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Inhibition of NOS enhances antigen-induced contractions and increases release of cysteinyl-leukotrienes in guinea pig lung parenchyma: NO as a protective factor.

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Abstract

Nitric Oxide (NO) in exhaled air is a biomarker of airway inflammation. However, the role of NO in the peripheral lung is not known. The aim of this study was to determine the role of endogenous NO in antigen-induced contractions of ovalbumin (OVA)-sensitized guinea pig lung parenchyma (GPLP). The contraction in this *in vitro* model of the peripheral lung closely resembles the corresponding response in human airways. Cumulatively increasing concentrations (10-10,000 μg/L) of ovalbumin (OVA) induced concentration-dependent contractions of the GPLP that were enhanced by the nitric oxide synthase (NOS) inhibitors N^{ω}-nitro-L-arginine (L-NOARG, 100 μM), N^{ω}-monomethyl-L-arginine (L-NMMA, 100 μM), N^{ω}-Nitro-L-arginine methyl ester (L-NAME, 100 μM) and 1400W (1 μM). The enhancement induced by L-NOARG was reversed by co-administration with the 5-lipoxygenase inhibitor BAYx1005 (3 μM), whereas co-administration of L-NOARG with the cyclooxygenase inhibitor indomethacin (10 μM) did not change the effect of L-NOARG alone. L-NOARG (100 μM) did not affect the cumulative concentration response relations for either leukotriene (LT) D_4 (0.1-100 nM) or histamine (1-30 μM). The NO donor NONOate (0.001-100 μM) was ineffective in GPLP but potently relaxed precontracted guinea-pig pulmonary artery. Furthermore, L-NOARG enhanced the release of LTE_4 and decreased the release of prostaglandin E_2 induced by OVA. In conclusion, endogenous NO exerts an inhibitory effect on antigen-induced contractions in the peripheral lung. The action of NO apparently involves inhibition of the release of mediators, rather than direct relaxation of airway smooth muscle. The findings support that endogenous NO has a protective anti-inflammatory effect in the airways.

Introduction

Nitric Oxide (NO) is measured in exhaled air and is considered as a biomarker of airway inflammation. Asthmatics have elevated levels of NO in their exhaled breath (Alving et al., 1993), but the function of NO in the airways is not completely known. NO is formed by three different nitric oxide synthases (NOS), the constitutively expressed neuronal (nNOS) and endothelial NOS (eNOS), and the inducible NOS (iNOS). It is known that iNOS is upregulated during inflammation and increased production of NO from iNOS may further aggravate the inflammation (Coleman, 2001; Ricciardolo et al., 2004). In addition, although NO is known to relax central airways in different animal models, including guinea pig (Gustafsson et al., 1991; Lei et al., 1993; Nijkamp et al., 1993), its role in the peripheral part of the lung has not been established. This is the purpose of the study here reported.

The major mediators of the early allergic airway response in humans are known to be cysteinyl-leukotrienes (CysLTs) and histamine (Roquet et al., 1997), but also products of the cyclooxygenase (COX) pathway are involved (Manning et al., 1991). It has been shown that antigen-induced contractions in small airways are more severe (Wohlsen et al, 2003) than in larger airways. The aim of this study was to determine the role of NO in antigen-induced contractions of the peripheral part of the lung. The study was performed in the lung parenchyma obtained from actively sensitized guinea pigs (GPLP), an *in vitro*-model for mast cell driven antigen-induced contractions. The mediators of this particular contraction response (Wikström Jonsson and Dahlén, 1994) are similar to those established for the anaphylactic contraction of human airways *in vitro* and *in vivo* (Björck et al., 1993; Roquet et al., 1997). A recent study in the isolated perfused and ventilated guinea pig lung indicates that in addition

to histamine and CysLTs one or several prostanoids contribute to the antigen-induced airway constriction in this particular species (Sundström et al., 2003).

Mast cells are able to produce NO (Gilchrist et al., 2002) and NO has been shown to inhibit the release of histamine from rat mucosal mast cells (Masini et al., 1991). A co-localization of NOS with 5-LO along the nuclear membrane has recently been suggested, providing a potential for interaction between NO and leukotriene synthesis in human mast cells (Gilchrist et al., 2004). In airway macrophages, NO may in fact act on 5-LO and suppress leukotriene synthesis (Coffey et al., 2000; 2002). The actions of NO on the COX pathway have not been completely elucidated but apparently involve both direct and indirect mechanisms (Watkins et al., 1997; Salvemini et al., 1993; Devaux et al., 2001).

One hypothesis to be tested was that NO also in the peripheral lung affects the antigen-induced contractions by relaxation of the airway smooth muscle. Another hypothesis was that NO affects the release of mast cell mediators, either globally or specifically via actions on COX or 5-lipoxygenase (5-LO) pathways for arachidonic acid metabolism. Different selective inhibitors of endogenous NOS, COX and 5-LO were used to study the impact of NO in the functional responses in the GPLP model. The tissue bath fluid was analysed by enzyme immuno assays (EIA) to determine if some of the key interventions affected the amount of released mediators during antigen-induced challenges.

Methods

Animals and OVA-sensitization

For studies of antigen-induced contractions, male *Dunkin Hartley* guinea pigs (300-350 g b.w.) were sensitized to Chicken Egg Albumin (OVA) at least four weeks prior to experiment. A stock solution of OVA was prepared by dissolving 500 mg OVA in 10 mL 0.9 % NaCl and 10 mL 2 % aluminium hydroxide gel and shaken for one hour. The guinea pigs were given a subcutaneous injection of 0.4 mL OVA (10 mg) in the neck and an i.p. injection of 0.4 mL OVA (10 mg). The study was approved by the regional committee of animal experimentation ethics (N14/02, N127/04) and in accordance with the Declaration of Helsinki.

