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Effects of Cigarette Smoke on Uterine Wall Contractions in Virgin Female Long Evans Rats

Kyle T. Gibbens
Department of Biology
The University of Nebraska at Kearney
905 West 25th Street
Kearney, Nebraska 68849 USA

Faculty Advisor: Dr. Janet Steele

Abstract

Premature birth is a major risk factor for women who smoke while pregnant, although the casual agents in the smoke are unknown due to the large number of chemicals and compounds composing it. There are different possible explanations for this effect that involve hormones such as oxytocin and progesterone, as well as nicotine, a major component of cigarette smoke. The effects of smoke on myometrial contractions can be seen by exposing virgin female Long Evans rats to cigarette smoke extract for a period of three weeks. The smoke was extracted into a liquid form that could be administered drop-wise onto the noses of the rats. A survival surgery was performed in order to remove the uterine horns, which were then cut into two cross-sections. These cross-sections, when placed in an isolated tissue bath and exposed to increasing concentrations of oxytocin, were forced to contract while a computer recorded the contractions. A trace was formed showing the strengths and frequencies of contractions per dose of oxytocin. The results showed that there was a significant difference in the frequencies and durations of contractions when compared to the control group, but not a significant change in force. This suggests that smoking prior to pregnancy can potentially predispose the myometrium to abnormal responses in the presence of oxytocin.

Keywords: Cigarette smoke, prior to pregnancy, uterine contractions

1. Introduction

Smoking cigarettes or exposure to second hand smoke while pregnant may lead to a greater risk of premature birth and death by Sudden Infant Death Syndrome (SIDS) [22, 15]. Maternal smoking is one of the highest risk factors for SIDS, because the risk of death is increased when a baby is born prematurely[22]. Premature birth is seen in up to 13% of pregnancies, and smoking may be one of the causes for this[9]. Preterm birth can also increase the chances of the infant being born blind, deaf, or with pulmonary and neurological disorders[3]. However, the mechanisms behind the effects of cigarette smoke, such as certain diseases and physiological defects, are not fully understood[10]. There are close to 4000 different toxic compounds that comprise cigarette smoke, each of which have different chemical properties and could potentially affect part of the reproductive system[7]. Currently, it is known is that when a human female smokes while she is pregnant, it increases the chance of shortening the gestation period and causing premature birth, increasing infant morbidity and mortality rates[24]. Additionally, any impact the smoke has on the fetus in the womb can increase its chances of being born with a low birth weight.

In order to discuss the effects of cigarette smoke on uterine muscle contractions, the physiology of these muscles must first be understood. The uterine muscle, the myometrium is stimulated to contract by a hormone called oxytocin[8]. This hormone is produced in the hypothalamus and is classified as a peptide hormone[1]. Once made, oxytocin is stored in and released from the posterior pituitary gland[13]. Although classified as a hormone because it

travels through the bloodstream to its target cells, oxytocin actually plays a role as a neurotransmitter. Since smoking can lead to premature birth, cigarette smoke may lead to more contractile sensitivity in the presence of oxytocin earlier in the pregnancy, as shown in previous studies[8, 18]. Oxytocin is one of the chemicals responsible for initiating the parturition process, so acting on the muscles too soon can cause premature birth[17]. Previous studies have used uterine tissue from pregnant rats in order to study the effects of cigarette smoke on a pregnant uterus[8, 18]. In this study, however, the rats were virgin females.

A possible explanation for how early oxytocin binding can occur deals with a second hormone called progesterone. During pregnancy, this hormone regulates myometrial contractions by blocking oxytocin binding sites until it is time for birth[6]. When progesterone is removed, oxytocin is free to bind to its receptors and act on the muscles, stimulating contraction. If oxytocin binds before the developmental period is complete, it could lead to premature birth. Progesterone also regulates the number of oxytocin receptors[17]. When progesterone secretion is reduced, the number of oxytocin receptors increases, which increases the probability that oxytocin will bind, leading to contraction. If exposure to the chemicals of cigarette smoke were able to affect progesterone and its regulation, it could lead to the previously mentioned problems experienced by women who smoke during pregnancy. The withdrawal of progesterone is one of the main initiators of labor because it allows oxytocin to bind and begin myometrial contractions[5]. Progesterone withdrawal is not fully understood, but some of the possible ways progesterone is withdrawn include inactivation in target cells or metabolite formation that prevents progesterone from binding to its receptors. It is possible that cigarette smoke can cause one or both of these to occur prematurely, withdrawing progesterone too soon and leading to premature contractions from oxytocin. Another way progesterone is thought to be withdrawn is through the production of prostaglandins, which have been suggested to mediate the initiation of labor[23]. If cigarette smoke somehow causes the production of prostaglandins, this could once again lower the progesterone levels in the uterus, leading to possible premature birth. In summary, if chemical compounds composing cigarette smoke decrease the progesterone levels in the uterus, this could provide causation for why, specifically, cigarette smoking increases the risk of premature birth in pregnant women.

