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Nitric oxide inhibits prostanoid synthesis in the rat oviduct

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Summary We have studied the effect of nitric oxide (NO) on the production of arachidonic acid ([^{14}C]-AA) metabolites in the rat oviduct. The basal synthesis of eicosanoids was measured by the conversion of ([^{14}C]-AA) to the different radiolabeled products of cyclooxygenase (COX). The oviducts incubated for 1h with the labeled substrate of COX were able to convert 3.3 ± 0.3 % of ([^{14}C]-AA) to 6-ceto-$PGF_{1\alpha}$, 10.7 ± 1.0% to $PGF_{2\alpha}$, 13.5 ± 1.2% to PGE_2 and 6.3 ± 0.5 % to TXB_2. The tissues were incubated with different doses of two NO donors: SIN-1 and Spermine NONOate. The results indicated that SIN-1 produces a significant decrease (50%; $P < 0.05$) in all prostanoids evaluated in a dose-response fashion. The inhibitory effect was completely reversed by addition of 20 μg/ml of hemoglobin (Hb), a NO scavenger. The addition of Spermine NONOate to the incubation medium diminished significantly (65%) the synthesis of COX metabolites suggesting that NO acts by inhibiting COX activity in the rat oviduct. However, NOS inhibitors, N^G-L-arginine-methyl-ester (L-NAME) nd N^G-L-monomethyl-arginine (L-NMMA) had no effect on basal production of the prostanoids. These results indicate that in the rat oviduct the synthesis of COX metabolites is negatively regulated by nitric oxide. © 2000 Harcourt Published Ltd

INTRODUCTION

Nitric oxide (NO) is a gaseous mediator, known to be produced by various cell types in different organs, including reproductive tissues.[1] NO is synthesized from L-arginine by the NO synthase (NOSs) enzymes.[2] There are at least three different isoenzymes of NOS. Two are constitutive (cNOS), Ca^{2+}/calmodulin-dependent responsible for basal NO synthesis, and can be inhibited by a Ca^{2+}-chelator such as EGTA. The other isoform, Ca^{2+}/calmodulin independent, is cytokine or endotoxin-inducible (iNOS). Under specific conditions, namely immune reactions, iNOS generates large amounts of NO. Both isoforms have been described in bovine and human fallopian tubes[3] and more recently in the rat oviduct.[4,5]

NO interacts with heme-containing enzymes affecting their activation state.[6] Binding of NO to the heme moiety of soluble guanylate cyclase results in an activation of this enzyme and enhanced production of cyclic GMP which mediates several cellular actions of NO.[7] Cyclooxygenase is a heme-containing enzyme catalyzing the formation of prostaglandins, prostacyclin and thromboxanes.[8] Depending on the cell type and experimental conditions used, NO has been found either to stimulate,[9–12] to inhibit[13–15] or not to influence[16,17] PGs synthesis.

We have recently reported that rat oviductal NOS may be regulated by $PGF_{2\alpha}$.[18] Dong et al.[19] showed that exogenous PGE_2 significantly inhibited $IL_{1\beta}$ induced but not constitutive, nitrite production.

The aim of this study was to investigate the effects of exogenous NO on the cyclooxygenase pathway in the rat oviduct.

MATERIALS AND METHODS

Drugs and chemicals

Prostaglandins, hemoglobin (Hb), N^G-L-monomethyl arginine (L-NMMA), N^G-nitro-L-arginine methyl ester (L-NAME) were purchased from Sigma Chemical Co. (St Louis, Mi, USA); [^{14}C]-arachidonic acid was from Amersham Corporation (Arlington Heights, IL, and USA). SIN-1 and Spermine NONOate were purchased from Cayman (Ann Harbor, MI USA).

All other chemicals were analytical grade.

