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Role of nitric oxide in the metabolism of arachidonic acid in the rat anterior pituitary gland

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Abstract

Nitric oxide (NO) affects cyclooxygenase (COX) and lipooxygenase (LOX) activities in several tissues. The aim of this study was to investigate the effect of NO on the AA metabolism in the anterior pituitary. LOX and COX products from anterior pituitaries of Wistar male rats were determined by [^{14}C]-AA radioconversion method. Sodium nitroprusside (NP, 0.5 mM) and DETA NONOate (1 mM), NO donors, decreased 5-hydroxy-5,8,11,14-eicosatetraenoic acid (5-HETE) synthesis ($P < 0.05$), effects that were reversed by hemoglobin. L-arginine also inhibited LOX activity. To the contrary, the inhibition of NO synthase by L-NAME (0.5 mM) or aminoguanidine (0.5 mM) increased 5-HETE production ($P < 0.05$). COX activity was slightly stimulated by NP and L-arginine. However, DETA NONOate induced a stimulation of the synthesis of all prostanoids ($P < 0.05$), this effect being reversed by hemoglobin. Neither NOS inhibitors nor hemoglobin modified basal prostanoids synthesis. These results indicate that NO inhibits LOX activity and stimulates COX activity in the anterior pituitary gland. The inhibition of LOX by NO may be another mechanism involved in the effects of NO on hormone release in the anterior pituitary. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Nitric oxide; Lipooxygenase; Cyclooxygenase; Anterior pituitary; Prolactin; 5-HETE

1. Introduction

Nitric oxide (NO), generated from L-arginine by NO synthase (NOS), is a free radical involved in the regulation of many physiologic functions, including endothelium-dependent vasodilation, apoptosis, neurotransmission and hormone secretion (Wolf, 1997). Immunocytochemical studies and in situ hybridization have demonstrated the presence all three types of NOS in rat anterior pituitary gland (Ceccatelli et al., 1993; Lloyd et al., 1995; Okere et al., 1998).

NO participates in the regulation of prolactin, adrenocorticotropin (ACTH), growth hormone (GH), and luteinizing hormone (LH) secretion and its effect can be directly exerted at the pituitary level (Kato, 1992; Brunetti et al., 1993; Ceccatelli et al., 1993; Duvilanski et al., 1996).

Several reports showed that arachidonic acid (AA) stimulates prolactin, ACTH, follicle-stimulating hormone (FSH), GH, LH, and thyroid-stimulating hormone (TSH) release from anterior pituitary cells, these effects depending mostly on the production of metabolites from the lipooxygenase (LOX) pathway (Canonico et al., 1984; Conte et al., 1986; Cowell et al., 1991; Ben Menahem et al., 1994; Roudbaraki et al., 1996). 5-hydroxy-5,8,11,14-eicosatetraenoic acid (5-HETE) is the most important LOX metabolite involved in FSH, LH and prolactin release (Koike et al., 1985; Dan Cohen et al., 1992; Ben Menahem et al., 1994), while 12-HETE and 12-hydroperoxy 5,8,11,14-eicosatetraenoic acid (12-HPETE) increase ACTH and GH secretion, respectively (Won and Orth, 1992; Roudbaraki et al., 1996). Leukotrienes, 15-HETE and epoxyeicosatrienoic acids are also involved in the secretion of these hormones (Cashman et al., 1987; Dan Cohen et al., 1992; Ben Menahem et al., 1994).

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On the other hand, the effects of cyclooxygenase (COX, prostaglandin-H synthase) products on anterior pituitary hormone release are controversial. One of these products, prostacyclin (PGI_2), stimulates basal release of TSH (Wright and Hedge, 1981).

NO regulates LOX and COX activities in several tissues, by direct effect on the heme group of these enzymes (Maccarrone et al., 1996; Goodwin et al., 1999). Depending on the cell type, NO can stimulate or inhibit LOX and COX activities. However, the effect of NO on the metabolism of AA in the anterior pituitary gland is not yet known.

Since NO affects anterior pituitary hormone release and AA metabolites participate in the process of hormone secretion, the aim of this study was to investigate the effect of NO on AA metabolism in the anterior pituitary.

2. Materials and methods

Male Wistar rats (200–250 g), kept on 12 h light-dark cycles and controlled temperature (20–25°C), were used. Food and water were supplied ad libitum. The animals were maintained in accordance with the NIH Guide for the Care and Use of Laboratory Animals. The animals were killed by decapitation and the anterior pituitary glands removed and placed in Krebs–Ringer bicarbonate buffer containing 10 mM glucose (KRB buffer), previously gassed for 20 min with 95% O_2–5% CO_2 and adjusted to pH 7.35–7.40.

