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Effects of Ponderosa Pine Needle Ingestion on Uterine Vascular Function in Late-Gestation Beef Cows

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ABSTRACT: Consumption of Ponderosa pine (Pinus ponderosa) needles (PN) by beef cows during late gestation results in premature delivery in association with profound constriction of the caruncular arterial bed. Further, PN extracts and plasma from PN-fed cows increase uterine arterial tone in vitro. Uterine arterial tone is a measure of the arterial resistance to stretch and controls the baseline rate of flow through the vascular bed. Uterine arterial tone results from the uptake of extracellular Ca²⁺ into smooth muscle cells through specific membrane channels called potential sensitive channels. Functional potential sensitive channels remain open for prolonged periods after activation, allowing a continuous uptake of Ca²⁺ and the maintenance of uterine arterial tone. Recent evidence from our laboratory has demonstrated that a group of estrogen metabolites produced by the placenta and/or endometrium, called catechol estrogens, inhibits Ca²⁺ uptake through the potential sensitive channels. During gestation, progressive decreases in uterine arterial tone are observed, with resultant increases in uterine arterial blood flow. Thus, the continuous production of catechol estrogens may be necessary to maintain the pronounced uterine vasodilation that is required for fetal survival. Ponderosa pine needle extracts exhibit antiestrogenic activity, as evidenced by their inhibition of estrogen-induced uterine hyperemia. Data from our laboratory show that after consumption of PN by beef cows during late gestation, uterine arterial blood flow progressively decreased to < 50% of prefeeding rates before premature delivery of a live calf. Studies are currently under way to determine whether the PN-induced decrease in uterine arterial blood flow is the result of reduced secretion of catechol estrogens by the gravid uterus or a direct effect on the uterine arterial smooth muscle cell. We postulate that a substance in PN (or a metabolite of it in plasma) produces abortions by disturbing the hormonal control of uterine arterial blood flow.

Key Words: Pinus Ponderosa, Uterus, Blood Circulation, Parturition, Cows


Introduction

Premature parturition in cattle due to the ingestion of Ponderosa pine (Pinus ponderosa) needles is a major economic problem in the western United States and Canada (Lacey et al., 1988). Abortions from pine needle ingestion generally occur when cows are in the last trimester of pregnancy and occur 1 to 3 d after pregnant cows have begun daily consumption of needles or buds, but abortion may be delayed 2 to 3 wk (James et
Figure 1. Sequence of events thought to result in a phasic contraction of the uterine arterial smooth muscle cell.

Control of Uterine Arterial Blood Flow

Background

The rate of uterine blood flow is a primary determinant of nutrient uptake by the gravid uterus and fetus of the cow (Ferrell et al., 1983) and ewe (Morris et al., 1980). Not only is there a 35-fold increase in uterine blood flow in late gestation, but the fraction of uterine blood flow distributed to the caruncles, the fetal-maternal interface, increases progressively throughout pregnancy, with 80 to 85% of the blood flow to the gravid uterine horn distributed to the caruncles at term (Makowski et al., 1988). These changes in uterine blood flow throughout pregnancy seem to be due to steroid-induced alterations in uterine arterial phasic contractility and tone (Ford, 1989). Phasic contractility is a transient reduction in luminal diameter in response to an acute maternal stress resulting in sympathetic nerve stimulation or adrenal release of catecholamines, which leads to short-term reductions in arterial flow. Tone is defined as the pressure exerted by an arterial segment against an intraluminal flow and is considered to set the baseline rate of flow to an organ or tissue.

Phasic contractility and tone seem to be regulated individually, with tone changes predominating during pregnancy (Ford, 1989). Phasic contractility of smooth muscle is ultimately due to the formation of cross-bridges between phosphorylated myosin and actin filaments, followed by the movement of the myosin relative to actin as

al., 1989). The term "abortion" may be a misnomer, however, because the calf is commonly born weak, but viable, suggesting a prematurely induced parturition. Secondary complications such as retained fetal membranes, metritis, peritonitis, and even maternal death are commonly seen following abortions induced by Ponderosa pine needles (Stevenson et al., 1972; James et al., 1977; Call and James, 1978). Pathologic studies have revealed accelerated bacterial decomposition of tissues of the placenta. However, careful studies of calves and cows immediately after delivery have not shown histologic evidence of infection or positive bacterial or fungal cultures. A finding that has been consistently observed and reported, however, is a profound vasoconstriction of the caruncular arteries, with an associated ischemic necrosis at the placental attachment site (Stuart et al., 1989). This may well be the cause of the premature parturition inasmuch as even brief bouts of uterine vasoconstriction during late gestation cause fetal distress and the fetal cortisol release that prompts labor in cattle (Challis et al., 1989).