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Animals and tissues

Estrogenized rats (1 ∓g/rat) of the Wistar strain (180–200g body weight) were used. Animals were kept under controlled illumination (14 h light and 10 h dark) and temperature (24°C). Pelleted food and water were supplied ad libitum. The rats were killed by a blow on the neck. The oviducts were removed immediately, cleaned of fat and rinsed thoroughly in cold Krebs Ringer Bicarbonate buffer (KRB).

Metabolism of [^{14}C]-arachidonic acid

The metabolism of exogenous arachidonic acid in rat oviduct was determined by incubating the tissue for 60 min in KRB containing 0.25 μCi of [^{14}C]-arachidonic acid at 37°C, bubbled with a mixture of 95% O_2/ 5% CO_2 and constant shaking. For each determination, two oviducts were used. Before adding [^{14}C]-arachidonic acid, the tissues were randomly incubated with different NO donors or NOS inhibitors for 60 min. The controls were incubated in medium alone. At the end of incubation, the tissues were removed and the cyclooxygenase products were extracted (three times) with 1 vol. of ethyl acetate. Pooled acetate extracts were dried under nitrogen. The residues were applied to silica gel TLC plates. Authentic prostanoids were applied to other plates, which were run in parallel. The plates were developed in the solvent system 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. Radioactivity from TLC zones for arachidonic acid and for different prostanoids was measured by liquid scintillation counting. The results were expressed as a percentage of the total radioactivity of the plates.

Statistics

Statistical significance was tested by Student–Newman–Keuls multiple comparison test for unequal replicates. Differences with $P < 0.05$ were considered significant.

RESULTS

To study the effect of different NO donors or NOS inhibitors on oviductal prostanoid production, we measured the basal PG synthesis in our system, by conversion of [^{14}C]-arachidonic acid ([^{14}C]-AA) to different labeled products of cyclooxygenase. After 1h, rat oviducts were capable to convert 3.3 ± 0.3% of ([^{14}C]-AA) to 6-keto-PG $F_{1\alpha}$, 10.7 ± 1.0% to $PGF_{2\alpha}$, 13.5 ± 1.2 to PGE_2 and 6.3 ± 0.5% to TXB_2. We considered only the metabolites where conversion was higher than 1% of cpm on TLC plate.

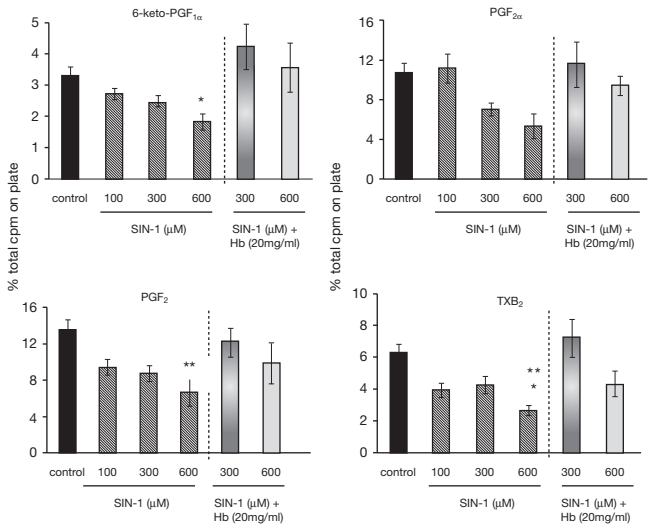

Fig. 1 Effect of a NO donor, SIN-1, on basal metabolism of [^{14}C] arachidonic acid to various cyclooxygenase metabolites: 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$, PGF_2, and TXB_2. Each column represents mean ± of $n = 6$ animals. *$P < 0.05$ vs control; **$P < 0.01$ vs control and SIN-1 (600 μM) + Hb.

Effect of exogenous nitric oxide on prostanoid synthesis

To study the effect of exogenous NO on prostanoid production, oviductal tissue was incubated for 60 min in KRB medium with SIN-1 (100, 300, 600 μM), a NO donor. As shown in Figure 1, the addition of SIN-1 produced a decrease, in a dose-dependent fashion ($P < 0.05$), of the synthesis of all prostanoids evaluated. This effect was completely reversed by addition of 20 μg/ml of Hb (for 60 min). This scavenged the NO released.

