 4AA. It is possible that the changes
in the other uterine tissues, such as the endometrium, do not play a significant role in the cervix. The cervix may be more prone to sympathetic innervation [11,12]. Differences in local PGF$_{2\alpha}$ prostacyclin production, and/or catabolism by the tissues lining the uterine cavity might be one such mechanism. In addition, 4AA is thought, to a great extent, to influence the blood flow to the uterus. It is quite possible that the results may also be due to a difference in blood flow, and thus in the distribution of 4AA to the tissue of the myometrium and cervix. Another mechanism may result from differences in the number of neurogenic receptors and in the number of gap junctions [13].

PGF$_{2\alpha}$ given at low dosages did not elicit contracture-like EMG activity in the cervix as viewed in the myometrium. This lack of responsiveness was less pronounced on estrogen replacement. This finding may result from more PGF$_{2\alpha}$ receptors in the myometrium. Alternatively, the greater response of the myometrium simply may reflect histological differences [14].

Although there was lack of statistically significant changes in plasma PGFM and 6-keto F$_{1\alpha}$ titers during estradiol replacement or 4AA administration, there appeared to be an inverse relationship between these two metabolites. It has been suggested that in some species, such as sheep and human, endogenous PGI$_2$ of uterine origin may be an inhibitor of myometrial activity in different states, including pregnancy. Such an inhibition might be overcome by elevated PGF$_{2\alpha}$ production at the tissue site [15]. However, as a result of the low statistical power of the test ($n = 3$), no conclusion is warranted here. Therefore, these PGFM:6-keto F$_{1\alpha}$ trends may only provide justifica-

**TABLE 4**
The Mean ± SD of the PGFM:6-keto F$_{1\alpha}$ Ratio from Three Non-Pregnant Ovariectomized Sheep With or Without Estradiol and During the Administration of 4-amino-antipyrine (Samples were collected five minutes within the PGF$_{2\alpha}$ ten-minute infusion.)

|                      | Without Estradiol: PGFM:6-keto F$_{1\alpha}$ Ratio | With Estradiol: PGFM:6-keto F$_{1\alpha}$ Ratio |
|----------------------|---------------------------------------------------|-----------------------------------------------|
| Control              | 1.1 ± 0.5                                         | 6.9 ± 7.8                                     |
| With 4AA             | No experiment                                     | 1.5 ± 1.1                                     |
| PGF$_{2\alpha}$ (µg · min$^{-1}$) |                                                   |                                               |
| .0                   | 2.4 ± 1.6                                         | 3.1 ± 1.6                                     |
| .2                   | 4.8 ± 4.5                                         | 7.7 ± 3.5                                     |
| .5                   | 11.7 ± 5.8                                        | 13.8 ± 5.3                                    |
| 1.0                  | 14.0 ± 14.2                                       | 18.5 ± 13.5                                   |
tion for generating a hypothesis concerning the interplay between PGF$_{2\alpha}$ and prostacyclin in relation to EMG activity.

In conclusion, we suggest that electrophysiological differences between the cervix and uterus may not result totally from mechanical properties or differences in blood flow but may possibly represent differences in response to regulatory mechanisms. For example, we have investigated the broad ligament (mesometrium) and found important differences in regard to the response to estradiol replacement [accepted for publication]. It is interesting to note that many of the (sympathetic postganglionic) fibers which travel via the broad ligament converge at the border of the cervix [11,12]. It is known that α-adrenergic stimulation increases myometrial contractility by further enhancing PGF$_{2\alpha}$ and E$_2$ synthesis while β-adrenergic stimulation reduces contractility by further enhancing 6-keto F$_{1\alpha}$ production in pregnant human myometrial strips [15]. Unfortunately, no investigations have been conducted recording simultaneous cervical and mesometrial EMG activity, measuring regional differences in PG metabolites under adrenergic stimulation or inhibition. Also, an appropriate in vitro model should be searched for, enabling experimenters to circumvent possible systemic effects and to allow one to focus at the tissue level. Such investigations may further enable us to elucidate or describe possible mechanisms of control and/or regulation.

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