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Uterine Arterial Responses to Arachidonate in Pregnant and Nonpregnant Rabbits (41712)

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Abstract. Several investigators have suggested that prostaglandins (PG) may play a major regulatory role in maintaining uteroplacental blood flow in pregnancy. The present study was undertaken to assess the response of the uterine artery from near-term pregnant and nonpregnant rabbits to the PG precursor Na-arachidonate (AA) (C 20:4). Isolated uterine arterial strips were equilibrated isometrically under their optimal resting tensions in physiologic salt solution. Uterine arteries from pregnant rabbits elicited significantly greater contractile responses to arachidonate over the dose-range studied (10^{-10}–10^{-3} *M*) than did arteries from nonpregnant rabbits. These contractions were seen whether the strip was relaxed or precontracted with potassium chloride (30 m*M*). The contractile responses to AA were antagonized in a competitive manner by pretreating the arteries with the cyclooxygenase inhibitors meclofenamate (10^{-5} *M*) or indomethacin (10^{-5} *M*), thus suggesting that the contractile response to AA was the result of its conversion to prostanoids by the cyclooxygenase pathway. The possibility that the AA response was a general fatty acid effect was ruled out since oleate (C 18:1) had no effect on the arteries. In addition, prostaglandins $F_2\alpha$ and E_2 (10^{-5} *M*) also contracted the uterine arteries from the pregnant group. It is concluded from these studies that the uterine arterial wall from near-term pregnant rabbits utilizes the PG precursor, AA, for the production of prostanoids which, in turn, cause uterine arterial constriction.

The precise mechanism(s) by which uterine blood flow is regulated during pregnancy has been open to question for some time. The notion that a modulatory role for endogenous prostaglandins (PG) is involved in the regulation of uteroplacental blood flow stems from early studies which found large concentrations of prostaglandin E (PGE) in uterine venous blood following infusion of angiotensin II (AII) to pregnant dogs (1) and monkeys (2). Concomitant with the appearance of PGE, uterine blood flow increased in the dog (1). This paradoxical AII vasodilation of the uterus was attributed to AII stimulation of vasodilating PG, particularly PGE. In more recent studies, Naden and Rosenfeld (3) demonstrated that AII is a uterine vasoconstrictor in the conscious term pregnant ewe since uterine vascular resistance increased in a dose-dependent manner to AII infusion. Anderson *et al.* (4) also observed a vasoconstrictor response to AII using the same preparation. Their data, however, further suggested that AII stimulated a vasodilating PG unique to the nonplacental myometrial vasculature. In addition, recent work from this laboratory demonstrated that AII constricted isolated uterine arteries from both pregnant and nonpregnant rabbits and also stimulated prostaglandin synthesis in the uterine arterial wall itself. However, the prostanoids synthesized from the AII stimulation had a vasoconstricting action (5).

To further characterize the nature of the uterine arterial wall PG synthesis, the present study was undertaken to evaluate: (a) the ability of the uterine arterial wall itself to synthesize prostanoids from exogenous precursor arachidonate, (b) the response of the uterine arterial wall to the prostanoids thus synthesized, and (c) the impact of pregnancy on the uterine arterial wall responses to exogenous arachidonate. The technique chosen for this study utilizes strips of small uterine arteries isolated from rabbits *in vitro,* thus providing a means to indirectly assess PG synthesis and effects which are intrinsic to the arterial wall and isolated from neural, humoral, tissue, or mechanical factors otherwise present *in vivo.*

Materials and Methods. New Zealand nonpregnant and near-term pregnant rabbits (27–29 days; gestation 30–31) were anesthetized with sodium pentobarbital (26.5 mg/kg, iv). Segments of first- and second-order uterine arteries (400–700 μm, o.d.) were excised, cut

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into helical strips, and suspended isometrically in 50 ml of physiological salt solution (PSS). Arterial responses to vasoconstricting stimuli were recorded after passively stretching the strips to their predetermined optimal resting tension for force generation which was similar for both groups of arteries. The PSS was aerated with 95% O_2–5% CO_2, maintained at 37°C, pH 7.4, and contained (m*M*): NaCl 119, KCl 4.7, $CaCl_2H_2O$ 1.6, KH_2PO_4 1.2, $MgSO_4$ $7H_2O$ 1.2, $NaHCO_3$ 22.6, dextrose 5.0, sucrose 25, and Ca-Na_2-EDTA 0.03 (pH 7.4).

