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IL1alpha augments prostaglandin synthesis in pregnant rat uteri by a nitric oxide mediated mechanism

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Summary This study aims to examine the possible relationship between cytokines, nitric oxide (NO) and prostaglandins in the pregnant rat uterus. Results indicate that 1) $IL_{1\alpha}$ enhances the synthesis of prostaglandins and augments NO production in pregnant rat uteri and 2) the effect of $IL_{1\alpha}$ on prostaglandin synthesis is abolished by NMMA, a NOS inhibitor, by aminoguanidine, an iNOS inhibitor, and by NS-398, a COX-2 inhibitor. These results suggest that there is an interaction between $IL_{1\alpha}$, NO and prostaglandins and that are involved COX-2 and iNOS in this interrelationship. This mechanism might be important in the regulation of uterine contractility during pregnancy and labor. © 2000 Harcourt Publishers Ltd

INTRODUCTION

A role for selected cytokines in the initiation of labor and the onset of preterm labor has been proposed on many occasions.[1] Interleukins are a family of inmunoregulatory polypeptides hormones produced by several cell types, such as macrophages, glomerular mesangial cells, etc[2,3] involved in the control of local and systemic events of the inmune response, inflammatory reactions, healing and haematopoiesis. Interleukin-1 is produced in the uterus during the preimplantation period, pseudopregnancy and oil-induced decidualization.[1] Interleukins are also present in amniotic fluid of women with intraamniotic infections and in women with spontaneous labor at term.[4,5]

Evidence for the involvement of prostaglandins in parturition is strong since inhibitors of prostaglandin synthesis delay labor, prostaglandins concentrations are high in amniotic fluid as labor advances, and the administration of prostaglandins can induce labor.[6] IL1α induces an increase in PG accumulation and a significant increase in cyclooxygenase activity in rat endometrial cells in vitro[7] and in estrogenized rat uteri.[8]

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Cyclooxygenase (COX) is the enzyme which converts arachidonic acid to PGS. COX has been found in two forms. The widely occurring constitutive enzyme (COX-1) accounts for the release of PGs involved in the regulation of physiological functions.[10] After stimulation with LPS or some cytokines, many cell types, including endothelial cells and macrophages, express the inducible isoform (COX-2) which is responsible for the production of large amounts of proinflammatory PGs at the inflammatory site.[11,12] IL-4 and IL-1α augment PGs production by inducing COX-2[13,14] and IL-1α enhances PGs production via COX-2 in estrogenized rat uteri.[8]

Nitric oxide (NO) is an inorganic free radical gas that is well recognized as the principal mediator of several functions including the relaxation of smooth muscles in a variety of tissues.[15] Nitric oxide is generated from L-arginine by NO synthase (NOS) and multiple isoforms of this enzyme have been reported.[16] Nerve fibers synthesizing NO have been demonstrated in the uteri of nonpregnant rats and mice by both colocalization with NADPH diaphorase, and by immunoreacitivity using antibodies raised against pig and rat neuronal NOsynthase (NOS) and inducible NOsynthase (NOS).[17,18,19]

We have demonstrated that NO enhances PGs production by isolated estrogenized rat uterus.[20] We have also shown that IL-1α augments PGs synthesis via NO[8] in the same model. The aims of this study were to further investigate the ability of $IL_{1\alpha}$ and IL_2 to modify PG production

in the pregnant rat uterus and then to explore the mechanism through which the cytokine acts.

MATERIALS AND METHODS

Time-mated pregnant rats of the Wistar strain (200–230 g body weight) were used. They were maintained on a 12:12-h light-dark schedule. Animals received an ad libitum supply of animal chow and water. Pregnant animals were killed on day 22 of gestation. Spontaneous term labor occurs on the night of the 22nd day. All animals were stunned by a blow on the neck. The uterus was removed immediately, cleaned of fat, placenta, fetuses, fetal membranes and blood vessels and rinsed thoroughly in cold Krebs Ringer Bicarbonate buffer (KRB).

Metabolism of (^{14}C) arachidonic acid

The metabolism of exogenous arachidonic and by rat uterine tissue was determined by incubating the tissue for 60 min in KRB containing 0.25μCi of (^{14}C) arachidonic acid in an atmosphere of 95% O_2 /5%CO_2 with constant shaking at 60 cycles per min at 37°C. For each determination 200 mg of uterine tissue was used. The uterine strips were randomly treated with IL-1α (10^{-10}, 10^{-11} and 10^{-12} M) or IL-2 (10^{-10}, 10^{-11} and 10^{-12} M) for 60 min. The controls were incubated in medium alone.

