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# Interaction of nitric oxide synthase inhibitors and their D-enantiomers with rat neutrophil luminol dependent chemiluminescence response

[1]M. Dikshit, S.S. Chari, P. Seth & R. Kumari

Pharmacology Division, Central Drug Research Institute, Lucknow 226001, India

**1** Formyl-methionyl-leucyl-phenylalanine (FMLP) or arachidonic acid (AA) induced luminol dependent chemiluminescence (LCL) response of rat polymorphonuclear leukocytes (PMNLs) was found to be inhibited by nitric oxide synthease inhibitors and their D-enantiomers.

**2** Rat PMNLs LCL response was inhibited by $N^G$-nitro-L-arginine methyl ester (L-NAME), D-NAME, $N^G$-monomethyl-L-arginine (L-NMMA) or D-NMMA, in a concentration- and time-dependent manner.

**3** It was observed that both L- and D-enantiomers of the arginine analogues (1000 $\mu$M) did not inhibit AA induced lucigenin-dependent chemiluminescence (LUCDCL) response and cytochrome c reduction, used for estimating the NADPH-oxidase activity in the cells and in the cell free system, respectively.

**4** None of the L- and D-enantiomers had any effect on either rat basal PMNLs or AA-induced oxygen consumption.

**5** In addition, neither the L nor D-enantiomers of NAME altered either AA-induced release or the activity of myeloperoxidase from rat PMNLs azurophilic granules.

**6** The results obtained indicate that the attenuation of the LCL response by L- and D-enantiomers of arginine analogues, is a non-specific effect as there was no inhibition of NADPH-oxidase and MPO activity, MPO release or oxygen consumption. Therefore, the data obtained indicate that these agents should be used with caution to analyse the role of nitric oxide in rat PMNLs LCL response.

**Keywords:** L-arginine analogues; polymorphonuclear leukocytes; Luminol- and lucigenin-dependent chemiluminescence; cytochrome c reduction; myeloperoxidase; oxygen consumption

## Introduction

Nitric oxide (NO) is synthesized from the terminal guanidino nitrogen atom of L-arginine in the presence of NO synthase (NOS). NO is known to participate in various physiological (vascular relaxation, inhibition of platelet aggregation and adhesion) and pathological responses (ischaemia-reperfusion injury and tumour necrosis factor-mediated cytotoxicity). Identification of the guanidino group of L-arginine as the precursor of NO led to the development of $N^{\omega}$-derivatives of L-arginine as potent inhibitors of NO formation (Moncada *et al.*, 1991). NOS inhibitors have been instrumental in establishing the role of NO in the regulation of blood pressure and vascular tone. Most of the NOS inhibitors are arginine derivatives and are known to be substrate analogue inhibitors of NOS. D-Arginine, an enantiomer of L-arginine which is not utilized by NOS has been used to establish the specificity of the response being analyzed (Moncada *et al.*, 1991; Seth *et al.*, 1994).

Many workers have used analogues of L-arginine to demonstrate the role of NO in polymorphonuclear leukocyte (PMNL) functions such as free radical generation, chemotaxis, aggregation, adhesion and microbicidal activity (Kaplan *et al.*, 1989; Rubanyi *et al.*, 1991; Kubes *et al.*, 1991; Malawista *et al.*, 1992; Belenky *et al.*, 1993). Recently inhibition of free radical generation from rat PMNLs by both endogenous and exogenous nitric oxide has been demonstrated by us (Seth *et al.*, 1994). However, we also observed that the NOS inhibitors ($N^G$-nitro-L-arginine methyl ester (L-NAME) and $N^G$-monomethyl-L-arginine (L-NMMA) inhibited free radical generation from rat PMNLs, suggesting nonspecificity of these agents. NOS inhibitors have also been shown to release NO, interfere with nitrate assays, inhibit the cyclo-oxygenase pathway, interact with muscarinic receptors and affect haemodynamic parameters in addition to inhibiting NOS (Archer & Hampl, 1992; Peterson *et al.*, 1992; Thomas & Ramwell, 1992; Westergaard *et al.*, 1993; Buxton *et al.*, 1993; Shimizu *et al.*, 1994; Greenberg *et al.*, 1995; Mitaka *et al.*, 1995; Nakaike *et al.*, 1995).

