British Journal of Pharmacology (1998) 125, 335–340 
http://www.stockton-press.co.uk/bjp

# Interactions between inducible isoforms of nitric oxide synthase and cyclo-oxygenase *in vivo*: investigations using the selective inhibitors, 1400W and celecoxib

[1]Lorna C. Hamilton & [1,2]Timothy D. Warner

Vascular Inflammation, The William Harvey Research Institute, St. Bartholomew's and the Royal London School of Medicine and Dentistry, Charterhouse Square, London, EC1M 6BQ

**1** Exposure of tissues to endotoxin (LPS) and/or cytokines leads to the induction of both inducible nitric oxide synthase (iNOS) and cyclo-oxygenase-2 (COX-2). It has previously been reported that there is 'cross-talk' between these two systems. However, such previous studies have been limited by the availability of highly selective inhibitors. Here we have investigated the interactions between iNOS and COX-2 *in vivo* using 1400W, an iNOS-selective inhibitor, and celecoxib, a COX-2-selective inhibitor.

**2** Infusion of LPS to rats for 6 h caused a time-dependent increase in the plasma concentrations of 6 keto-prostaglandin $F_{1\alpha}$ (6 keto-$PGF_{1\alpha}$) and nitrite/nitrate ($NO_2/NO_3$), consistent with the induction of iNOS and COX-2. Bolus injection of arachidonic acid (AA) at $t=6$ h resulted in a further increase of circulating levels of 6 keto-$PGF_{1\alpha}$ in LPS-treated animals.

**3** Treatment of rats with 1400W or the non-selective NOS inhibitor $N^G$-monomethyl-L-arginine (L-NMMA) inhibited the increase in plasma $NO_2/NO_3$ but were both without effect on the plasma concentration of 6 keto-$PGF_{1\alpha}$ before or after AA.

**4** Treatment with the non-steroidal anti-inflammatory drugs (NSAIDs), A771726 or diclofenac, or with celecoxib significantly reduced the increase in circulating 6 keto-$PGF_{1\alpha}$ caused by LPS, and the large increase in 6 keto-$PGF_{1\alpha}$ following injection of AA. None of the COX inhibitors affected the increase in plasma $NO_2/NO_3$. Dexamethasone, however, significantly inhibited both the increase in 6 keto-$PGF_{1\alpha}$ and the increase in $NO_2/NO_3$.

**5** In conclusion, the use of selective inhibitors does not support the concept of cross talk *in vivo* between iNOS and COX-2.

**Keywords:** Nitric oxide; nitric oxide synthase; prostanoids; cyclo-oxygenase-2; 1400W; celecoxib; lipopolysaccharide; rat

## Introduction

In many *in vitro* and *in vivo* systems exposure to inflammatory mediators such as cytokines and endotoxin (LPS) leads to the induction of both cyclo-oxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) (Stadler *et al*., 1993; Salvemini *et al*., 1993, 1994, 1995; Vane *et al*., 1994; Swierkosz *et al*., 1995; Bishop-Bailey *et al*., 1997). This co-induction has encouraged researchers to look for cross talk between the NOS and COX systems. We and others have previously reported from *in vitro* and *ex vivo* experiments that the production of large amounts of nitric oxide (NO) decreases COX-2 expression and activity (Kanner *et al*., 1992; Stadler *et al*., 1993; Swierkosz *et al*., 1995; Hulkower *et al*., 1996; Amin *et al*., 1997; Habib *et al*., 1997). Conversely others using similar experimental models have found NO to increase the activity and expression of COX-2 (Corbett *et al*., 1993; Inoue *et al*., 1993; Salvemini *et al*., 1993, 1994, 1995; Franchi *et al*., 1994; Tetsuka *et al*., 1996). In support of these latter studies experiments in LPS-treated rats have led to the suggestion that NO produced by iNOS *in vivo* also increases the production of COX-2 metabolites (Salvemini *et al*., 1995). Unfortunately these previous investigations were limited by the lack of highly selective iNOS inhibitors. This is particularly relevant to studies *in vivo*, where all three isoforms of NOS are present (Moncada, 1997), and where NO produced by endothelial NOS is central to the regulation of regional blood flow and systemic blood pressure (Moncada, 1997). Recently, however, the novel compound 1400W has been described, which in rat tissues is more than 1000 fold selective for iNOS over endothelial NOS (eNOS) (Garvey *et al*., 1997). Here we have used 1400W and the selective COX-2 inhibitor, celecoxib (Penning *et al*., 1997) to investigate the *in vivo* interactions between iNOS and COX-2 in LPS-treated rats. For comparison the effects of the non-selective NOS inhibitor $N^G$-monomethyl-L-arginine (L-NMMA) and the non-selective COX inhibitors, diclofenac and A771726, as well as dexamethasone were also examined. Some of these results have been presented to the British Pharmacological Society (Hamilton *et al*., 1997a, 1998).