At the end of the incubation period, tissues were removed and the incubation medium was acidified to pH 3.0 with 1.0 M HCI in 1 vol. of ethyl acetate and extracted twice for PGs. Pooled acetate extracts were dried under nitrogen. The residues were then applied to silica gel TLC plates. The plates were developed in a solvent system of benzene/dioxane/glacial acetic acid (60:30:3). The position of the authentic eicosanoids was visualized by spraying the dried plates with 10% phosphomolybdic acid. Redioactivity from TLC zones for arachidonic acid and for different prostanoids was measured by liquid scintillation counting. The results were expressed as percentages of the total radioactivity of the plates.

Determination of NOS activity

For determination of NO release from incubated uterine strips, we used a modification of the method of Bredt & Snyder[21] which measures the conversion of [^{14}C]-arginine into [^{14}C]-citrulline since citrulline remains in the sample, whereas the equimolar amounts of NO produced are rapidly destroyed. Slices of tissue were incubated at 37°C in a buffer that contained 20mM HEPES, 10 uM [^{14}C]-arginine (0.3uCi) and 0.5mM NADPH. After 15 min of incubation, samples were homogenized. The samples were centrifuged for 10 min at 10 000 r.p.m. and then they were applied to a 1 ml DOWEX AG50W-X8 column (Na+form) and [^{14}C]-citrulline was eluted in 3 ml of water. The radioactivity was measured by liquid scintillation counting. Enzyme activity is reported in cpm/100mg wt weight.

Drugs and chemicals

Prostaglandins, interleukins, aminoguanidine (AG) and N-monomethyl-L-arginine (NMMA) were purchased from Sigma Chemical Co. (St Louis, Mi, USA); [^{14}C]-arginine and [^{14}C]-arachidonic and were from Amersham Corporation (Arlington Heights, II, USA). All other chemicals were analytical grade.

Statistics

Statistical significance was tested by ANOVA test. Differences between means were considered significant at $P < 0.05$.

RESULTS

Interleukin action on nitric oxide synthase activity

Fig. 1 shows that pregnant uterus has NO synthase activity. IL-1α increases NOS activity in the uteri obtained from rats during pregnancy on day 22. IL-1α stimulated NOS only in a dose of 10^{-11} M. Addition of IL-2 (10^{-12} to 10^{-10}M) did not modify the conversion of arginine to citrulline (data not shown).

In order to discriminate which isoform of NOS is implicated in the stimulatory effect of IL-1α we preincubated the tissues with aminoguanidine (500uM AG), a selective inhibitor of inducible NOS (iNOS). Fig. 2 shows that the stimulatory effect of IL-1α was greatly reduced in the presence of AG. These results suggest the participation of iNOS in the IL-1α-induced NO production.

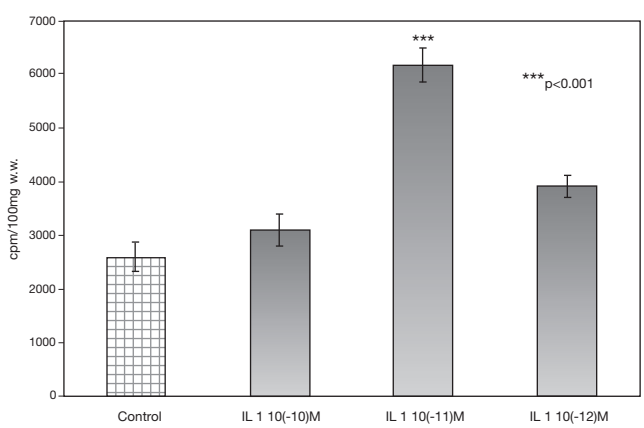

Fig. 1 Effect of 60 min preincubation with IL-1α on the conversion of arginine in citrulline by pregnant rat uterus. Bars represent mean ± SEM of six uterus.

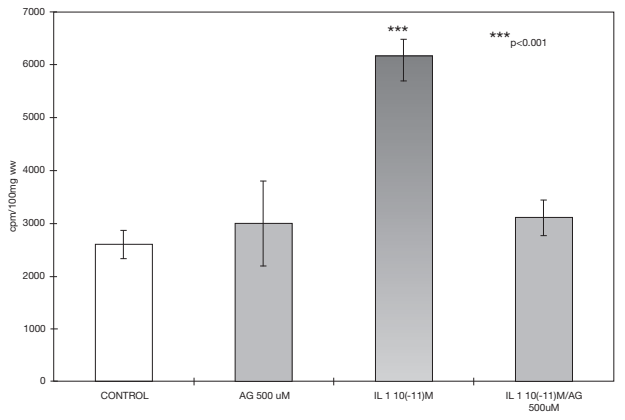

Fig. 2 Effect of aminoguanidine (500 uM) on IL-1α effect on the conversion of arginine in citrulline by pregnant rat uterus. Bars represent mean ± SEM of six uterus.