The present investigation was therefore undertaken to study the effect of commonly used NOS inhibitors (L-NAME and L-NMMA) and their D-enantiomers on the luminol and lucigenin-dependent chemiluminescence response, release of myeloperoxidase (MPO), oxygen consumption and NADPH-oxidase and MPO activity in rat PMNLs.

## Methods

### *Isolation of PMNLs*

Rat blood PMNLs were obtained from male Sprague-Dawley rats (130-150 g) by the method of Boyum (1968). Blood was collected by cardiac puncture in to sodium citrate (0.129 M, pH 6.5, 9:1, v/v) under ether anaesthesia from normal animals. Platelet-rich plasma was obtained by centrifugation at 150 *g* for 20 min, at 20°C and the buffy coat was subjected to dextran sedimentation as described in detail previously (Dikshit *et al.*, 1993). Cells obtained were suspended in Hanks' balanced salt solution (HBSS) and were counted under a microscope. The viability of the cells was tested by a trypan blue exclusion test in some experiments and was never less than 95%. PMNLs ($1-5 \times 10^7$ cells $ml^{-1}$) were kept at 4°C till the time of experimentation, which was never more than 2-3 h.

### *Measurement of luminol (LCL) and lucigenin (LUCDCL) chemiluminescence*

Free radical generation from rat neutrophils ($1-5 \times 10^6$ cells $ml^{-1}$) stimulated with formyl-methionyl-leucyl-phenylalanine

[1]Author for correspondence.

(FMLP; $1 \times 10^{-6}$ M) or arachidonic acid (AA; $1 \times 10^{-5}$ M) was measured, at 37°C with constant stirring at 900 r.p.m. by use of a dual channel lumiaggregometer (Model 560 Chronolog Corp. Havertown, PA, U.S.A.) and has been presented as LCL and LUCDCL units. The LCL or LUCDCL units have been defined as the maximum output (obtained from stimulated PMNLs) divided by the 'gain setting' of the instrument at the time of response (Seth *et al.*, 1994). The assay mixture (1000 μl) contained $1-5 \times 10^6$ PMNLs, 10 μM luminol or 50 μM of lucigenin, test substance (L- and D-arginine analogues) and FMLP ($1 \times 10^{-6}$ M) or AA ($1 \times 10^{-5}$ M).

### *Preparation of membrane and cytosol fractions of PMNLs*

Rat PMNLs ($5 \times 10^7$ cells $ml^{-1}$) were suspended in ice-cold extraction buffer (100 mM HEPES), 100 mM NaCl, 5 mM $MgCl_2$, 0.1 mM PMSF and 0.1 mM DTT, pH 7.4). Cells were disrupted by sonication and centrifuged at $100\,000 \times g$ (4°C) for 1 h, the two fractions (pellet and supernatant) were suspended in the extraction buffer (Ding *et al.*, 1994). Both the fractions were either used immediately or stored at −70°C for not more than 24 h for measuring superoxide release (Ding *et al.*, 1994). NADPH-oxidase activity induced by AA ($1 \times 10^{-5}$ M) and NADPH (0.3 mg $ml^{-1}$) was estimated in duplicate in the cell free system as superoxide dismutase (SOD) inhibitable ferricytochrome c (cyt c) ($1 \times 10^{-4}$ M) reduction at 550 nm. The test substances (L- and D-NAME or sodium nitroprusside (SNP), 1 mM) were preincubated with the cell free system for 30 min before the activation by AA. In some sets these agents were added after AA addition; however, the rate of cyt c reduction remained unaltered in these sets.

### *Measurement of myeloperoxidase (MPO) activity*

Experiments were performed to determine the effect of L- and D-NAME (300 and 1000 μM) on the granular release from the PMNLs. AA ($1 \times 10^{-4}$ M) induced release of enzyme MPO from the control and NAME pretreated PMNLs was estimated in the supernatant; the remaining enzyme activity was also estimated in the lysed PMNLs preparations by methods described previously (Dikshit *et al.*, 1992). The amount of enzyme released (in %) was calculated from the total activity; total activity was obtained by adding the activity estimated in the supernatant and in the lysate in each set.