Lung parenchymal strips

The animals were sacrificed by an overdose of inhaled CO_2 and the heart-lung-package was quickly removed and placed in ice-cold Tyrode's solution (prepared each day, containing NaCl 149.2 mM, KCl 2.7 mM, $NaHCO_3$ 11.9 mM, glucose 5.5 mM, $CaCl_2$ 1.8 mM, $MgCl_2$ 0.5 mM, NaH_2PO_4 0.4 mM). The lung parenchyma was cut parallel to the peripheral margins, yielding four to eight strips, each having a size of 2x2x20 mm and a weight of approximately 50 mg.

Organ bath experiments

The lung parenchymal strips were set up at a resting tension of 2.5 mN (0.25 g) in 5 mL organ baths filled with Tyrode's solution, bubbled with carbogen gas (6.5 % CO_2 in O_2) to keep a pH of 7.4 and the temperature was kept at 37 °C. Changes in smooth muscle tension, i.e. contractions and relaxations, were recorded via isometric force-displacement transducers connected to a Grass polygraph and responses were

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displayed via a chart-recorder or by using the IOX data acquisition system (EMKA, France). Data were analysed either manually from charts or by the software program Dataanalyst (EMKA, France). After an equilibration period of 90 min and washes each 15 min, histamine (1-30 μM) was added cumulatively as a control of the GPLP reactivity. Preparations displaying contraction responses less than 1.0 mN to the highest concentration of histamine were excluded from further experiments. Another wash and equilibration period between histamine and treatment period was performed. The enzyme inhibitors indomethacin (10 μM) and BAY x1005 (3 μM) were given 30 min before and all other drugs were given 15 min before the challenges. OVA was added as cumulative challenge of increasing concentrations (1-10,000 μg/L) every 10 min without changing bath fluid. For study of NO-donors, the GPLP was precontracted with a single dose of LTD_4 (10 nM). Maximum contractions of the preparation were determined with histamine (1 mM), acetylcholine (1 mM) and KCl (50 mM) at the end of each experiment, and other responses were expressed as percent of maximum contractions. Guinea pig pulmonary artery (GPPA) was prepared as rings with the endothelium gently removed, and then mounted in the organ baths under a resting tension of 15 mN (1.5 g). After an equilibration period of 60 min and washes each 10 min, noradrenaline (10 μM) was added and drugs were given at the plateau to study relaxations.

Drugs

NaCl, KCl, $CaCl_2$, $MgSO_4$, $NaHCO_3$, KH_2PO_4 and glucose were obtained from VWR International (West Chester, PA). Histamine dihydrochloride, noradrenaline, acetylcholine, indomethacin, ovalbumin (OVA, chicken egg albumin, grade II), L-NMMA (N^{ω}-monomethyl-L-arginine), L-NAME (N^{ω}-Nitro-L-arginine methyl ester),

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L-arginine and dimethylsulfoxid (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO). BAY x1005 ((R)-2-[4-(quinolin-2-yl-methoxy)phenyl]-2-cyclopentyl acetic acid) was from Bayer AG (Wuppertal, Germany). L-NOARG (N^{ω}-Nitro-L-arginine), 1400W (N-(3-(aminomethyl)benzyl)acetamidine) and diethylamine NONOate were purchased from Calbiochem (San Diego, CA). Leukotriene D_4 was from Cascade Biochemicals Ltd. (Reading, UK). Celecoxib (Celebrex ®) was obtained from Pfizer (CA). SC560 (5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-(trifluoromethyl)-1H-pyrazole), prostaglandin E_2 (PGE_2) and the EIA kits for leukotriene E_4 (LTE_4), thromboxane B_2 (TXB_2), leukotriene B_4 (LTB_4), prostaglandin D_2 (PGD_2)-mox and PGE_2 were obtained from Cayman Chemicals (Ann Arbor, MI).

Indomethacin was dissolved in equal parts of ethanol and 1 M, pH 8.0 Tris, and then diluted in 0.9 % NaCl. Stock solutions of 1 mM LTD_4 were dissolved in 50 % ethanol-water and then diluted in 20 % ethanol-water. The concentration and purity of LTD_4 was checked by UV spectroscopy. L-arginine was dissolved in 1 M HCl. BAYx1005, SC560 and celecoxib were dissolved in DMSO. OVA and L-NOARG were dissolved in 0.9 % NaCl. The other drugs were dissolved and diluted in Tyrode's solution or millipure water. Dilutions of drugs were freshly made from the stocks for each experiment. The drugs were present in the organ bath fluid during the remaining experiment.

Measurements of released mediators

Fluid (1 mL) was collected from each organ bath and immediately frozen at –20°C. The samples were taken at the end of the equilibration period to obtain basal mediator release from the tissue and at the obtained contractile plateau after challenge with OVA 100 μg/L. Enzyme immunoassay (EIA) analyses of the different mediators

CysLTs, LTB_4, TXA_2, PGD_2 and PGE_2 were performed according to the manufacture's instructions. TXA_2 was measured as the stable metabolite TXB_2. Cys-LTs were measured as LTE_4, the end metabolite of LTC_4 and LTD_4. The assay detection limits in the bath fluid levels for the different mediators were 7.8 pg/mL for TXB_2, LTE_4, PGE_2 and PGD_2 and 3.9 pg/mL for LTB_4. Results below detection limits were set as zero in the statistical evaluation. The EIA specificity for the different mediators to interfere with each other was less than 0.01 %, with the exception of the TXB_2 EIA that cross reacted with PGD_2 (0.53 %) and with PGE_2 (0.09 %). The LTE_4 EIA was performed with the cysLT antiserum and cross reacted with both LTC_4 (50 %) and LTD_4 (100 %). Histamine was measured as described previously (Shore et al., 1959; Bergendorff et al., 1972). Duplicates of 300 µL were placed in 96-wells plates and the amount of histamine was analysed by a fluorometer at the wavelength 450 nM. The detection limit for histamine was 3.9 ng/mL. The data were expressed as molar amounts per gram wet tissue (fmol/g or pmol/g).