To confirm the inhibition of cyclooxygenase activity by NO and to eliminate a non-specific action of SIN-1, we tested the effect of Spermine NONOate, the other donor, on PGs and thromboxane B_2 production. The oviducts were incubated with 600 μM of the NO donor for 60 min. The results (Fig. 2) indicated that the incubation with Spermine NONOate diminished significantly the prostanoid production. As with SIN-1, the addition of Hb (20 μg/ml) also produced a complete reversion of the donor inhibition (Fig. 2).

Effect of L-NAME and L-NMMA on cyclooxygenase activity

To study the effect of different NOS inhibitors on basal prostanoid production, the oviducts were preincubated with 600 μM of L-NAME or L-NMMA for 30 min. There was no significant effect of the NOS inhibitors on the basal synthesis of either the PGs or thromboxane evaluated; similarly to the addition of the NOS inhibitors, Hb alone had no effect on basal COX activity (Fig. 3).

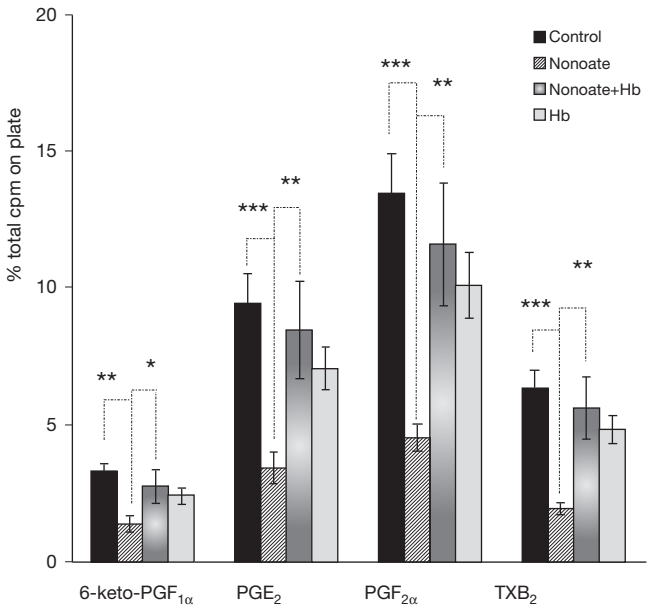

Fig. 2 Effect of a NO donor, Spermine NONOate (600 μM) on basal metabolism of [^{14}C] arachidonic acid to various cyclooxygenase metabolites. Each column indicates the mean ± SEM of $n = 6$ animals. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Hb (20 μg/ml).

DISCUSSION

The main purpose of the present study was to investigate whether exogenous or endogenous NO is able to modify the metabolism of labeled arachidonic acid in the rat oviduct. We demonstrate that exogenous NO in vitro decreases prostanoid synthesis in this organ.