In another set the effect of these agents on myeloperoxidase (MPO) activity was also assessed. Cells were sonicated in 2 ml HBSS and equal amounts of homogenate were distributed to different test tubes, which were incubated for 30 min at 37°C with L-NAME (1 mM), D-NAME (1 mM), SNP (100 μM) or HBSS. After 30 min, 2 ml (50 mM) acetic acid containing 0.5% hexadecyl trimethyl-ammonium bromide (HTAB) were added and incubated for 15 min at 37°C in a shaking water bath. Colour was developed as described earlier (Dikshit *et al.*, 1992).

### *Measurement of the oxygen consumption by rat PMNLs*

Oxygen consumption of rat PMNLs was recorded by use of a Clark's oxygen electrode (Gilson Oxygraph, Gilson Medical Electronics, USA) as described by Babior and Cohen (1981). The cells in the mixing vial were maintained at 37°C by a water bath circulator. Cells were continuously stirred by a magnetically driven teflon stir bar. Neutrophils ($4 \times 10^6$ cells $ml^{-1}$) suspended in the Hanks' balanced salt solution supplemented with glucose (10 mM) were added to the mixing vial (2 ml) and allowed to equilibrate for 4 min, and for 10 min the basal oxygen consumption was followed. After 10 min AA ($5 \times 10^{-4}$ M) was added to induce the oxygen consumption which was recorded for 10–20 min. To assess the effect of test compounds, cells were preincubated at 37°C with the test agent for 30 min before the cells were transferred to the mixing vial. The same protocol was followed as in the control to evaluate the effect of L-NAME, D-NAME and SNP. The rate of oxygen consumption has been presented in μmol per $10^6$ cells $h^{-1}$.

### *Chemicals*

FMLP, arachidonic acid (AA) ferricytochrome c, superoxide dismutase (SOD), dextran-500, L-arginine, D-arginine, L-NAME, phenylmethylsulphonyl fluoride (PMSF), dithiothreitol (DTT), O-dianisidine, lucigenin and luminol were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). L-NMMA, D-NMMA and D-NAME were obtained from Research Biochemicals International (U.S.A.). All other chemicals used in the present study were of analytical grade (SRL, Bombay, India).

### *Statistical analysis*

The results are presented as means ± s.e.mean. Comparisons of the differences between the groups were performed by one-way analysis of variance. When the $F$ ratio was significant, differences between individual groups were calculated with $t$ test. Differences were considered to be statistically significant when the $P$ value was less than 0.05.

## Results

### *Effect of NOS inhibitors on the luminol- and lucigenin-dependent chemiluminescence response*

Table 1 summarizes the effects of the L- and D-enantiomers of NAME and NMMA on the PMNLs FMLP-induced LCL response. The response was found to be inhibited by the NOS inhibitors following preincubation for 60 min at concentrations of 300 and 1000 μM. The D-enantiomers of NAME and NMMA were also found to be equally effective in inhibiting the LCL response. L- and D-NAME inhibited the LCL response by approximately 35% and 50% at 300 and 1000 μM, respectively. However, attenuation of the LCL response by L-NMMA and D-NMMA was around 40–70% at 300 and 1000 μM, following 60 min incubation.

To rule out the possibility that the inhibitory effect of these agents was specific to FMLP, the effects of L-NAME and D-NAME were also tested on the AA-induced LCL response and a similar degree of inhibition was observed (Figure 1a). In normal rat peripheral PMNLs AA-induced LCL was 905.27 ± 63.63 units, which was found to be significantly attenuated ($P<0.01$) in the presence of both L-NAME (694.40 ± 12.93 units) and D-NAME (618.75 ± 31.66 units) both at a concentration of 1 mM.