All drugs were obtained from Sigma Chemical Co., St Louis, MO, except DETA NONOate (Alexis, San Diego, CA), [^{14}C]-arachidonic acid (New England Nuclear, Buenos Aires, Argentina), 5-hydroxy-5,8,11,14-eicosatetraenoic acid (5-HETE) and 12-HETE (Paesel, Frankfurt). All drugs were freshly prepared in KRB buffer. In NO scavenging experiments, 2 μM oxyhemoglobin (Hb) was used. For oxygenation, 40 mg/ml hemoglobin was mixed with an excess of sodium dithionite, and gassed with O_2 for 5 min. Then, sodium dithionite was removed from the solution with a Sephadex G-25 column. Concentrations of oxy- and methemoglobin were calculated from their values of absorbance found at 577 and 630 nm.

2.1. Incubation of anterior pituitary glands

Two pituitaries per tube were preincubated for 45 min in 2 ml KRB buffer in an atmosphere of 95% O_2–5% CO_2 with constant shaking at 60 cycles per min at 37°C. Afterwards, tissues were incubated for another 60 min in 2 mL of fresh KRB buffer containing 2 μL [^{14}C]-arachidonic (55 mCi/mmol) acid and the different drugs being studied. When hemoglobin or NOS inhibitors were used, they were present during both preincubation and incubation periods. DETA NONOate was prepared one hour before the beginning of experiments to allow NO concentration to reach a plateau. At the end of the incubation time, the tissues were removed from the tubes and the media immediately processed to measure the products of LOX and COX. Tissues were homogenized in water and protein content was determined by the Lowry et al., method.

2.2. Determination of [^{14}C]-arachidonic acid metabolites

LOX and COX metabolites from [^{14}C]-AA were determined as previously described (Franchi et al., 1994). Briefly, LOX products were extracted from the medium three times with 2 ml ethyl acetate. Pooled extracts were dried under nitrogen. The residue was suspended in 0.2 ml of chloroform/ethanol (2:1 vol/vol) and applied to a silica gel thin layer chromatography (TLC) plate. Standard 5-HETE and 12-HETE were run in parallel. The plates were developed in the solvent system ethyl acetate/isooctane/acetic acid/water (76:67:20:100, vol/vol). The spots were visualized with iodine vapor and scraped off the plates. Average R_f values for 5-HETE, AA and 12-HETE were 0.70, 0.85 and 0.92, respectively. This method was not able to discriminate between 15-HETE and AA, because these lipids had similar R_f. In our experimental conditions, 12-LOX products were not detectable.

After extraction of LOX products, the remaining medium was acidified to pH 3.0 with 1.0 N HCl in 1 volume of ethyl acetate and extracted three times for prostaglandins (PGs) determination. Pooled acid ethyl acetate extracts were dried under nitrogen. The residues were suspended in chloroform/methanol and applied to silica gel TLC plates. The plates were developed in a solvent system of benzene/dioxane/glacial acetic acid (60:30:3, vol/vol). Standard prostanoids were run in parallel. The position of COX products was visualized by spraying the dried plates with 10% phosphomolybdic acid in ethanol followed by heating at 110°C for 10 min. Average R_f values were 0.30 for 6-keto-$PGF_{1\alpha}$, 0.35 for $PGF_{2\alpha}$, 0.47 for PGE_2, 0.57 for thromboxane B_2 (TXB_2) and 0.80 for AA.

Radioactivity was measured by liquid scintillation counting. Results were expressed as cpm per μg of protein.

2.3. Statistics

The results were expressed as mean ± SEM and evaluated by Student's t-test or one or two-way analysis of variance (ANOVA) followed by Student-Newman–Keuls multiple comparison test for unequal replicates or Dunnett's test depending on the experimental design. Differences between groups were considered significant

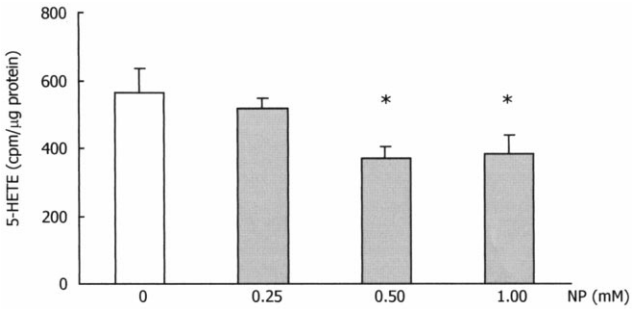

Fig. 1. Effect of increasing concentrations of NP on 5-HETE production. Anterior pituitary glands were incubated with increasing concentrations of NP for 60 min. Bars represent the mean ± SEM ($n = 6$). *$P < 0.05$ vs control without NP (Dunnett's test).

if $P < 0.05$. Results were confirmed by at least three independent experiments.