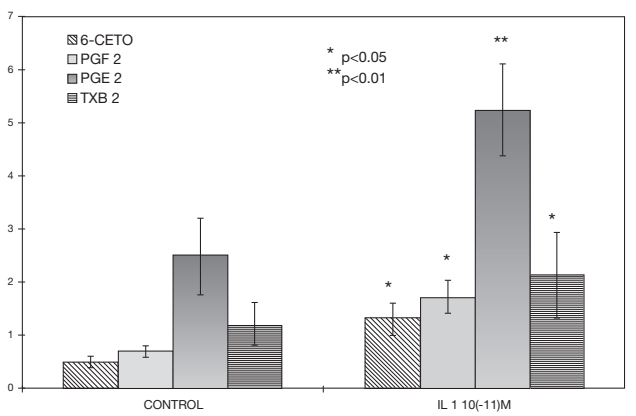

Fig. 3 Effect of 60 min preincubation with IL-1α on the arachidonic acid metabolism by pregnant rat uterus. Bars represent mean ± SEM of six uterus.

Effect of Interleukins on arachidonic acid metabolism

Incubation for 60 min with IL-1α caused an increase in the conversion of arachidonic acid to all the prostanoids evaluated only at a dose of 10^{-11}M, the same dose that increase NO production (Fig. 3). The other doses (10^{-12} and 10^{-10} M) did not change prostaglandin synthesis (data not shown).

We found that IL-2 added in vitro to the incubation medium of uterine tissue did not alter the metabolism of arachidonic acid (data not shown).

Effect of NMMA and AG on IL-1α -induced arachidonic acid metabolism

We investigated whether the IL-1α-induced increase in uterine PGs production is modulated by NO use of an inhibitor of NO synthesis (NMMA). Tissues were preincubated with or without NMMA (300uM) in the presence or absence of IL-1α.

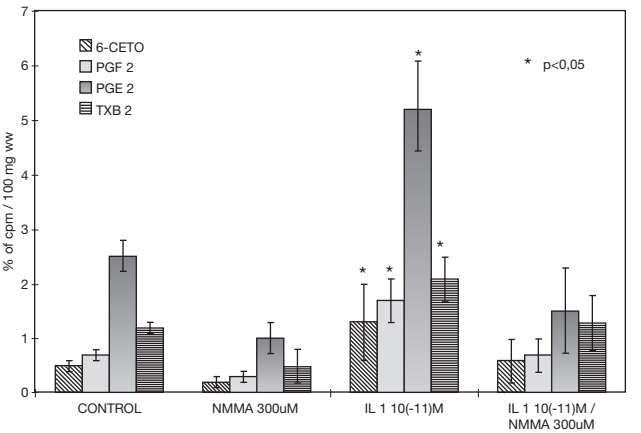

Fig. 4 Effect of NMMA on IL-1α effect on arachidonic acid metabolism by pregnant rat uterus. Bars represent mean ± SEM of six uterus.

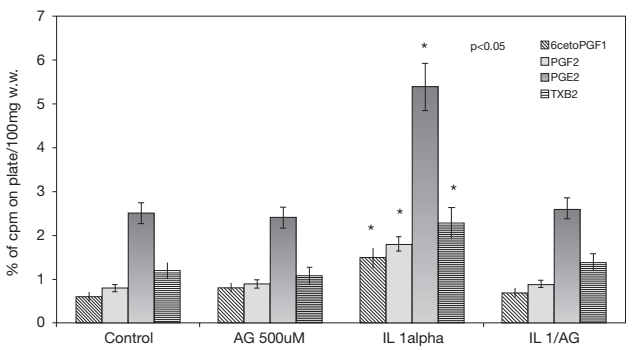

Fig. 5 Effect of aminoguanidine (500 uM) on IL-1α effect on arachidonic acid metabolism by pregnant rat uterus. Bars represent mean ± SEM of six uterus.

The stimulatory effect of IL-1α was greatly reduced in the presence of NMMA. NMMA alone did not have any effect (Fig. 4). These results indicate a positive effect of endogenous NO on PGs production in the pregnant uterus.

The stimulatory effect of IL-1α was also reduced in the presence of AG (500uM), suggesting that the enzyme involved in the effect of NO on PGs synthesis was iNOS (Fig. 5).

Effect of NS-398 on IL-1α-induced arachidonic acid metabolism

In order to discriminate between the activities of COX-1 and COX-2, we used a COX-2 inhibitor, NS-398. The uterus was incubated with or without NS-398 (30nM) in the presence or absence of IL-1α. The stimulatory effect of IL-1α on prostaglandin synthesis was prevented in the presence of the COX-2 inhibitor NS-398. By itself, NS-398 had no effect on basal prostaglandin synthesis (Fig. 6). These results suggest the participation of COX-2 in the IL-1α-induced PGs production.