The effect of L-NAME and D-NAME was also investigated on the PMNLs LCL response at different time

**Table 1** Attenuation of FMLP-induced rat PMNLs immunol dependent chemiluminescence (LCL) response by L- and D- enantiomers of arginine analogues

| | | L (enantiomer) D | | | |
|---|---|---|---|---|---|
| *Agents* | *Control* | *300 μM* | *1000 μM* | *300 μM* | *1000 μM* |
| NAME | 144.19 | 96.61* | 71.76* | 88.65* | 68.34* |
| ($n=12$) | ±18.45 | ±6.88 | ±12.54 | ±12.74 | ±15.88 |
| NMMA | 272.22 | 122.20* | 129.02 | 107.89* | 74.15* |
| ($n=15$) | ±25.13 | ±20.55 | ±10.16 | ±23.94 | ±11.27 |

Values (LCL units) are mean ± s.e.mean. Time of incubation with each test substance was 60 min. $n$ = number of observations. $F$ ratio for comparison between control and L-NAME is 7.5; $F$ ratio for comparison in between control and D-NAME is 18. *$P<0.01$ in comparison to the respective controls.

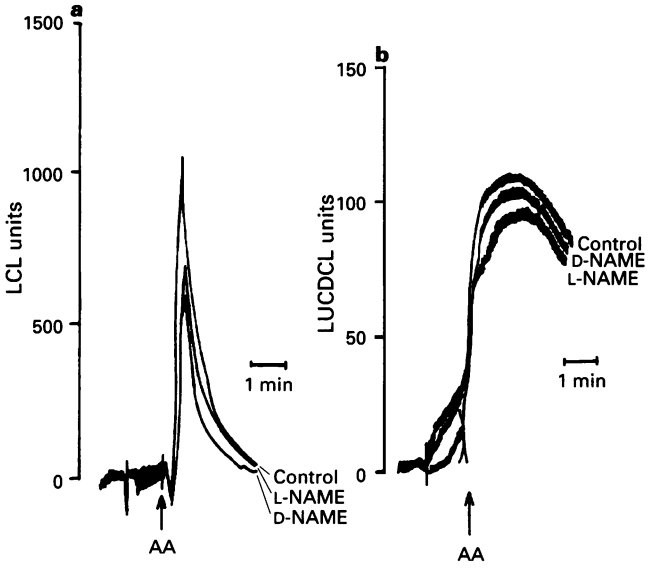


**Figure 1** Effect of L-NAME (1 mM) and D-NAME (1 mM) on PMNLs (a) luminol dependent chemiluminescence (LCL, $1 \times 10^6$ cells $ml^{-1}$) and (b) lucigenin dependent chemiluminescence (LUCDCL, $5 \times 10^6$ cells $ml^{-1}$) response induced by arachidonic acid (AA, $5 \times 10^{-5}$ M).

**Table 2** Modulation of the FMLP induced lumiminol dependent chemiluminescence pretreatment with L-NAME or D-NAME at different time intervals

| | *Preincubation time (min)* | | | | |
|---|---|---|---|---|---|
| *Treatment* | *5* | *15* | *30* | *45* | *60* |
| Control | 250.09 ±20.88 | 250.70 ±12.32 | 218.97 ±30.51 | 203.79 ±31.11 | 188.39 ±23.52 |
| L-NAME | 206.74 ±17.89 | 184.75* ±15.92 | 157.22* ±25.99 | 131.66* ±20.42 | 129.94* ±6.35 |
| D-NAME | 219.08 ±16.13 | 210.38* ±26.41 | 182.44* ±18.03 | 143.77* ±33.31 | 129.57* ±28.73 |

Values (LCL units) are mean±s.e.mean. $n=6$ observations, concentration of each agent used was 300 μM. *$P<0.01$ in comparison to control.

intervals (15, 30, 45 and 60 min, Table 2). It was observed that both enantiomers of NAME attenuated the free radical generation in a time-dependent manner and there was no significant difference between the inhibitory effects of these two enantiomers.

The effect of different concentrations (30, 100, 300, 1000 μM) of L- and D-NAME was also investigated, following 60 min incubation, on the FMLP-induced PMNLs LCL response. PMNLs free radical generation or LCL was found to be significantly inhibited by both L- and D-NAME in a concentration-dependent manner (Table 3). A 300 μM concentration of L- or D-NAME was found to reduce significantly the FMLP-induced LCL response.