3. Results

3.1. Effect of NO donors on LOX activity

In order to investigate the effect of NO on LOX activity in the anterior pituitary, we examined the effect of different NO donors on 5-HETE synthesis. Sodium nitroprusside (NP, 0.5 and 1 mM), a NO donor, significantly decreased 5-HETE production, while a lower concentration of NP (0.25 mM) had no effect (Fig. 1). DETA NONOate (1 mM), another NO donor, also significantly decreased 5-HETE production. (Control: 918 ± 33 cpm/μg protein, DETA NONOate: 752 ± 57, $n = 6$; $P < 0.05$, Student's t-test). Hemoglobin (Hb, 2 μM), a NO scavenger, did not affect basal 5-HETE synthesis but reversed the inhibitory effect of NP (Fig. 2). Hemoglobin also blocked the inhibitory effect of DETA NONOate on 5-HETE synthesis (data not shown).

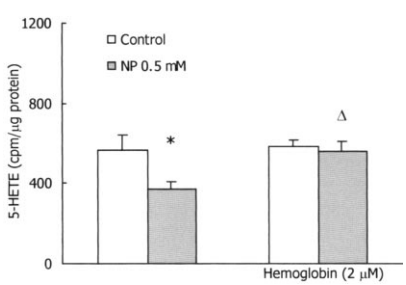

Fig. 2. Effect of hemoglobin on the NP-induced decrease of 5-HETE concentration. Anterior pituitary glands were preincubated with or without 2 μM hemoglobin for 45 min, then incubated with hemoglobin and/or NP for 60 min. Bars represent the mean ± SEM ($n = 6$). *$P < 0.05$, vs respective control without NP; $^{\Delta}P < 0.05$, vs respective control without hemoglobin (Student–Newman–Keuls test).

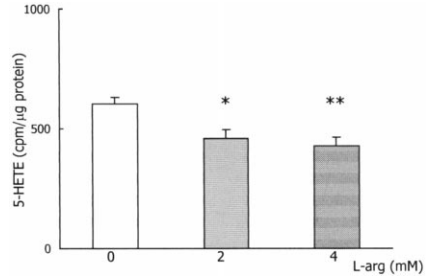

Fig. 3. Effect of L-arginine on 5-HETE synthesis. Anterior pituitary glands were incubated with increasing concentrations of L-arginine (L-arg) for 60 min. Bars represent the mean ± SEM ($n = 5$-6). *$P < 0.05$, **$P < 0.01$ vs control (Dunnett's test).

3.2. Effect of endogenous NO on LOX activity

Since NO is synthesized by anterior pituitary cells, we evaluated the participation of endogenous NO on 5-HETE synthesis. L-arginine (2 and 4 mM), a substrate of NOS, decreased 5-HETE production as did the NO donors (Fig. 3). Hemoglobin reversed the inhibitory effect of L-arginine (Control: 707 ± 35 cpm/μg protein, L-arginine: 558 ± 50, L-arginine + Hb: 790 ± 62, $n = 6$; $P < 0.05$, Dunnett's test). To the contrary, L-NAME (0.5 mM) and aminoguanidine (0.5 mM), NOS inhibitors, enhanced 5-HETE production (Fig. 4).

3.3. Effect of NO donors on COX activity

In order to investigate COX activity, the production of 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$, PGE_2, TXB_2 was measured. Table 1 shows that 1 mM NP stimulated $PGF_{2\alpha}$ synthesis and produced a non significant increase of the other prostanoids. Lower NP concentrations did not modify prostanoids synthesis. DETA NONOate (1 mM) induced a significant increase of all prostanoids studied (Fig. 5). Hemoglobin (2 μM) did not affect PGs and TXB_2 synthesis per se, but completely reversed the stimulation evoked by DETA NONOate (Fig. 5).

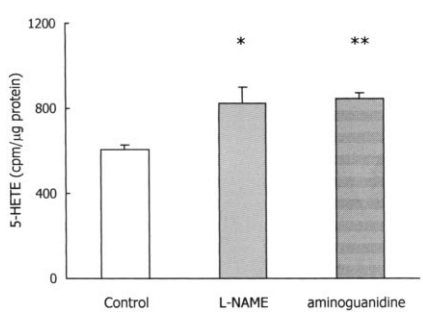

Fig. 4. Effect of NOS inhibitors on 5-HETE production. Anterior pituitary glands were preincubated with or without 0.5 mM L-NAME or 0.5 mM aminoguanidine for 45 min, and then incubated for another 60 min in the same conditions. Bars represent the mean ± SEM ($n = 6$). *$P < 0.05$, **$P < 0.01$ vs. control (Dunnett's test).