Calculations and Statistics

All data are presented as mean ± standard error of the mean (SEM). Statistical analyses were made for paired and unpaired observations by Student's t-test or analyses of variances (ANOVA) followed by Tukey's t-test or Bonferroni's t-test. A p-value of less than 0.05 was considered significant.

Results

Effects of NOS inhibitors on antigen-induced contractions

Challenge with cumulative concentrations of OVA (10-10,000 μg/L) induced concentration-dependent contractions of sensitized GPLP (fig 1). Pre-treatment with the unselective NOS-inhibitor L-NOARG (100 μM) did not affect the basal tone of the preparation nor the maximal contractile response (control 2.29±11 mN; n=5, and L-NOARG 2.43±21 mN; n=5; ns). However, L-NOARG (100 μM) significantly ($p<0.001$) shifted the concentration-response relation for OVA to the left (fig 1A). Three additional and structurally different NOS-inhibitors, L-NAME (100 μM), L-NMNA (100 μM) and 1400W (1 μM), respectively, mimicked the enhancement of the OVA response induced by L-NOARG (100 μM) (fig 1B).

The NO substrate, L-arginine (100 μM), reversed the L-NOARG-enhanced contractions to control level. This concentration of L-arginine did not by itself significantly affect the OVA-induced contractions (fig 1A); nor did a higher concentration of L-arginine (300 μM) (59±6.7 %, n=4) compared to control (58±9.7 %, n=5; ns) at OVA 1000 μg/L.

Direct effects of NO on smooth muscle

L-NOARG (100 μM) did not alter the contractions induced by cumulative challenge with either LTD_4 (0.1-100 nM) (fig 2A) or histamine (1-30 μM) (fig 2B). Nor did the NO-donor diethylamine NONOate (0.1-100 μM) relax the parenchyma precontracted with LTD_4 (10 nM) (fig 3A), whereas NONOate (0.001-100 μM) dose-dependently relaxed the guinea pig pulmonary artery precontracted by noradrenaline (10 μM) (fig 3B).

Release of mediators during ovalbumin-induced contractions

Basal levels of mediators in the organ bath were below detection limits, except for TXB_2 (60.9±50 fmol/g) and PGE_2 (2.4±0.2 fmol/g), whereas measurable levels were obtained for all mediators (LTE_4, LTB_4, TXB_2, PGE_2, PGD_2 and histamine) after challenge with OVA 100 μg/L (fig 4). The release of TXB_2 was increased 15-fold and PGE_2 was increased five-fold above basal levels. Inhibition of endogenous NO with L-NOARG (100 μM) significantly increased the release of LTE_4 induced by OVA 100 μg/L ($p<0.05$, fig 4A), whereas the release of LTB_4 (fig 4B), TXB_2 (fig 4C), PGD_2 (fig 4E) and histamine (fig 4F) were not significantly increased. In contrast, L-NOARG (100 μM) significantly reduced the release of PGE_2 after OVA 100 μg/L ($p<0.05$, fig 4D).

Effects of COX, PGE_2 and NOARG on OVA-induced contractions

Pretreatment with the unselective COX inhibitor indomethacin (10 μM) significantly enhanced ($p<0.05$) the cumulative concentration response to OVA (1-10,000 μg/L) (fig 5A). This response was mimicked by the selective COX-1 inhibitor SC560 (5 μM) ($p<0.01$), whereas the selective COX-2 inhibitor celecoxib (3 μM) had no effect on the contractions induced by OVA compared to control (fig 5B). The combination of L-NOARG (100 μM) and indomethacin (10 μM) shifted significantly the concentration-response induced by OVA (1-10,000 μg/L) to the left ($p<0.01$), however the response did not change the effect of L-NOARG (100 μM) alone (fig 5C). Pretreatment with exogenous PGE_2 (100 nM) significantly ($p<0.05$) reduced the contraction induced by OVA 1000 μg/L. After combined pretreatment with indomethacin (10 μM) and PGE_2 (100 nM), the enhanced OVA response was reversed to control level (Fig 5D). The release of PGE_2 was significantly reduced after

pretreatment with indomethacin (10 μM) (2.3±0.6 fmol/g, n=4; p=0.001) at OVA 1000 μg/L.

Effect of 5-LO and L-NOARG on OVA-induced contractions

The enhanced contractions to OVA by L-NOARG (100 μM) were reversed by co-administration with BAYx1005 (3 μM). The 5-LO inhibitor BAYx1005 (3 μM) alone had no significant effect on the cumulative concentration response to OVA (1-10,000 g/L) (fig 6). The release of LTE_4 during the antigen-induced contractions (19±6.1 fmol/g) was abolished after pretreatment with BAYx1005 (3 μM) (<1.5 fmol/g, n=4, p=0.001) at OVA 1000 μg/L.

Discussion

In the present study, inhibition of endogenous NO enhanced antigen-induced contractions of peripheral airways. The effect of NO in the peripheral lung appears to be at the level of mediator release since inhibition of endogenous NO or application of exogenous NO had insignificant relaxant activity at the level of the airway smooth muscle. The mechanism apparently involves increased release of contractile CysLTs and decreased release of PGE_2.

Pretreatment with the unselective NOS-inhibitor L-NOARG caused an enhancement of the contraction evoked by challenge with cumulative doses of ovalbumin. This enhancement by the competitive substrate analogue inhibitor L-NOARG was reversed when L-NOARG was given together with the substrate L-arginine. Moreover, the enhancement of the antigen response by L-NOARG was reproduced by three other NOS inhibitors, L-NAME, L-NMMA and 1400W. L-NAME, L-NMMA and L-NOARG are unselective inhibitors of eNOS, nNOS and iNOS, whereas 1400W has been documented to selectively inhibit iNOS (Garvey et al., 1997). There was no significant difference in effect of the four different NOS-inhibitors tested. Taken together, the main enzyme generating NO in this particular model may be iNOS, as supported by the effect of 1400W.