Nicotine, a major component in cigarette smoke, may also be responsible for the results seen in women who smoke during pregnancy. When nicotine enters the body, it binds to nicotinic acetylcholine receptors, which mediate its effects[2]. One of the functions of these receptors is to stimulate the release of neurotransmitters[4]. Since nicotine binds to these receptors, they are thought to mediate the actions of nicotine such as the release of neurotransmitters such as oxytocin, although the exact mechanism as to how this occurs is unknown [19, 12]. The result is increased contractility of the myometrium[19]. Increased contractility can lead to problems such as premature delivery or spontaneous abortions. If nicotine is the component responsible for these problems, the risk factors can be reduced by the use of filtered cigarettes. Filtered cigarettes should have a different effect than non-filtered cigarettes because they reduce the amount of nicotine and tar taken into the body by as much as 50% when compared to non-filtered cigarettes[20]. Overall, filtered cigarettes have a smaller percentage of total particulate matter. If any of this matter is responsible for premature myometrial contractions, these effects can be reduced by using filtered cigarettes. However, with thousands of different compounds making up cigarette smoke, it is difficult to identify if one component is responsible or if a combination of components causes the problems found in women who smoke during pregnancy. Even in cases where smoking does not cause premature labor, there is still a risk of the infant being born underdeveloped. A study has shown that rats that were exposed to smoke had a decrease in uterine blood flow[14]. This would lead to a decrease in the amount of oxygen available to the developing fetus and could impair its growth.

From the above discussion, it can be concluded that much is known about the effects of smoking during pregnancy on the causation of premature birth, but little to nothing is known about the effects of smoking prior to pregnancy. Therefore, the purpose of this study is to determine if smoking prior to pregnancy can affect the uterine tissue of a virgin in the same ways that smoking affects the uterus during pregnancy. To study the effects of cigarette smoke prior to pregnancy, the tension generated by isolated rings of uterine wall muscles was examined. It was hypothesized that the cigarette smoke extract would have an effect on spontaneous myometrial contractions in virgin females, potentially predisposing the tissue to abnormal responses to oxytocin. This information is important to women of child-bearing age who believe the negative effects smoking can have on their child can be eliminated if they stop smoking during pregnancy.

2. Methods and Materials

2.1 Subjects And Animal Care

This project was approved by the University of Nebraska at Kearney Institutional Animal Care and Use Committee (protocol #052512). The experiment was conducted using 16 virgin female Long Evans rats. Early on in the study, one of the control rats developed a liver tumor and died, reducing the total number to 15. Seven of the rats served as controls while the other eight were designated as the experimental group. These rats were 12 months of age at the start of this study. They were kept in cages with a bedding of Premium Pine Shavings and fed Lab Diet 5001 Rodent Chow *ad libitum*.

2.2 Cigarette Smoke Extract Preparation

To produce the extract, an unfiltered Marlboro® cigarette was inserted into a vacuum pump apparatus created by Dr. Thomas Freeman. This pump was used to simulate the act of breathing using a hole in the tubing, which was repeatedly covered and uncovered with the thumb. The smoke entered a flask filled with about 80 ml of water. The smoke mixed with the water, creating the cigarette smoke extract in liquid form, which was administered drop wise into the noses of the eight experimental rats. Two cigarettes created enough smoke to produce the extract.

2.3 Exposure To Smoke Extract

During the procedure, the eight experimental rats were exposed to cigarette smoke extract for a period of three weeks, in order for the exposure to be considered subchronic and to give the muscles enough time for an adaptive response[11]. The seven rats in the control group were given saline nose drops. Using a pipette, 50 µl of liquid, which is about the size of one drop, was squirted into each nostril of the rats. This was done once daily for the length of the exposure period. At the end of the three weeks, that particular rat was finished with nose drops. Two new rats were started on nose drops each week in order to stagger the surgeries.