All arterial strips were equilibrated for 2 hr prior to experimentation. The mechanical response of the arterial smooth muscle to cumulative additions (15 and 30 μl) of vasoactive agonists were quantitated using a Grass force transducer (FT.03) and recorded on a Grass Model 7 polygraph.

The drugs used in this study were sodium arachidonate (Sigma), L-norepinephrine (Sigma), indomethacin (Sigma), meclofenamate (Parke-Davis), oleate (Sigma), and prostaglandins E_2 and $F_{2\alpha}$ (Upjohn). With the exception of indomethacin and oleate, all drugs were dissolved in double-distilled, deionized water and expressed as final bath concentration. Indomethacin and oleate were dissolved in ethanol. The bath concentration of ethanol was less than 0.05% which, in separate control experiments, was without effect alone and did not interfere with the contractile response of the strips to various agonists.

Since norepinephrine (NE) (10^{-4} *M*) elicited large contractile responses in these blood vessels, individual arterial responses to the agents examined were normalized with respect to their percentage maximal NE response. This method of normalizing arterial response data corrects for individual differences in strip geometry which inevitably results from the preparation of arterial strips (6). Data were analyzed using two-way analysis of variance (2-way ANOVA) and analysis of covariance (ANCOVA) (7). Significance was designated at the level of $P < 0.05$. In all cases, *n* represents the number of animals studied with two uterine arteries (left and right) serving as duplicate trials in each animal.

Results. Helical strips of uterine arteries from nine near-term pregnant rabbits exhibited dose-dependent contractions in response to exogenous arachidonate (Fig. 1). The onset of the contraction was rapid, occurring within 40 sec after the addition of arachidonate. The contractions to arachidonate were observed whether the artery was relaxed under optimal resting tension or precontracted with 30 m*M* potassium chloride. At no concentration did arachidonate cause relaxation over the dose-range studied (10^{-10} to 10^{-3} *M*). However, the contractile response of uterine arteries from eight nonpregnant rabbits to arachidonate differed from those of the pregnant group. The mean contractile response of the uterine artery from nonpregnant rabbits was significantly less than that seen in uterine arteries from pregnant rabbits for each concentration of arach-

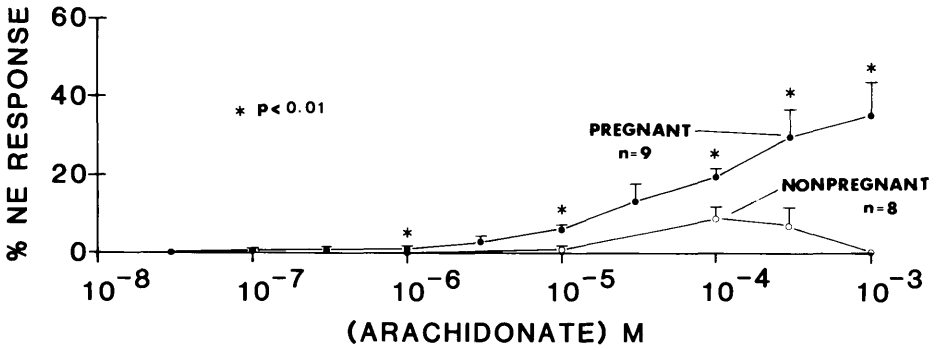

FIG. 1. Response of helical strips of uterine artery from near term pregnant and nonpregnant rabbits to cumulative addition of arachidonate. Uterine arteries from pregnant rabbits elicited greater contractile responses to arachidonate than did their counterparts from nonpregnant rabbits. Values are mean ± SE of artery responses from pregnant and nonpregnant rabbits to arachidonate; *n*, number of rabbits. *Statistical differences between the groups at $P < 0.01$ (2-way ANOVA).