Table 1
Effect of increasing concentrations of NP on prostanoids synthesis[a]

	Control	NP (mM)		
		0.25	0.50	1.00
6-keto $PGF_{1\alpha}$	631 ± 58	574 ± 57	552 ± 30	712 ± 32
$PGF_{2\alpha}$	800 ± 84	733 ± 42	933 ± 43	1136 ± 69*
PGE_2	1078 ± 127	902 ± 90	909 ± 44	1144 ± 69
TXB_2	340 ± 55	352 ± 24	311 ± 31	412 ± 42

[a] Anterior pituitary glands were incubated with increasing concentrations of NP for 60 min. Bars represent the mean ± SEM ($n = 6$). *$P < 0.05$ vs. control without NP (Dunnett's test).

3.4. Effect of endogenous NO on COX activity

L-arginine (2 and 4 mM), stimulated $PGF_{2\alpha}$ synthesis without significantly affecting the synthesis of other COX metabolites (Table 2). On the other hand, L-NAME (0.5 mM) or aminoguanidine (0.5 mM), did not modify any of the COX products studied (data not shown).

4. Discussion

The present study shows that NO inhibits LOX activity in rat anterior pituitary gland. Several reports indicate that, depending on the tissue type, NO is able to increase or inhibit LOX activity by interacting with the heme group of this enzyme (Hotter et al., 1996; Maccarrone et al., 1996). 5-HETE is the most important AA metabolite involved in the secretion of prolactin, FSH and LH (Koike et al., 1985; Dan Cohen et al., 1992; Ben Menahem et al., 1994). NP and DETA NONOate, two NO donors with different molecular

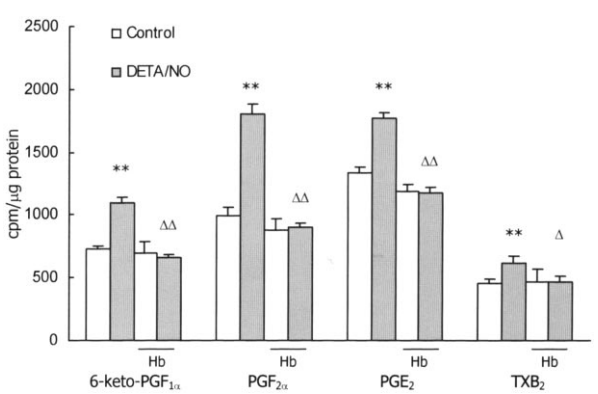

Fig. 5. Effect of DETA NONOate and hemoglobin on COX activity. Anterior pituitary glands were preincubated with or without 2 µM hemoglobin (Hb) for 45 min, then incubated with hemoglobin and/or 1 mM DETA NONOate (DETA/NO) for 60 min. Bars represent the mean ± SEM ($n = 6$). **$P < 0.01$ vs. respective control without DETA/NO; $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$ vs. respective control without hemoglobin (Student–Newman–Keuls test).

Table 2
Effect of L-arginine on PGs and thromboxane production[a]

	Control	L-arg 2 mM	L-arg 4 mM
6-keto $PGF_{1\alpha}$	597 ± 39	819 ± 35	684 ± 98
$PGF_{2\alpha}$	719 ± 28	966 ± 85*	955 ± 73*
PGE_2	989 ± 40	1285 ± 84	1197 ± 112
TXB_2	247 ± 7	270 ± 31	311 ± 34

[a] Anterior pituitary glands were incubated with L-arginine (L-arg) for 60 min. Bars represent the mean ± SEM ($n = 5$–6). *$P < 0.05$ vs. respective control without L-arginine (Dunnett's test).

structures, share only their ability to release NO. Both NP and DETA NONOate decreased 5-HETE production in the anterior pituitary gland. Additionally, L-arginine, the natural substrate of NOS, also decreased 5-HETE production. Since NO donors and L-arginine effects were blocked by hemoglobin, a NO scavenger, it can be surmised that these effects were exerted by NO. 5-HETE is a mediator of cellular responses with a short half life and its concentration can be considerated an indicator of its velocity of synthesis; therefore our results indicate that NO may directly inhibit 5-LOX activity in the anterior pituitary gland.

The inhibition of NO synthesis by L-NAME and aminoguanidine induced an increase of 5-HETE, suggesting that endogenous NO exerts an inhibitory effect on basal LOX activity.