2.4 Surgery

Each rat was anesthetized with a 100 mg/kg ketamine, 20 mg/kg xylazine mixture, which was injected using a 23 gauge needle and a 1 ml tuberculin syringe[21]. The second rat that underwent surgery had a bad reaction to the anesthetic, which killed it quickly. From then on, only half the dose was administered to each rat, regardless of weight, and was increased if necessary. During the surgery, the rats were placed on a heating pad in order to maintain body temperature. The abdomen of each rat was shaved using standard hair clippers and a midabdominal incision was made in order to expose the uterus using a scalpel. The intestines were removed and placed to the side with a Krebs-Henseleit solution buffer-soaked cloth to prevent them from drying out. Black braided silk was then tied to the uterine horn. One was tied off at the base of the horn while another was tied off at the ovary. The horn was then cut from the body and placed in buffer. After the horn was removed, the abdominal muscles were sutured using 5-0 black braided silk with a C-1 taper needle. Once the muscles had been sutured, the skin was sutured using 3-0 black braided silk with a FS-1 cutting needle. The rats were monitored until the anesthesia had worn off and allowed to remain in their cage in the lab overnight. The next day, the animal was returned to its home cage.

2.5 Isolated Tissue Bath

The uterine tissue removed from the rats was cut into two sections and placed between two triangle ring clamps. A string was tied to the clamps and the tissue was placed in an isolated tissue bath filled with Krebs-Henseleit solution buffer and allowed to equilibrate while the rat was sewn up. Once this was completed, the buffer in the bath was drained and 9 ml of fresh buffer was added so there was a known amount. The tissue was cut into two pieces, which allowed for two sets of data to be collected from the same animal. Oxytocin was added to the two compartments in 9 µl increments, starting with 10^{-6} M and increasing in concentration until 1 M was added. When the tissue contracted, it pulled on the string and generated tension. A force transducer measured this tension and recorded it in a trace on the computer as shown in Fig 1.

2.6 Statistical Analyses

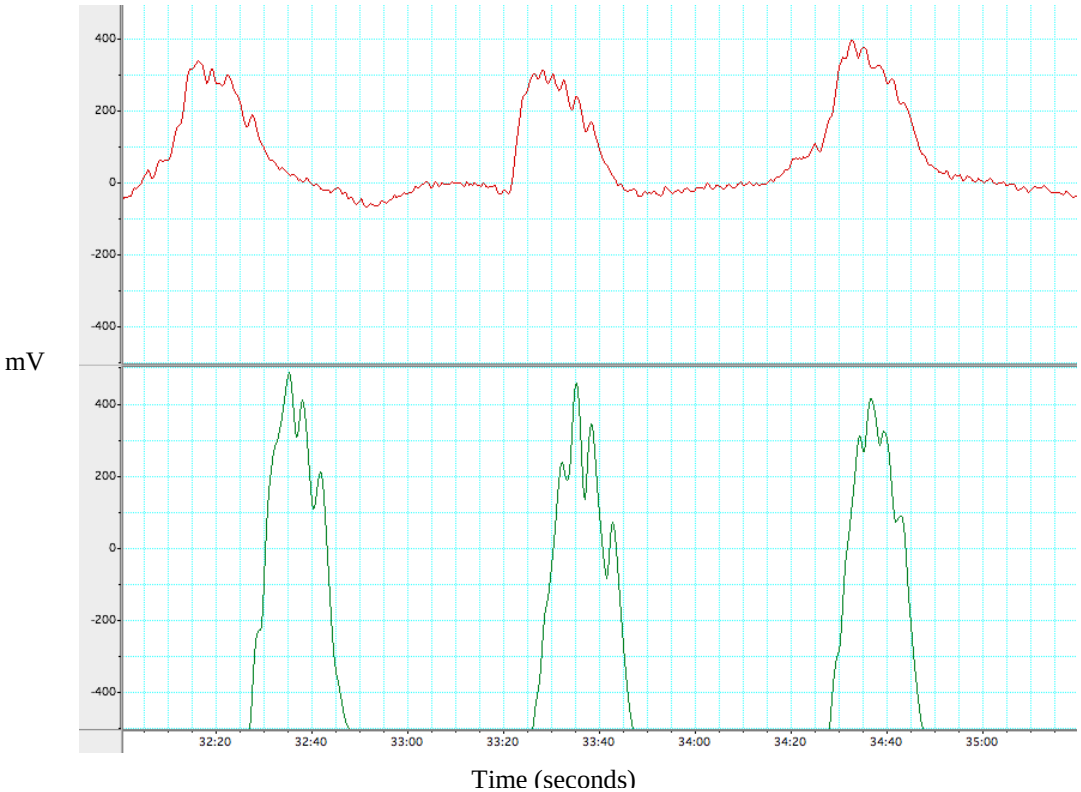

Figure 1. Contraction trace of rat uterine tissue

Figure 1. Example trace produced by the contractions of uterine tissue. The force transducer measures strength in mV on the y-axis while time elapsed is on the x-axis. The peaks are where the muscle contracts and the areas between peaks represent the baseline for contraction. Both tissues start out at the same base line, but over time, some may establish a new base line. This can be seen in the top tracing of this figure.