The AA-induced ($5 \times 10^{-5}$ M) generation of superoxide radicals, as measured by LUCDCL in rat peripheral PMNLs was 105.74±15.48 units ($n=4$). In L-NAME or D-NAME pretreated (1 mM, 30 min) PMNLs, there was no significant reduction in $O_2$-generation (75.48±9.24 and 88.06±14.24 units, respectively; Figure 1b).

### *Effect of NOS inhibitors on superoxide generation in the cell free systems*

L-NAME, D-NAME and other agents (all 1 mM) were preincubated with PMNLs membrane and cytosol fractions for 30 min. It was observed that superoxide radical generation was not inhibited by these agents as estimated by cyt c reduction. In control preparations cyt c reduction was 6.44±0.76 mmol/$5 \times 10^7$ cells 10 $min^{-1}$. Treatment with L-NAME or D-NAME resulted in values of 6.26±0.23 and 6.39±0.31 mmol cyt c/$5 \times 10^7$ cells 10 $min^{-1}$, respectively. To validate the cell free system we also investigated the effect of the NO-donor sodium nitroprusside (SNP) on superoxide radical generation. SNP was found to inhibit significantly (50%) the superoxide radical generation; inhibition of the superoxide radical generation in the cell free systems by NO is well documented.

### *Effect of L- and D-NAME on myeloperoxidase release from PMNLs*

AA-induced release of MPO from the specific as well as azuorphilic granules of the PMNLs remained unaltered in the presence of L-NAME and D-NAME (300 and 1000 μM). Release of MPO induced by arachidonic acid in the supernatant was 24.15±4.28%. In the presence of L-NAME (1 mM) the release of MPO induced by AA was 31.01±6.75%. Similarly, AA-induced release of MPO was 30.53±6.04% in the presence of 1 mM D-NAME. In addition neither L- nor D-NAME (1 mM) had any effect on the MPO activity tested in the PMNLs homogenate.

### *Effect of L- and D-NAME on the oxygen consumption of PMNLs*

Basal oxygen consumption by rat PMNLs was found to be 5.37±0.31 μmol/$10^6$ cells $h^{-1}$ and after the addition of AA ($5 \times 10^{-4}$ M) it was augmented to 18.22±1.15 μmol/$10^6$ cells

**Table 2** Modulation of the FMLP induced lumiminol dependent chemiluminescence pretreatment with L-NAME or D-NAME at different time intervals

| | *Preincubation time (min)* | | | | |
|---|---|---|---|---|---|
| *Treatment* | *5* | *15* | *30* | *45* | *60* |
| Control | 250.09 ±20.88 | 250.70 ±12.32 | 218.97 ±30.51 | 203.79 ±31.11 | 188.39 ±23.52 |
| L-NAME | 206.74 ±17.89 | 184.75* ±15.92 | 157.22* ±25.99 | 131.66* ±20.42 | 129.94* ±6.35 |
| D-NAME | 219.08 ±16.13 | 210.38* ±26.41 | 182.44* ±18.03 | 143.77* ±33.31 | 129.57* ±28.73 |

Values (LCL units) are mean±s.e.mean. $n=6$ observations, concentration of each agent used was 300 μM. *$P<0.01$ in comparison to control.

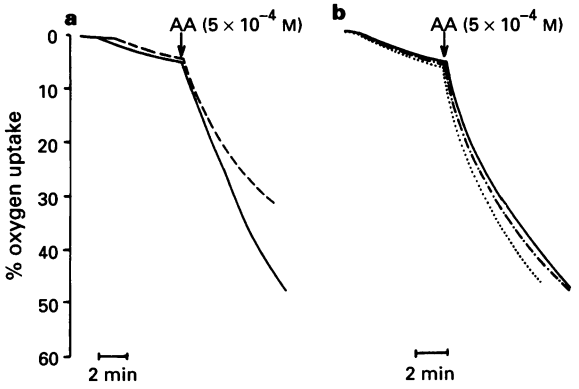


**Figure 2** Effect of (a) sodium nitroprusside (SNP, 100 μM; broken line); (b) L-NAME (1 mM; dotted line) and D-NAME (1 mM; broken and dotted line) oxygen consumption of rat PMNLs ($4 \times 10^6$ cells $ml^{-1}$). Solid lines show control oxygen consumption before and after the addition of arachidonic acid (AA, $5 \times 10^{-4}$ M).