The anterior pituitary is a heterogeneous cellular system, composed of several types of endocrine and non-endocrine cells. It is known that NO is synthesized by gonadotropes and folliculo-stellate cells (Ceccatelli et al., 1993). NO can act in an autocrine (Kato, 1992), or a paracrine manner (Duvilanski et al., 1996) on anterior pituitary endocrine cells. Therefore, the changes in LOX activity induced by NO donors in this system can reflect the action of NO on the NO-producing cells themselves or on neighboring cells. In the present study, NOS inhibitors increased 5-HETE synthesis, whereas hemoglobin did not modify it, perhaps the result of the fact that hemoglobin scavenges only extracellular NO without affecting NO action within the NO-producing cells. These results suggest that, in basal conditions, NO may be acting mainly on LOX activity in the cells that synthesize NO. Since there are anatomical relationships between cells with and without NOS activity (i.e. folliculo-stellate cells and lactotropes) (Morand et al., 1996), a juxtacrine effect of NO on LOX activity of cells that do not produce NO cannot be discarded.

Several studies indicate that NO modulates COX activity (Goodwin et al., 1999). The present results show that NO could stimulate COX activity in the anterior pituitary gland. Whereas NP and L-arginine produced only a significant increase of $PGF_{2\alpha}$, DETA NONOate induced a stimulation of the synthesis of all the prostanoids studied.

NO seems not to be involved in regulation of basal COX activity since the inhibition of NO synthesis did not modify the activity of this enzyme. Basal LOX, but not COX activity was affected by endogenous NO, suggesting that LOX and COX enzymes may have different affinities to NO. Recent studies with purified PGH synthase, have established an activation constant (K_a) of NO of 0.65 μM and 0.85 μM for COX and for peroxidase activities respectively (Maccarrone et al., 1997). The inhibition constant (K_i) of NO for purified soybean LOX is 0.40 μM (Maccarrone et al., 1996). In our experiments, the effective concentration of NO released from 1 mM NP was 0.87 μM, as detected electrochemically with a NO-sensitive electrode (World Precision Instruments, Sarasota, FL) (Poderoso et al., 1996). Similar submicromolar NO effective concentrations were obtained with 1 mM DETA NONOate. Therefore, we have used NO concentrations able to act on both LOX and COX activities.

The different capabilities of NP and DETA NONOate to stimulate COX activity could be explained by their abilities to release NO. In aqueous solution, NP immediately reaches a maximum of effective NO concentration that decreases with a half life time ($t_{1/2}$) of aproximately 20 min at 37°C. To the contrary, DETA NONOate release NO slowly, reaching a plateau of concentration at 30 min ($t_{1/2} > 500$ min). It is possible that a continuous but not a transient increase of NO concentration is necessary to stimulate COX activity.

It has been described that HPETEs, produced by LOX, inhibit COX activity (Maccarrone et al., 1997). Since NO inhibited LOX activity, the increase in COX activity might be due to a decrease in HPETE levels. Also, the effect of NO on COX activity could be exerted indirectly by a high offer of substrate (AA), probably as a consequence of the simultaneous inhibition of LOX activity by NO. Likewise, an effect of NO on AA metabolism through another heme-containing protein as well cytochrome P-450 should not to be discarded.

AA and its metabolites are involved in the process of hormone secretion in the anterior pituitary gland (Canonico et al., 1984; Conte et al., 1986; Cowell et al., 1991; Ben Menahem et al., 1994; Roudbaraki et al., 1996). NO inhibits the increase of ACTH, GH and LH release induced by their respective secretagogues, whereas prolactin is inhibited under basal conditions (Kato, 1992; Brunetti et al., 1993; Ceccatelli et al., 1993; Duvilanski et al., 1996). Therefore, NO could be affecting similar intracellular pathways in different anterior pituitary cell types.

The intracellular mechanisms by which NO modulates prolactin release in the anterior pituitary have not been completely elucidated. We have previously reported that NO stimulates guanylyl cyclase increasing cGMP levels (Duvilanski et al., 1996). NO, through the cGMP, stimulates cGMP-dependent protein kinase (PKG) and cGMP-stimulated phosphodiesterase (PDE2), decreasing cAMP levels and inhibiting prolactin release (Velardez et al., 2000). Furthermore, NO decrease intracellular calcium concentration in rat anterior pituitary gland (Duvilanski et al., 1998).

The present study demonstrates that NO decreases LOX metabolites, while increases COX products. Since AA through 5-HETE stimulates prolactin release, our results suggest that the LOX pathway may be involved in the inhibitory effect of NO on prolactin release.

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