We postulated that a substance in Ponderosa pine needles, or a metabolite of it in blood, may produce abortions by disturbing the hormonal control of uterine blood flow. During the first part of this article, we will attempt to describe our current hypotheses about the physiologic factors controlling blood flow to the gravid bovine uterus. We will then present data relating how ingestion of Ponderosa pine needles may interfere with these controls.
Figure 2. Sequence of events thought to result in the tonic contraction of the uterine arterial smooth muscle cell.

ATP is hydrolyzed (Reimer and Roberts, 1986; Rasmussen et al., 1987). The biochemical events hypothesized to precede this movement are diagrammed in Figure 1.

Activation of the alpha1-adrenergic receptors on the vascular smooth muscle membrane has been shown to cause hydrolysis of the membrane phospholipid, phosphatidylinositol diphosphate, by phospholipase C at the inner leaflet of the plasma membrane. Both the products of this hydrolysis, inositol trisphosphate and diacylglycerol, have a role in subsequent Ca\textsuperscript{2+} messenger events. Diacylglycerol contributes to the activity of the Ca\textsuperscript{2+}-producing Ca\textsuperscript{2+} messenger system regulated by protein kinase C (PKC), to be discussed later, whereas inositol trisphosphate triggers the activity of the Ca\textsuperscript{2+}-calmodulin system, which results in phasic contraction. Inositol trisphosphate activates the calmodulin system by triggering the sarcoplasmic reticulum to release its Ca\textsuperscript{2+} into the cytosol. There, the released Ca\textsuperscript{2+} associates with calmodulin, forming a complex that interacts with and activates a number of intracellular kinases. One of these, myosin light chain kinase, provides the phosphorylation of myosin, which ultimately leads to the movement of myosin along the actin chain. The resulting contraction is phasic because the myosin is rapidly dephosphorylated by phosphatases (Rasmussen et al., 1987), and Ca\textsuperscript{2+} is sequestered and/or extruded from the cytosol (Carsten and Miller, 1987). Meisher et al. (1981) have shown that subsequent phasic contractions can be obtained if the Ca\textsuperscript{2+} stores of the sarcoplasmic reticulum are reconstituted. Evidence suggests that the Ca\textsuperscript{2+} for this purpose enters the cell via receptor-operated Ca\textsuperscript{2+} channels that are linked directly with the sarcoplasmic reticulum (Putney, 1986). As might be expected, it is possible to obtain an initial phasic contraction without the need for extracellular Ca\textsuperscript{2+}. However, subsequent phasic responses cannot be elicited if extracellular Ca\textsuperscript{2+} is unavailable (e.g., when subsequent responses are tested in Ca\textsuperscript{2+}-free media or when the receptor-operated Ca\textsuperscript{2+} channels are blocked by a drug such as amrinone (Meisher et al., 1981; Stice et al., 1987b).

The only sex steroid shown to directly modulate the alpha1-receptor/calmodulin system is progesterone (Ford et al., 1984). Progesterone of endogenous or exogenous origin increases numbers of alpha1-adrenergic receptors on uterine arterial smooth muscle cells (Ford et al., 1977, 1984) and, thus, during the luteal phase of the estrous cycle and during pregnancy, alpha1-adrenergic receptors and phasic contractile response remain high. This is confirmed by the retained ability of the uterine arterial bed to phasically contract throughout pregnancy in the pig (Guenther et al., 1988) and cow (Sauer et al., 1989).