$h^{-1}$. Pretreatment of PMNLs with SNP (100 $\mu$M) attenuated the oxygen consumption and it was found to be $14.11 \pm 0.35$ $\mu$mol/$10^6$ cells $h^{-1}$, which was significantly ($P<0.001$) lower than control (Figure 2a). However, in the presence of 1 mM L-NAME ($18.85 \pm 1.27$ $\mu$mol/$10^6$ cells $h^{-1}$ or D-NAME ($17.66 \pm 1.02$ $\mu$mol/$10^6$ cells $h^{-1}$) the oxygen consumption was not altered significantly (Figure 2b).

## Discussion

The results obtained in the present investigation demonstrate inhibition of the LCL response, by both L- and D-enantiomers of NAME and NMMA (arginine analogues). The almost equal effect of the inactive D-enantiomer indicates that the inhibition is non-specific. Such an observation has not been published previously and indicates that these agents should be used with caution to delineate the role of NO in PMNLs free radical generation estimated by luminol.

In our previous study, while investigating the role of endogenous as well as exogenous NO on rat PMNLs free radical generation, we observed that NO from both sources inhibited, as well as scavenged, the free radicals (Seth *et al.*, 1994). Similar observations were also obtained by others in human PMNLs (Rubanyi *et al.*, 1991; Clancy *et al.*, 1992). However, we observed that L-NAME and L-NMMA both inhibited the LCL response, suggesting the non-specificity of these inhibitors. Concentrations of these two compounds used by us have also been used by many other investigators to study the involvement of NO in the functions of PMNLs (Kaplan *et al.*, 1989; Kubes *et al.*, 1991; Malawista *et al.*, 1992; Belenky *et al.*, 1993). Interestingly, Pou *et al.* (1992) have demonstrated that brain NOS can generate superoxide radicals in the absence of L-arginine and that this response was found to be inhibited by the NOS inhibitor L-NAME. They suggested that L-NAME interferes with the NADPH-dependent reduction of molecular oxygen and it is well known that the electron transfer reaction is basically catalysed by both NOS and NADPH-oxidase. McCall *et al.* (1991) demonstrated that L-NNA and L-NAME inhibit NO generation from PMNLs by around 50% at a concentration of 300 $\mu$M. It has also been found that PMNLs have to be incubated for at least 30–60 min to achieve inhibition of the intracellular NOS activity by the arginine analogues (McCall *et al.*, 1991; Malawista *et al.*, 1992). On the other hand Schmidt *et al.* (1993; 1994) have shown that NOS inhibitors in the endothelial cells are taken up by different channels. L-NMMA is taken up by the arginine transporter, while L-NAME is transported by the leucine transport system. Therefore, we used L-NAME, L-NMMA and their D-enantiomers to investigate their role on the LCL response at different concentrations. In addition, we investigated the effect of L-NAME and D-NAME on the PMNLs LCL responses at different time intervals. We observed that both enantiomers produced a similar degree of inhibition of LCL, suggesting that NAME-mediated inhibition is non-specific (Tables 1 and 2) and is not mediated by NOS inhibition.