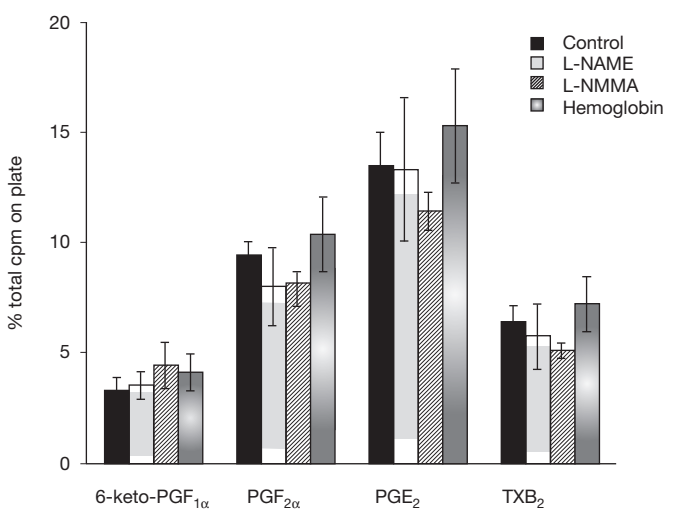

Fig. 3 Effect of NOS inhibitors on basal metabolism of [^{14}C] arachidonic acid to various cyclooxygenase metabolites. Each column represents mean ± SEM of $n = 6$ animal. L-NMMA (600 μM), L-NAME (600 μM), Hb (20 μg/ml).

We have found that SIN-1 or Spermine NONOate, two slow releasing-donors of NO, diminished significantly the synthesis of 6-keto-$PGF_{1\alpha}$, PGE_2, $PGF_{2\alpha}$, and TXB_2. The maximal decreases observed were 50% and 65% with 600 μM of SIN-1 or NONOate respectively, being the highest for TXB_2. On the other hand, the NO scavenger Hb, when added simultaneously with NO donors, completely reversed the inhibitory action of NO.

Previous work demonstrated the participation of NO in the basal synthesis of PGE_2 in rat hypothalamic fragments.[20] We have found that NOS inhibitors, L-NAME or L-NMMA did not modify the basal prostanoid production in estrogenized rat oviduct. These results are in according with the findings of Franchi et al.[9] who did not find an effect of those inhibitors on the basal production of prostanoids, except for PGE_2, in the estrogenized rat uterus.

NO interacts with heme-containing enzymes affecting their activation stage.[6] The chemical interaction between NO and the heme moiety of COX has been demonstrated.[21] Several studies have found that NO could influence prostanoid synthesis by inhibiting COX activtiy and/or its expression.[13,14] Recently, Kosonen et al.,[15] found that, in human endothelial cells, NO-donors inhibited the activity of COX-2 but did not alter the expression of this enzyme.

Conversely, the COX metabolites have been reported to influence iNOS induction. In a recent study, we found that $PGF_{2\alpha}$ increased NO synthesis by inducing the Ca^{2+}-independent NO synthase activity in the rat oviduct.[18] The role of endogenously produced PGs as enhancers of NO production was reported in rat Kuppfer cells[22] and other authors reported the regulation of iNOS by metabolites of COX.[19,23]

Recent data from our laboratory and others indicated that a NO system exists in the rat oviduct[4,5] and that NO plays a role as a mediator of tubal $PGF_{2\alpha}$-induced contractility.[18] Several agents that act as stimulators or inhibitors of oviductal smooth muscle contraction and ciliar activity regulate oviductal function. Different pathways participate in that regulation. The oviduct of different mammalian species produces PGs and their effects are intimately related to the regulation of oviductal motility and ciliary activity.[24] Riehl et al.[25] found differential affinity to binding sites for these PGs in different portions of the organ. There was also demonstrated a correlation between the distribution of binding sites for PGs and the oviductal smooth muscle relaxation during ova transport towards the uterus.

The relaxing effect of NO has been reported in a variety of smooth muscle organs in different animals as well as in humans. In vitro experiments showed that NO mediates relaxation of the pregnant rat uterus, supporting the notion that NO may contribute to the maintenance of uterine quiescence during pregnancy.[26] Furthermore, our previous results[27] indicated that in the rat oviduct, NO

inhibition increases tubal motility that results in an accelerated egg transport suggesting that NO could act as an inhibitory mediator between different layers of the oviductal wall.

The fact that NO decreases PG synthesis in the rat oviduct suggests that this molecule could be regulating the synthesis of contractile agents, such as $PGF_{2\alpha}$, so as to avoid the early entrance of the embryos to the uterus. Thus, NO may contribute to the maintenance of the oviductal quiescence during the fertilization process or to control the ova passage towards the uterus. This study provides evidence that nitric oxide could be involved in the regulation of rat oviductal function.

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