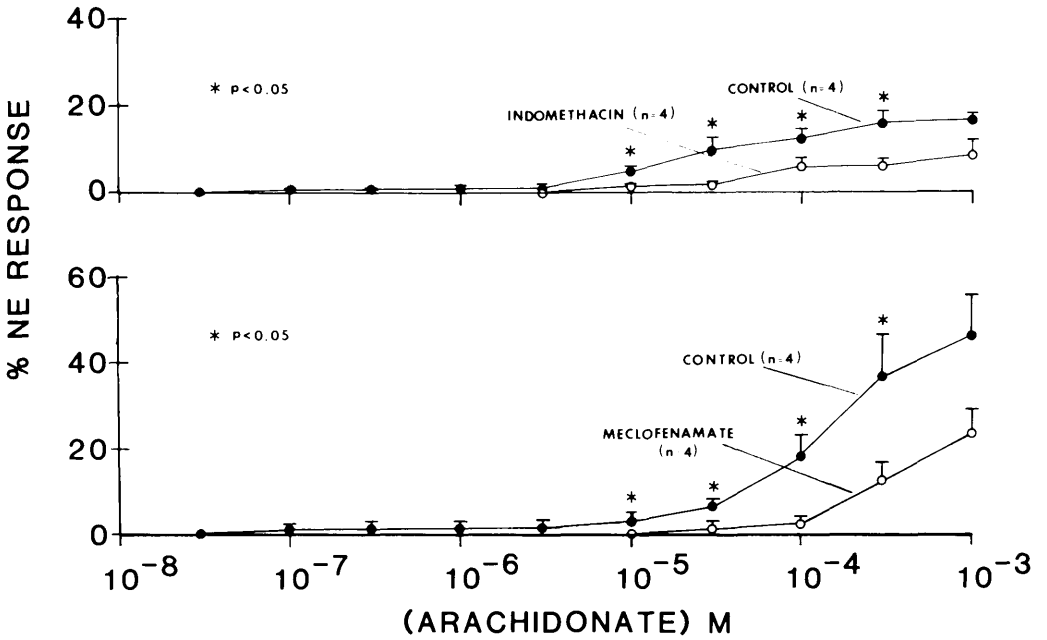

FIG. 2. Response of uterine arteries from pregnant rabbits to arachidonate in the presence of cyclooxygenase inhibitors. Pretreatment of uterine strips with 10^{-5} *M* meclofenamate or indomethacin produced a parallel shift (ANCOVA) in the arachidonate dose–response relation. Values are mean ± SE of the artery response to arachidonate; *n*, number of rabbits. *Statistical differences between treated and untreated strips at $P < 0.05$ (2-way ANOVA).

idonate ($P < 0.01$). Interestingly, uterine arteries from nonpregnant, but not pregnant rabbits, relaxed when exposed to the highest concentration of arachidonate (10^{-3} *M*).

To determine if the arterial responses to arachidonate was a result of PG synthesis via the cyclooxygenase pathway in the arterial wall, uterine arteries from pregnant rabbits were preincubated with the competitive cyclooxygenase inhibitors meclofenamate (10^{-5} *M*) or indomethacin (10^{-5} *M*) 20 min prior to exposure to exogenous arachidonate. Both meclofenamate and indomethacin produced a significant parallel shift to the right in the arachidonate dose–response relationship ($P < 0.05$) (Fig. 2).

The contractions elicited by arachidonate in these arteries do not appear to be a nonspecific fatty acid effect since oleate (C 18:1) (10^{-6} to 10^{-4} *M*), which is not a substrate for the cyclooxygenase enzyme, had no effect on the uterine artery (not shown).

The actions of $PGF_2\alpha$ and PGE_2 (10^{-5} *M*) are illustrated in Fig. 3. Both compounds elicited contractions; the response to $PGF_2\alpha$ was 25 ± 6% of the maximum NE response while the response to PGE_2 was 15 ± 5%.