Piper *et al.* (1994) used 500 $\mu$M L-NAME, added only 5 min before the addition of a NO donor compound, to investigate the modulator role of endogenous NO on reactive oxygen species production in canine PMNLs. They suggested that endogenous NO might increase neutrophil activation. Inhibition of opsonized zymosan-induced LCL by L-NAME has also been observed by Piper *et al*, which they explained on the basis of the nonavailability of endogenous NO. However, according to our observations this hypothesis is unlikely to be true, as they did not use D-NAME. It is probable that the effect of L-NAME was in fact not due to the inhibition of NOS, but to a nonspecific effect of NAME. Similarly, Carreras *et al.* (1994) obtained a concentration-dependent increase in the human PMNLs LCL response by phorbol 12-myristate 13-acetate (PMA). They also observed a concentration-dependent increase in NO release, oxygen uptake and hydrogen peroxide production by PMA. PMA-induced LCL responses in PMNLs preincubated with L-NMMA (1 mM) for 30 min was found to be attenuated. This was explained by them totally on the basis of the inhibition of peroxynitrite synthesis in PMNLs during PMA-induced respiratory burst. However, this may not necessarily be so since, in our studies, both L- and D-enantiomers of NMMA inhibited the LCL response at this concentration to a similar extent. Antonini *et al.* (1994) have demonstrated the inhibition of PMA-induced LCL response of the rat lung phagocytic cells by 1 mM L-NAME, which was also attributed to the inhibition of peroxynitrite synthesis. On the other hand, Chen *et al.* (1994) demonstrated an increase in superoxide radical generation induced by PMA in the presence of 1 mM L-NMMA in human PMNLs following 60 min preincubation, which implies the scavenging of superoxide radicals by NO. However, since we have not tested the effect of these inhibitors on the PMA-induced response, there is no immediate explanation for this discrepancy. It is possible that the difference might be due to the use of PMNLs from different species. In addition, Shimizu *et al.* (1994) have demonstrated a reduction of hydrogen peroxide-induced endothelial injury in bovine aortic endothelial cell cultures by L-NNA at concentrations of $10^{-3}$ and $10^{-4}$ M which is not due to NOS inhibition.

Some workers have used very low concentrations (30–100 $\mu$M) of NOS inhibitors (Simonet *et al.*, 1993; Hecker *et al.*, 1994; O'Murchu *et al.*, 1994; Vanheel *et al.*, 1994) in different preparations, therefore we though it was worth investigating the effect of L-NAME at different concentrations on the PMNLs LCL response after 60 min incubation (Table 3). We observed a concentration-dependent inhibition of the LCL response by L- as well as D-NAME. Since almost similar results were obtained with NAME and NMMA on the LCL response of rat PMNLs, we investigated the effect of L- and D-NAME in detail on different parameters which individually participate in the LCL response, such as NADPH-oxidase and MPO activity, MPO release and oxygen consumption.

It is known that the LCL response is dependent on reactive oxygen species and MPO (Takahashi *et al.*, 1991), therefore the release of MPO induced by AA from the PMNLs in the presence of L-NAME and D-NAME was investigated. However, the release of MPO remained unaltered by both L-NAME and D-NAME, suggesting that the inhibition of LCL is not due to inhibition of MPO release. In order to investigate the action of these inhibitors on the LCL response futher, their effect was also studied on the activity of MPO in PMNL homogenates, this was found to be unaffected by both L-NAME and D-NAME. In addition, the activity of NADPH-oxidase was also estimated in the cell free system where the membrane and cytosol fractions were co-incubated with these agents before the induction of free radical generation with AA. During activation of the enzyme NADPH-oxidase, cytosolic components translocate to the membrane and lead to free radical generation (Dewald *et al.*, 1979). Addition of these agents prior to the activation of the NADPH oxidase with AA did not decrease cytochrome-c reduction, suggesting that L-NAME, as well as the D-enantiomer, does not interfere with the enzyme activation, as the interaction of the subcellular components is necessary for the generation of superoxide radicals. Superoxide radical generation in the presence of L-NAME and D-NAME was also estimated in intact PMNLs as lucigenin-dependent chemiluminescence. We did not observe any significant alteration in the PMNLs lucigenin dependent chemiluminescence response in the presence of L-NAME and D-NAME, suggesting that inhibition of the LCL response could also not be explained on the basis of oxygen consumption since we did not observe any changes in the oxygen consumption by the PMNLs in presence of L-NAME and D-NAME.

In conclusion, these studies have demonstrated inhibition of the LCL response in the rat PMNLs by NOS inhibitors (both L- and D-enantiomers) in a non-specific manner. This suggests caution should be exercised in interpreting data from studies in which NOS inhibitors have been used to study the role of NO in the rat PMNLs LCL response.

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