Both a one-way analysis of variance (ANOVA) across all treatments within groups and an unpaired t-test for each dose of oxytocin between groups were used to determine if there were significant differences in contraction force, frequency and duration. Significance was ascribed for $p < 0.05$.

3. Results

There were some surprising results that were discovered during the first surgery. When the tissue was placed in the buffer, it began to contract rhythmically before the oxytocin was even added. This suggests that non-pregnant uterine muscles undergo peristalsis, much like the esophagus and intestines.

The contraction traces produced on the computer were used to measure the force, frequency, and duration of contractions for both the experimental and control groups. Averages were calculated in each category and can be found in Table 1. These are the values that were used to determine significant difference between the two groups. An ANOVA test was performed in order to determine variance within each category. The results of this test showed variance within the contraction force and frequency categories, which suggests that the concentration of oxytocin plays a role for these, but not for duration.

To compare contractions between the control and experimental groups, and unpaired t-test was used. Instances of significance ($p<0.05$) can be seen in Table 1. Significant difference can only be seen in frequency and duration, but not in force.

Table 1. Average force, frequency, and duration of uterine contractions in response to oxytocin in virgin female rats. The experimental group (n=8) was exposed to a cigarette smoke extract drop-wise into the nose for 3 weeks prior to study while the control group (n=7) received only saline. SD designates standard deviation. * significantly different, $p < 0.05$.

Oxytocin Concentrations (M)	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	10^{0}
Control							
Force (V)	0.871	0.982	1.016	1.058	0.893	0.637	0.428
SD	0.370	0.389	0.482	0.331	0.391	0.291	0.227
Frequency (Hz)	0.011	0.011	0.010	0.012	0.016	0.017	0.020
SD	0.004	0.005	0.004	0.004	0.005	0.006	0.015
Length (s)	66.04	64.11	64.84	59.04	53.87	55.57	63.68
SD	16.96	20.17	18.77	20.67	15.89	17.27	12.45
Experimental							
Force (V)	1.086	1.456	1.436	1.448	1.274	0.703	0.541
SD	0.353	0.465	0.356	0.434	0.423	0.340	0.235
Frequency (Hz)	0.014	0.013	0.013*	0.015	0.019	0.022*	0.021
SD	0.007	0.005	0.005	0.006	0.007	0.007	0.008
Length (s)	50.85*	52.40	48.75*	50.53	45.97	44.72*	45.40*
SD	15.50	17.25	10.60	14.37	13.16	12.91	15.09

4. Discussion

The results show that cigarette smoke impacts the virgin female uterus in terms of frequency and duration of contraction, but not force. These results are consistent with those of previous studies involving pregnant rats done by Egawa[8] and Nakamoto[18], in which the frequency (or contractile sensitivity) increased, but the force did not. A third category, duration, was added for this study and was shown to decrease when the uterine muscle was exposed to cigarette smoke. Because the rats were 12 months of age at the beginning of the study, age cannot be dismissed as a possible effect. The data from this procedure support the hypothesis that smoking prior to pregnancy can potentially predispose the myometrium to abnormal responses in the presence of oxytocin. These results still do not show what components of cigarette smoke are responsible for the problems seen in women who smoke while pregnant, nor will they shed light on the mechanisms of how these contractions are caused prematurely, but they provide support for the correlation between cigarette smoking and contractions of the uterine wall prior to pregnancy. The results of the project can lead to even more research on the subject and public warnings, based on the findings. Since smoking can potentially predispose the tissue to abnormal responses to oxytocin, warnings may have to be issued in order to inform the public of this new danger for women who smoke and want to have children. Statistics show that 13-21% of women who are pregnant smoke, however 39% of smokers quit when they become pregnant[16, 25]. Our results support the hypothesis that smoking prior to pregnancy can cause abnormal responses to oxytocin, which can impact a large number of people.

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