Rasmussen et al. (1987) presented evidence that the tone of vascular smooth muscle may result from activation of PKC and phosphorylation of proteins that regulate the length of the actin filaments (Figure 2). By altering the length of this portion of the contractile apparatus, it is possible to change the overall length (tone) of the smooth muscle cell without changing its response to phasic contractile stimuli. Like calmodulin, PKC activity is linked to a hormone receptor on the membrane, to a membrane phospholipid, and to Ca\textsuperscript{2+}, which is necessary for kinase activity (Niedel and Blackshear, 1986). However, in each instance, the specific component tied to PKC differs from that associated with calmodulin activity. Recent studies have shown that the membrane receptor associated with generation of arterial tone is the alpha2-adrenergic receptor (Van Zwieten et al., 1987; Ford et al., 1990). The membrane phospho-
lipid in the PKC system is phosphatidylserine (PS), which is concentrated at the inner leaflet of the lipid bilayer. There, four PS molecules are arranged about a chelated Ca\(^{2+}\) (Ganong et al., 1986) and provide a site for the membrane association of PKC. The kinase activity of this ternary complex is minimal until it is stimulated by the diacylglycerol produced by phosphotidylinositol diphosphate hydrolysers. The active PKC complex phosphorylates proteins in the "actin domain" of the cell, causing shortening (tone) of the smooth muscle. In addition, PKC increases entry of extracellular Ca\(^{2+}\) via the potential sensitive channel (Kikkawa et al., 1986). Calcium entering via the potential sensitive channel cycles through the submembrane portion of the cell and functions in the continued activation of PKC before being extracted from the cell by Ca\(^{2+}\) pumps. Should the potential sensitive channel be blocked by a drug or a biologically active "channel blocker," tone falls, and the vessel dilates. Direct evidence for the role of PKC in tone generation in the caruncular artery is the previously reported ability of 12-O-tetradecanoylphorbol-13-acetate (TPA) to increase caruncular arterial tone in vitro without altering the phasic contractile response to the alpha\(_{1}\)-adrenergic agonist phenylephrine (Sauer et al., 1987). It has been shown that TPA activates PKC (Castagna et al., 1982) and that it induces a slowly developing, but sustained, Ca\(^{2+}\)-dependent contraction in a variety of vascular tissues, including the rabbit ear arteries (Rasmussen et al., 1984).

In all species examined to date, the hydroxylation of the phenolic A ring of estrogens at the 2 or 4 position is a significant metabolic pathway (Barnea et al., 1984). The resulting 2- and 4-OH estrogens (catechol estrogens) seem to be the metabolites responsible for the characteristic uterine vasodilation associated with estrogen dominance, as during estrus and pregnancy (Ford and Stice, 1985). These compounds cause uterine arterial relaxation (decreased tone) by blocking the entry of Ca\(^{2+}\) into the vascular smooth muscle cell through the potential sensitive channel (Stice et al., 1987a,b). Catechol estrogens in blood plasma and urine have been shown to increase progressively throughout human pregnancy (Barnea et al., 1984). Further, the gravid uterus seems to be a major source of these compounds because extremely high concentrations of catechol estrogens are found in uterine lymphatic fluid of both the sow (Van Orden et al., 1988) and cow (Ford, unpublished data) during late gestation. Data from our laboratory suggest that catechol estrogens are produced locally by a soluble uterine peroxidase (Van Orden et al., 1988; Farley et al., 1989). The importance of peroxidases and catechol estrogens in the maintenance of uterine blood flow was described only recently; thus, it is not surprising that no study has specifically examined the catechol estrogen pathway in pine needle abortion. Nevertheless, Allison and Kitts (1964) used the uterine edema response in castrated rats to test for toxic substances in Ponderosa pine needles. The results are exciting in light of current knowledge. It is now known that the edema response to estrogen depends on the increased activity of uterine peroxidase after estrogen administration and is negative when an animal is treated with a substance that blocks enzymatic activity of peroxidase (Van Orden et al., 1988). Because Allison and Kitts (1964) found that extracts from Ponderosa pine needles blocked the edema response in the test rats, a suppression of catechol estrogen synthesis or activity by components in pine needles seems likely. Lack of catechol estrogen production or activity would fit the clinical picture of a vasoconstricted uterine vasculature and stressed fetus in pine needle abortion.

Experimental Findings

We have recently modified our in vitro uterine artery perfusion systems so that we can study, individually, the controls of phasic contractility and tone. In this modified technique, individual placentalmores were excised from the uterine wall of a cow during late gestation (220 to 260 d) and the maternal caruncular artery was cannulated and attached to a constant-flow peristaltic pump (Sauer et al., 1989). The bovine placentome model was developed so that we might investigate the reactivities of the resistance vessels of the gravid uterus without the overlay of systemic or central nervous system factors. A pulse similar to that present in vivo was superimposed on the intraarterial flow through use of a physiological perfusion pump (Medical Engineering Consultants, Los Angeles, CA). Pulse pressures were held at 25% of the perfusion pressure, and the pulse rate was maintained at 80 beats/min. Drugs were delivered into the arterial lines, and baseline perfusion pressure (a measure of tone) and phasic contractions (short-term elevations in perfusion pressure above baseline) were recorded from a pressure transducer.