Furthermore, the role of NO in the early allergic airway response appears to be protective by reducing the contraction response in the peripheral lung. Studies in a transgenic mouse model with an overexpressed iNOS also indicated that NO had no proinflammatory effects on the lung and decreased the airway responsiveness (Hjoberg et al., 2004). However, data from murine models with different deletions of NOS are conflicting (Xiong et al., 1999; De Sanctis et al., 1999), and these particular mice studies also used measurements of pulmonary function that presumably measure

central airway function. It remains to establish how these in vivo observations in mice relate to our current findings in the peripheral lung of the guinea pig.

In any event, one possible explanation of the inhibitory effect of endogenous NO on the antigen-induced contraction would be relaxation of airway smooth muscle, as has been shown in more proximal airways (Persson et al., 1993). However, endogenous or exogenous NO did not induce any significant relaxant effect in the GPLP model, suggesting that the peripheral lung may be less responsive to the direct smooth muscle relaxant effects of NO. L-NOARG had no significant effect on contractions induced by either of the direct smooth muscle stimulants histamine and LTD_4. The lack of effect on LTD_4 contractions was also seen in a previous study (Sakata and Bäck, 2002), and is in distinct contrast to the effect of NO inhibition on the responses induced by agonists in vascular preparations (Bäck et al, 2002). The finding that NO is devoid of smooth muscle relaxing properties in the peripheral lung was further strengthened by the fact that the NO-donor NONOate had no relaxant activity even at 100 μM bath concentration in LTD_4–precontracted GPLP. In contrast, 30 nM of NONOate caused 50 % relaxation of guinea pig pulmonary artery precontracted by noradrenaline. Taken together, it is not likely that the inhibitory effect of NO on the antigen-induced contractions was related to effects at the level of the airway smooth muscle. There is in fact one report where nitroglycerin did not relax bovine lung parenchyma (Gruetter and Lemke, 1985), suggesting that the lack of NO sensitivity on airway smooth muscle is a general characteristic of the peripheral lung.

On the basis of the initial findings, the study therefore tested the other hypothesis that the effects of the NO inhibitors were exerted at the level of release of proinflammatory mediators. Challenge with ovalbumin markedly increased release of

LTE_4, LTB_4, PGD_2, TXB_2, PGE_2 and histamine from GPLP. The NOS-inhibitor L-NOARG had no significant effect on the release of histamine after OVA challenge. Other studies have shown that inhibition of endogenous NO caused increased release of histamine from activated mast cells (Masini et al., 1991) and that NOS inhibition enhanced the antigen-induced mast cell degranulation (Coleman, 2002). However, a recent study indicated that NOS inhibition had no effect on the on-set of mast cell degranulation (Swindle et al., 2004). We therefore speculated that NO, in this particular lung model, predominantly inhibits a more specific pathway than mast cell activation in general.

Influence of 5-LO and 5-LO products on effect of NOS inhibition

Pretreatment with L-NOARG significantly enhanced the OVA-induced release of LTE_4. To further investigate the role of CysLTs and NO, the 5-LO inhibitor BAYx1005 was used. Pretreatment with BAYx1005, as expected, abolished the release of LTE_4. Moreover, the enhancement of the OVA-response by L-NOARG was not seen in preparations also treated with BAYx1005, suggesting that the effect of NO inhibition is exerted at the level of 5-LO. This interaction is supported by the findings suggesting that eNOS are co-localized with 5-LO in human mast cells nucleus and that endogenous NO acts as a regulator of LT-synthesis (Gilchrist et al., 2004). Likewise, NO appears to be able to suppress 5-LO activity in airway macrophages (Coffey et al., 2000; 2002).

It may seem surprising that BAYx1005 alone did not significantly affect the contractile response to OVA. However, this lack of effect of 5-LO inhibition alone has been explained previously in detailed studies of antigen-induced contractions in the guinea pig airways (Wikström Jonsson and Dahlén, 1994; Sundström et al., 2003).

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Neither inhibition of LTs, histamine or prostanoids separately affects the antigen-induced contraction, whereas combined inhibition of these synergistically acting mediators almost completely abolishes the antigen-induced contraction.

Influence of COX-products on effect of NOS inhibition

The unselective COX inhibitor indomethacin (10 μM) enhanced the concentration response relation to OVA. The enhancement was mimicked by the selective COX-1 inhibitor SC560, whereas the selective COX-2 inhibitor celecoxib had no effect, suggesting that it is a COX-1 generated product that is involved in the observed modulation of the early allergic airway response. Exogenous PGE_2 shifted the concentration response curve for OVA to the right, and reversed the enhancement induced by indomethacin, suggesting that endogenous PGE_2 inhibits the antigen response in this particular model. The effect may accordingly in part relate to relaxation of airway smooth muscle but inhibition of mast cell mediator release (Raud et al., 1988; Tilley et al., 2001) may also be involved.

The release of PGE_2 was significantly decreased in preparations treated with L-NOARG, which raised the possibility that endogenous NO stimulated PGE_2 release. However, on the functional level, when L-NOARG was given to indomethacin treated preparations, the enhancement of the concentration response relation to OVA caused by L-NOARG was the same. The current observations therefore suggests that the two regulatory mechanisms (NO and PGE_2) in this particular model predominantly represent different or at least not immediately connected pathways. The effect of L-NOARG on PGE_2 release may be indirect and further studies are required to resolve the relation between COX and NO pathways in this particular model.