Discussion. Previously we suggested that AII stimulates synthesis of prostaglandins which have a net constricting action on the isolated uterine artery obtained from the near-term pregnant rabbit (5). The present study further supports the suggestion that the uterine arterial

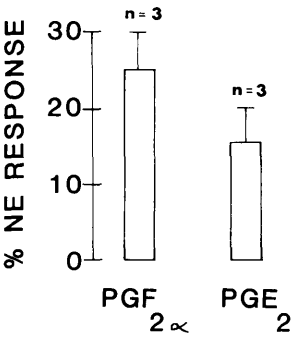

FIG. 3. Response of uterine arteries from pregnant rabbits to $PGF_2\alpha$ and PGE_2 (10^{-5} *M*). Values are mean ± SE of the artery response to the agonist; *n*, number of rabbits.

wall from rabbits in this stage of gestation possesses an active cyclooxygenase pathway which utilizes arachidonate to produce prostanoid products which, in turn, have a net vasoconstricting action in the small uterine artery. Utilization of the cyclooxygenase pathway is indicated as pretreatment of these arteries with either meclofenamate or indomethacin antagonized the contractile response to arachidonate in a competitive fashion consistent with the mechanism of action of these cyclooxygenase antagonists. However, it cannot be determined from these data whether the constriction to arachidonate was due to its direct conversion to PG or that it served to release endogenous arachidonate which, in turn, was converted to PG as is suggested to occur by Mahony and Clyman in the lamb ductus arterious (8).

The finding that arachidonate at all active concentrations contracts the uterine artery in near-term pregnant rabbits does not support the results of previous studies which suggest that vasodilating prostaglandins, particularly those of the E series, are synthesized by the uterine vasculature in pregnancy and subserve physiologic uterine vasodilation (1, 2, 9). It must be pointed out though, that the earlier studies measured the uterine venous effluent levels of prostaglandins, and consequently, the actual site of synthesis and/or the action of the PG could not be determined. In the present study, the direct response of the small uterine artery to arachidonate was evaluated and found to be contraction, not relaxation. Hence, it is possible that PG synthesis observed *in vivo* occurred in the periarterial myometrium, and in turn, acted on the vasculature in a local manner. The uterus is well-known to produce prostaglandins which are suspected to be involved in uterine physiology (10).

It should be pointed out that the arterial segments used in our studies were obtained from arteries which, though small, may have included segments proximal to the uterine resistance arterioles. Resistance vessels, however, have been classically described to range up to 200–500 μm in diameter (11, 12). However, Clark *et al.* (13) recently presented *in vivo* data supporting the present finding. Using the instrumented, conscious, near-term pregnant ewe, they showed that exogenous arachidonate infused into the uterine artery produced an increase in uterine vascular resistance. This would suggest that arachidonate was being metabolized to prostanoids whose net response leads to vasoconstriction.

The effect of the individual prostaglandins on the uterine vasculature during pregnancy have been examined previously. PGE_2 and $PGF_2\alpha$ decrease uterine blood flow in the sheep (13–15). PGD_2 has vasodilating properties in the rabbit (16) and sheep uterine vasculature (13). Curiously, PGI_2 has been shown both to increase (13) and decrease (17) uterine blood flow in the sheep. In the present study, PGE_2 and $PGF_2\alpha$ contracted the uterine artery from the pregnant rabbit. Thus, it seems unlikely that PGE_2 is a physiologic vasodilator of the uterine artery at this stage of gestation.

In summary, the uterine arterial wall from the near-term pregnant rabbit is capable of utilizing the prostaglandin precursor, arachidonate, in the cyclooxygenase pathway. The products synthesized in the arterial wall to arachidonate stimulation elicit a dose-dependent contractile response which is antagonized by cyclooxygenase inhibitors, thus suggesting that the uterine artery synthesizes prostanoids which have a net vasoconstricting effect at this stage in gestation.

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