As shown previously for the porcine uterine artery (Ford et al., 1989), a constant 60-min perfusion of phenylephrine (alpha\(_{1}\)-adrenergic agonist) into the caruncular artery results in a rapid increase from baseline perfusion pressure, which peaks at 5 min then declines progressively to the original perfusion pressure by 25 min, where it remains for the rest of the perfusion (phasic response). A tonic response is obtained by a 60-min perfusion of epinephrine (alpha\(_{1}\) - and al-
pha2-adrenergic agonist). This catecholamine induces a progressive increase in perfusion pressure during the first 20 min, followed by a maintenance of contraction throughout the rest of the perfusion period. Continuous perfusion of clonidine (alpha2-adrenergic agonist) with phenylephrine results in a slowly developing, maintained contraction similar to that produced by epinephrine, whereas perfusion of yohimbine (alpha2-adrenergic antagonist) with epinephrine results in a phasic contractile response similar to a phonylphenylephrine response. Perfusion of prazosin (alpha1-adrenergic antagonist) with epinephrine prevents both the phasic and tonic contractile responses. When yohimbine is added during an ongoing tonic response to epinephrine, perfusion pressure declines to the original basal level. These pharmacologic studies show that phasic contraction requires activation of only the alpha1-adrenergic receptor. Tonic contraction requires the combined and sustained stimulation of alpha1- and alpha2-adrenergic receptors. An understanding of controls of uterine arterial tone during pregnancy is imperative inasmuch as we have previously demonstrated that the phasic contractile response of the caruncular artery to phenylephrine remains unchanged throughout gestation, whereas the tonic contraction of the artery to epinephrine declines progressively in association with an increase in vessel diameter and blood flow (Guenther et al., 1988; Sauer et al., 1989).

Preliminary data from our laboratory suggest that a component in the plasma from cows fed pine needles may reverse the normal catechol estrogen-induced uterine arterial vasodilation (Christenson et al., 1989). Plasma from control cows and cows fed pine needles was tested for vasoactivity in the in vitro perfused bovine placental preparation established in our laboratory. As previously stated, this model system responds to tone-producing agents (i.e., epinephrine) with a gradual rise in baseline perfusion pressure, followed by a sustained plateau that continues throughout infusion of the agent. Further, bolus depolarizing doses of KCl can be administered to measure the level of potential sensitive channel activity before and during perfusion of tone-producing agents (Stice et al., 1987a,b).

In preliminary experiments using the bovine placenta, a specific dose-dependent effect of intraarterial perfusions of a plasma pool from beef cows fed pine needles during late gestation (250 d) on increasing baseline perfusion pressure and response to depolarizing doses of KCl was observed for the caruncular artery (Christenson et al., 1989). This same plasma pool had no effect on altering caruncular arterial responsiveness to phenylephrine. The cotyledonal arterial vasculature of the preparations exhibited no increases in baseline perfusion pressure or response to KCl in response to perfusion with the same pool of pine needle plasma. Plasma collected from control cows during the last 3 d of a normal gestation exhibited no similar effects on augmenting caruncular arterial tone. Further, the same plasma pool from cows fed pine needles in this study did not alter the tone of control mesenteric arteries. The uterine vasoconstrictive effects of Ponderosa pine needles in vivo was recently confirmed in beef cows during late gestation in our laboratory (Christenson et al., 1990). After the initiation of daily feeding of Ponderosa pine needles on d 250 of gestation, uterine arterial blood flow declined progressively through d 258 (> 56% decrease), followed by the birth of a live calf on d 259 ± 1.

The selectivity of an agent(s) in pine needles to affect uterine arterial tone is consistent with an effect on the steroid hormone-controlled protein kinase C/potential sensitive channel/Ca2+ uptake mechanism that controls uterine arterial blood flow. The clinical picture and available experimental results suggest that pine needle abortion is due to a failure of the mechanism that produces uterine vasodilation in pregnancy. Accordingly, we postulate that a component in Ponderosa pine needles (or metabolite thereof) may block uterine peroxidase activity, resulting in subnormal catechol estrogen production. This would prompt reversal of the normal catechol estrogen-induced potential sensitive channel blockade and allow entry of the Ca2+, which increases uterine arterial tone, thus decreasing uterine blood flow. Studies are currently under way to identify the active component in pine needle plasma that augments caruncular arterial tone.

Implications

Currently, there is no clinical diagnosis to positively identify a cow at risk of aborting in response to pine needle consumption, or even to determine that an abortion has resulted from pine needle ingestion. Further, the causative agent or agents are unknown. We have presented evidence that a circulating agent(s) in blood, resulting from ingestion of Ponderosa pine needles by beef cows during late gestation decreases blood flow to the gravid uterus and fetus by increasing the tone of the caruncular arterial vasculature. The long-range goal of our research is to identify the circulating agent(s) and to determine its specific mechanism of action. We anticipate that the successful isolation of this agent(s) will lead to the development of a treatment regimen that will prevent pine needle-induced early parturition in the beef cow.
Literature Cited