In conclusion, inhibition of endogenous NO enhanced the antigen-induced contractions in this model of the peripheral lung. The mechanism apparently involves modulation of the release of mediators, rather than direct relaxation of airway smooth muscle. Accordingly, the NO-donor did not relax the airway smooth muscle but there were increased release of contractile CysLTs and reduced release of PGE_2 after inhibition of NOS in the GPLP. Based on these findings, we propose that endogenous NO in the peripheral lung exerts an inhibitory effect on antigen-induced contractions by reducing the release of CysLTs. Studies of human airways *in vitro* indicate that the early airway response after antigen stimulation is more severe and long lasting in the smaller airways (Wohlsen et al., 2003), which further implies that NO has an important protective role in the peripheral lung as a beneficial modulator of the early allergic airway response.

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Footnotes

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Legends for Figures

Fig 1. Drug effects on the concentration-response to OVA (1-10,000 µg/L) in sensitized lung parenchymal strips. **A,** effect of pretreatment with NOS inhibitor L-NOARG 100 µM (n=5), L-arginine 100 µM (n=5), and the combination L-NOARG 100µM and L-arginine 100 µM (n=5) on the concentration response to OVA (10-10,000 µg/L) compared to control (n=5). **B,** effect of the NOS-inhibitors L-NAME 100 µM (n=5), L-NOARG 100 µM (n=9), L-NMMA 100 µM (n=5) and1400W 1 µM (n=5) on the concentration response to OVA (1-10,000 µg/L) compared to control (n=9). Data are expressed as mean ± S.E.M. *, P<0.05; **, P<0.01; ***, P<0.001.

Fig 2. Drug effects on concentration responses to agonists in non-sensitized GPLP. **A,** effect of pretreatment with L-NOARG 100 µM (n=5), L-arginine 100 µM (n=5), and the combination L-NOARG 100 µM and L-arginine 100 µM (n=5) on cumulatively increasing doses (0.1-100 nM) of LTD_4 (n=5). **B,** effect of pretreatment with L-NOARG 100 µM (n=5), L-arginine 100 µM (n=5), and the combination L-NOARG 100 µM and L-arginine 100 µM (n=5) on cumulatively increasing doses (1-30 µM) of histamine (n=5). Data are expressed as mean ± S.E.M.

Fig 3. Effect of NO-donor NONOate (0.0001 - 100 µM) on precontracted non-sensitized guinea pig lung parenchymal strips (GPLP) and pulmonary arteries (GPPA). **A,** effect of cumulative concentrations of NONOate (0.01 - 100 µM) (n=5) on 10 nM LTD_4 precontracted GPLP compared to control (n=6), ns. **B,** effect of cumulative concentrations of NONOate (0.0001 - 100 µM) (n=4) on 10 µM noradrenaline precontracted GPPA compared to control (n=4). Data are expressed as mean ± S.E.M. ns, P>0.05; ***, P<0.001.

Fig 4. Effect of pretreatment with L-NOARG 100μM (black, n=5) compared to control (white, n=6) on mediator release from sensitized GPLP after challenge with OVA 100 μg/L. Release of **A,** LTE_4. **B,** LTB_4. **C,** TXB_2. **D,** PGD_2. **E,** PGE_2. **F,** Histamine. All samples were collected at baseline and then at the plateau after OVA 100 μg/L. The parenchymal strips had been pretreated 15 min with either Tyrode's-solution or L-NOARG 100 μM. All data are expressed as means ± S.E.M. Concentrations are expressed as mol/gram wet lung parenchymal strip. *, $P<0.05$.

Fig 5. Drug effects on the concentration-response to OVA (1-10,000 μg/L) in sensitized lung parenchymal strips. **A,** effect of pretreatment with indomethacin (10 μM, n=10) compared to control (n=12). **B,** effect of pretreatment with SC560 (5 μM, n=6) and celecoxib (3 μM, n=7) compared to control (n=7). **C,** effect of combined treatment with indomethacin (10 μM) and L-NOARG (100 μM, n=10) compared to control (n=12) and L-NOARG 100 μM (n=10). **D**, effect of pretreatment with PGE_2 (100 nM, n=6) and combined treatment with indomethacin (10 μM) and PGE_2 (100 nM) (n=6). Data are expressed as mean ± S.E.M. *, $P<0.05$; **, $P<0.01$.

Fig 6. Drug effects on the concentration-response to OVA (1-10,000 μg/L) in sensitized lung parenchymal strips. Effect of pretreatment with BAYx1005 (3μM) (n=8), L-NOARG (100 μM, n=10) and combined treatment with BAYx1005 (3 μM) and L-NOARG 100 μM (n=5) compared to control (n=10). Data are expressed as mean ± S.E.M. ***, $P<0.001$.