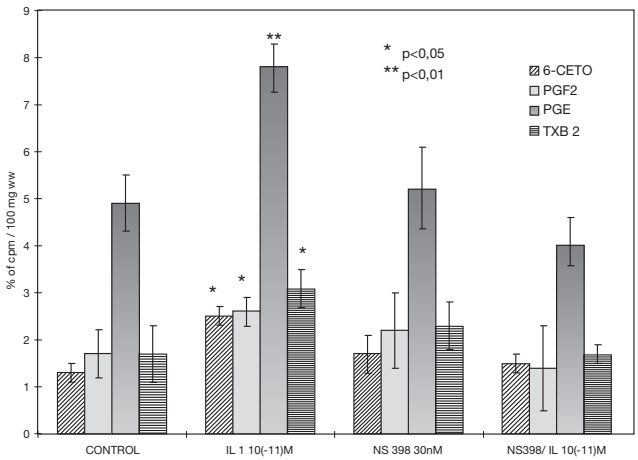

Fig. 6 Effect of NS-398 on IL-1α effect on arachidonic acid metabolism by pregnant rat uterus. Bars represent mean ± SEM of six uterus.

DISCUSSION

The main purpose of the present study was to investigate the effect of two interleukins on prostanoid synthesis and on NOS activity in the pregnant rat uterus. Recently we demonstrated that NP, a classic NO donor, increased metabolism of labeled arachidonic acid in the oestrogenized uterus[20] and that IL-1α augmented PGs production in the same model.[8] In the present study, the action of NO on cyclooxygenase activity and the effect of IL-1α on NO and PG synthesis were confirmed. We also showed that aminoguanidine, an inhibitor of Ca independent-NOS, was able to diminish the effect of IL-1α on NO and PG synthesis, suggesting that a Ca^{2+} independent-NOS is responsible for the enhancement in NO output by cytokines.

This work shows that IL-1α but not IL-2 stimulated the prostaglandin synthesis and the production of NO. Previous studies have demonstrated that the proinflammatory cytokine IL-1α caused enhanced production of PGs in sensitized rat endometrial stromal cells in vitro[22] and in uterine epithelial cells.[11] Nitric oxide metabolites have been found to be higher than normal in patients with preterm labor and premature rupture of membranes, a situation where inteleukins are augmented.[23]

In the present experiment IL-1α stimulated PG production in isolated pregnant uterus. We analyzed the effect of a COX-2 inhibitor, NS-398, in order to investigate the mechanism by which IL-1α enhanced the production of PGs in pregnant uteri. The COX-2 antagonist abolished the effect of IL-1α on PG production, suggesting that COX-2 is responsible for the enhancement in PG output by cytokines. We have recently shown that the effect of IL-1α on PG synthesis in estrogenized rat uterus is via COX-2.[8] It has been reported that IL-1β regulates COX-2 in amnion-derived WISH cells[13] and in cultured amnion cells[24] and that IL-4 induces COX-2 in intact human amnion tissue.[14]

We have demonstrated that nitroprusside, a classical donor of NO, increased PG and 5-HETE synthesis in estrogen-treated rat uteri[20] and that the IL-1α effect on PG production in estrogenized rat uterus was mediated by NO.[8] Others showed that both endogenous and exogenous NO increase PGE_2 production by the pregnant rat uterus.[25] It has been also reported that cytokines enhanced NO synthesis in several cell types[25,26] so we investigated whether the IL-1α effect on PG synthesis was modulated by endogenous NO by use of L-NMMA, a NOS inhibitor. L-NMMA inhibited IL-1α-induced PG production in the pregnant uterus.

As we have shown in this paper, IL-1α enhanced NOS activity at a concentration of 10^{-11} M. Thus, in this tissue, NO seems to directly activate COX resulting in an overproduction of PGs. This effect of NO on PG production has been reported in Swiis fibroblasts,[27] ex vivo rabbit kidney perfusion[28] and the pregnant rat uterus[25] etc.

We have also investigated which NOS isoenzyme was involved in the IL-1α-effect on NO synthesis. Our results suggest the participation of iNOS in this effect.

Spontaneous labor was associated with a three fold increase in uterine COX-2, suggesting that increased expression of COX-2, may be involved at term with increased uterine contractility. We have shown a positive effect of IL-1α on PG synthesis via COX-2 on term pregnant uterus. An increase of IL-1 activity in human amniotic fluid in third trimester with a further increase at parturition has been demonstrated.[15] Therefore, it is possible that an increased IL-1α activity at term or preterm could provide the stimulus for increased COX-2 expression and PG production in uterine tissues.

In this study we demonstrated an effect of IL-1α on term pregnant uterus prostanoid production and that this action is mediated by NO. This mechanism may be involved in regulation of labor.

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