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Figure 1

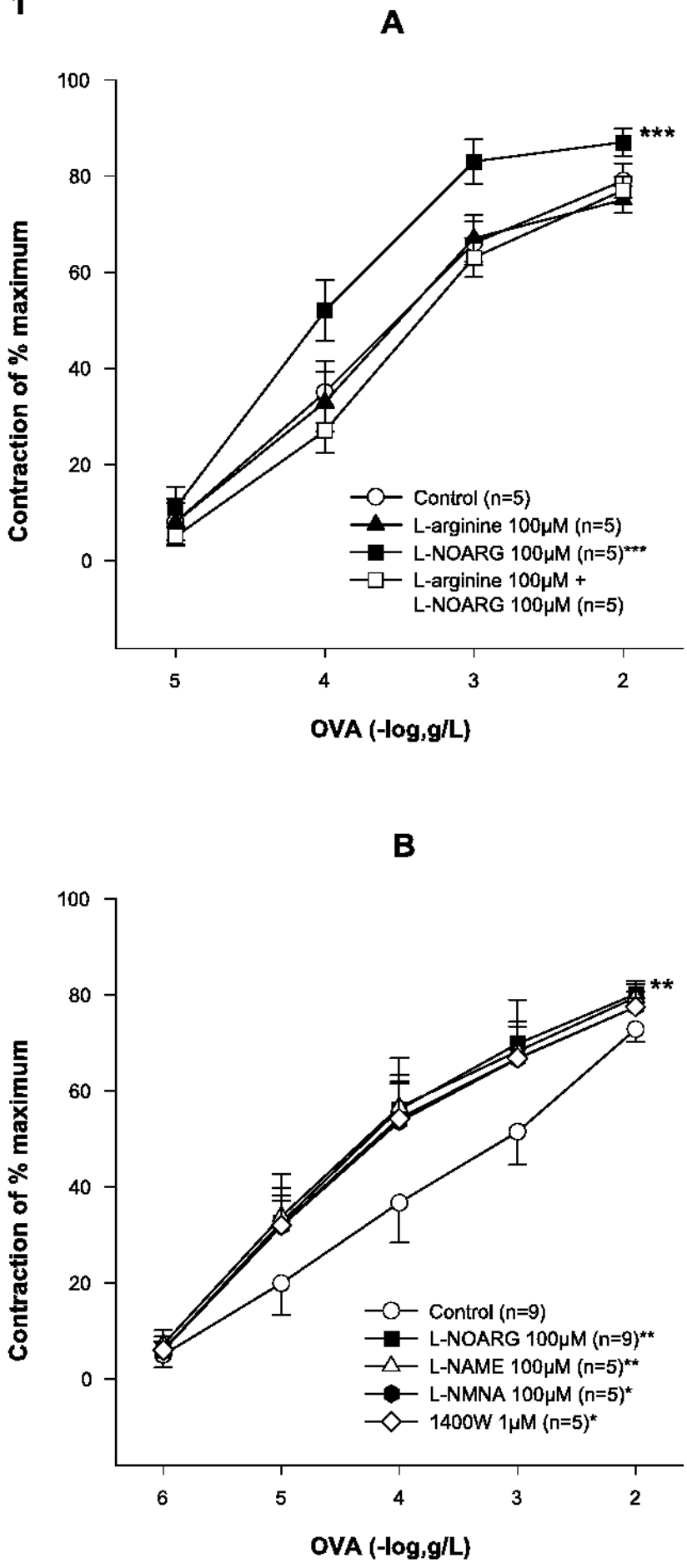

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Figure 2

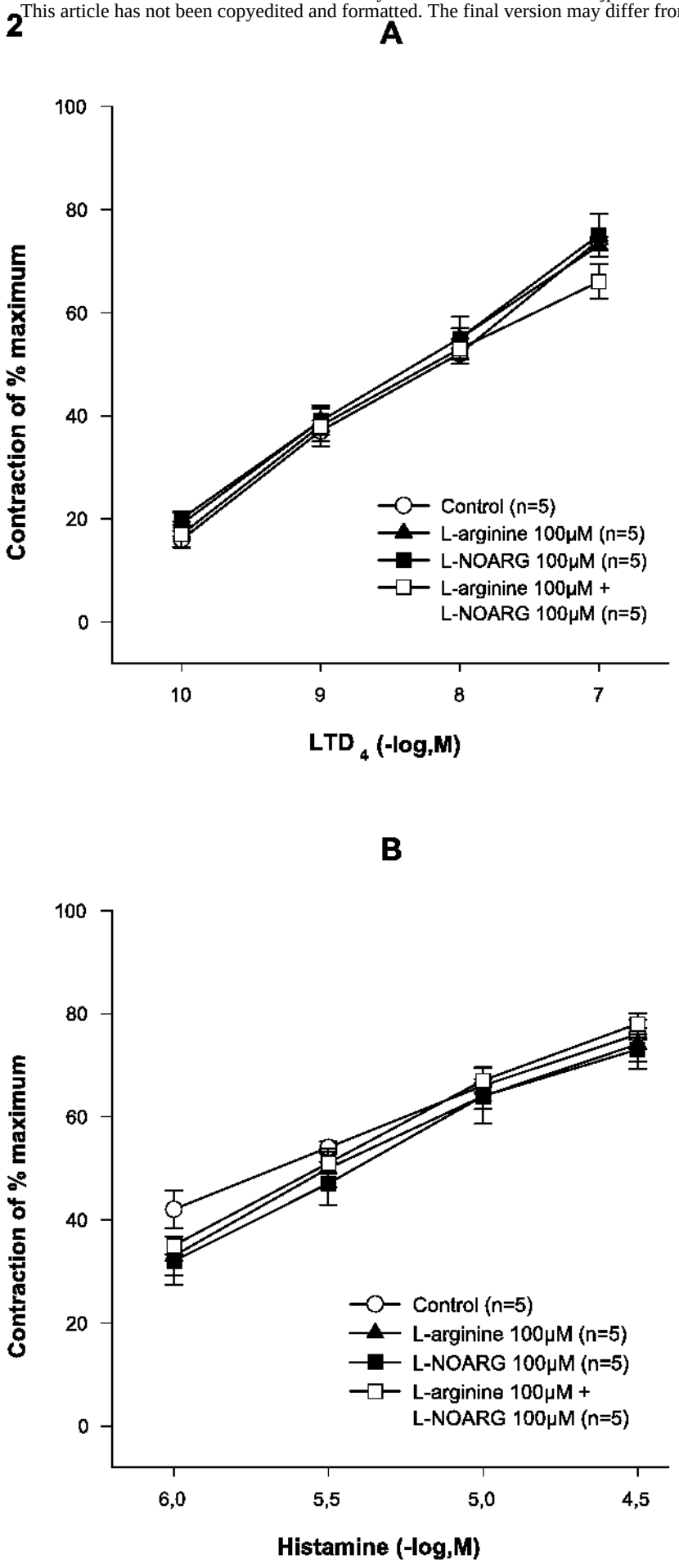

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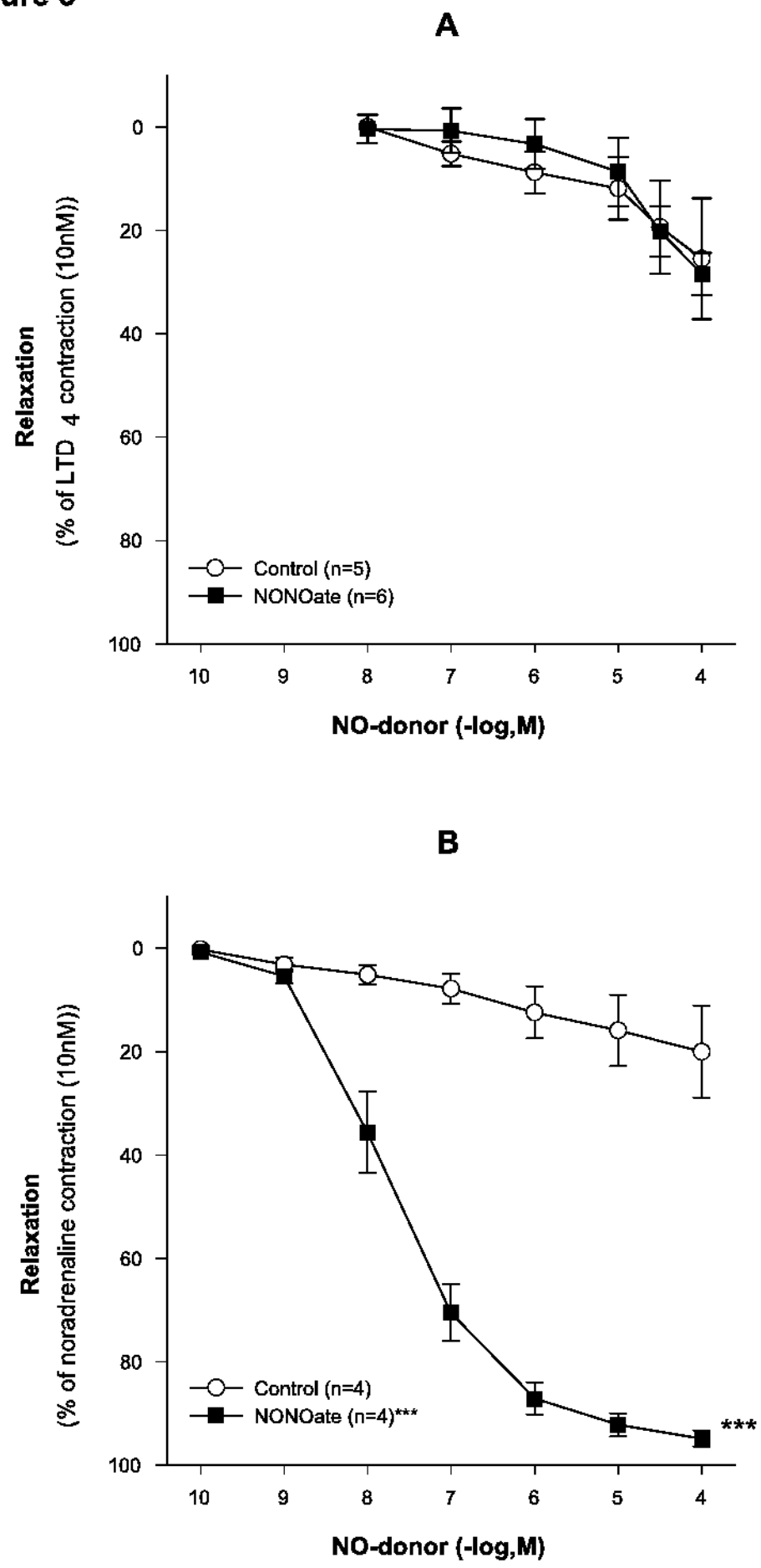
Figure 3



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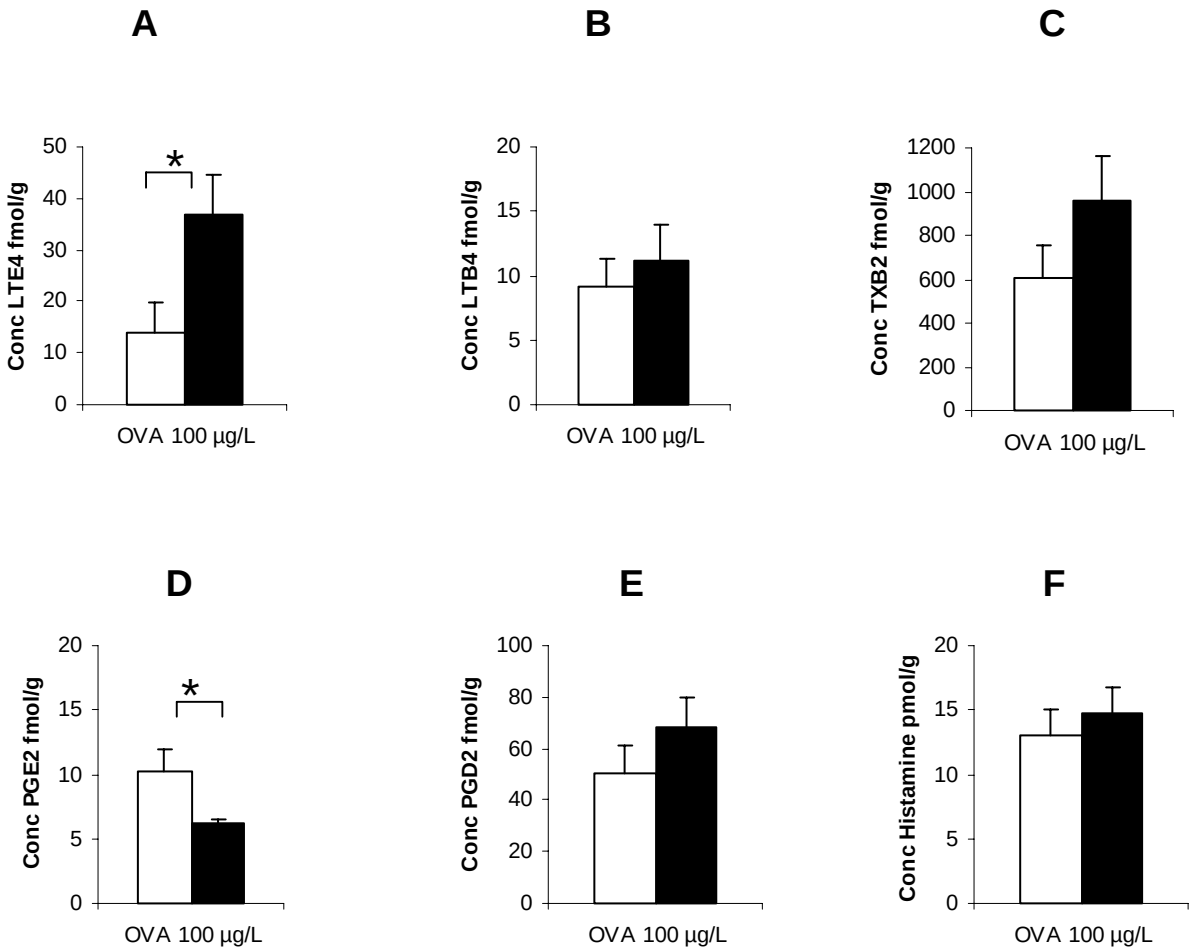

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Figure 5

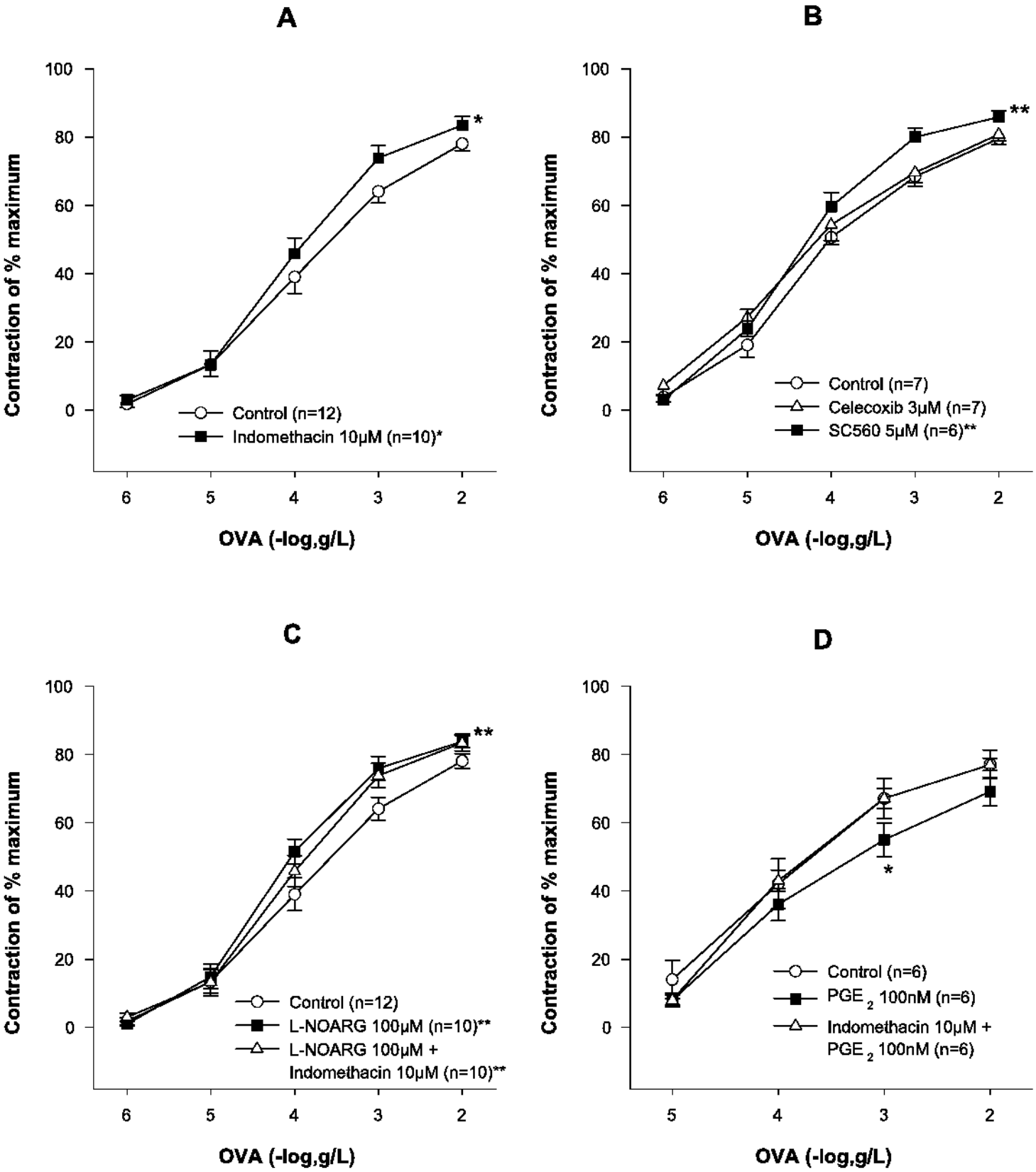

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Figure 6