## Methods

### *Surgical procedure*

Male Wistar rats (220–250 g; Tuck, U.K.) were anaesthetized with thiobutabarbital sodium (Inactin; 120 mg $kg^{-1}$, i.p.). The trachea was cannulated to facilitate respiration. The right carotid artery was cannulated and connected to a pressure transducer (Elcomatic Type 750) for the measurement of systemic blood pressure which was recorded on a Graphtec Linearcorder (Type WR 3101). Mean arterial pressure (MAP) was calculated as the diastolic pressure plus one third of the pulse pressure. Haemodynamic parameters were measured throughout the experiments. A cannula was also introduced into the left jugular vein for the administration of drugs. Body temperature was maintained at 37°C by use of a homeothermic

[2] Author for correspondence.

blanket regulated by a rectal thermometer (Biosciences, Sherness, Kent).

Upon completion of the surgical procedure, animals were left for 15 min to allow stabilization of the cardiovascular parameters. Commencing 1 h later ($t=0$) animals received a 6 h continuous infusion of lipopolysaccharide (LPS, serotype 0127:B8, 0.2 mg $kg^{-1}$ $h^{-1}$) or LPS vehicle (saline). Animals were either treated with A771726 (50 mg $kg^{-1}$, i.p.), celecoxib (3 mg $kg^{-1}$ i.p.), diclofenac (10 mg $kg^{-1}$, i.p.) L-NMMA (3 mg $kg^{-1}$ $h^{-1}$) or drug vehicle (DMSO, 10% w/v) 1 h before the start of the LPS infusion or were given the selective iNOS inhibitor 1400W (10 mg $kg^{-1}$ i.v. bolus at $t=0$ h, followed by infusion of 10 mg $kg^{-1}$ $h^{-1}$ i.v. at times $t=2-4$ h) (Wray *et al.*, 1998). Following the 15 min equilibration period, some animals were treated with dexamethasone (3 mg $kg^{-1}$, i.p.) with LPS infusion commencing 2 h later.

At the end of the infusion period with LPS or vehicle ($t=6$ h) animals were challenged with a final bolus of arachidonic acid (3 mg $kg^{-1}$ i.v.), bradykinin (10 $\mu$mol $kg^{-1}$, i.v.) or L-arginine (10 mg $kg^{-1}$ i.v.). Blood samples (500 $\mu$l) were taken *via* the carotid artery cannula at $t=0$, 2, 4 and 6 h, and 1 min after administration of the final bolus of arachidonic acid or bradykinin. The animals were then killed by overdose of anaesthetic and organs were snap frozen, under hexane, for immunohistochemical analysis of COX-2 and iNOS expression.

### *Measurement of plasma 6 keto-$PGF_{1\alpha}$*

The plasma concentration of 6 keto-prostaglandin (PG) $F_{1\alpha}$, the stable hydrolysis product of prostacyclin ($PGI_2$), was measured by specific radioimmunoassay as a determinant of COX activity, using commercially antibodies and tritiated prostanoids. The detection limit of this assay for 6-keto-$PGF_{1\alpha}$ is approximately 10 pg per tube.

### *Measurement of plasma nitrite($NO_2$)/nitrate($NO_3$)*

As a measure of NO formation plasma total concentrations of nitrite/nitrate ($NO_2/NO_3$) were determined (Schmidt *et al.*, 1992). Briefly, $NO_3$ present within plasma was stoichiometrically reduced to $NO_2$, by incubation (15 min, 37°C) of samples (10 $\mu$l) with nitrate reductase (1 iu $ml^{-1}$), NADPH (500 $\mu$M) and flavine adenine dinucleotide (FAD, 50 $\mu$M) (final volume 80 $\mu$l). Following $NO_3$ reduction unused NADPH was oxidized by addition of lactate dehydrogenase (100 iu $ml^{-1}$) and sodium pyruvate (100 mM) (final volume 100 $\mu$l) and incubation for 5 min at 37°C. Total $NO_2$ concentration was assayed by adding 100 $\mu$l of Griess reagent (4% sulphanilamide and 0.2 % naphtylenediamide in 10% phosphoric acid) to each 100 $\mu$l sample which forms a purple azo dye in the presence of nitrite. The formation of this dye was measured spectrophotometrically (Molecular Devices, Richmond, CA, U.S.A.). Total $NO_2$ concentrations (nmol $ml^{-1}$) were calculated by comparison with the optical density of standard solutions of sodium nitrite and sodium nitrate (also stochiometrically reduced to $NO_2$) prepared in plasma.

### *Materials*

[$^3$H] 6-Keto $PGF_{1\alpha}$ was bought from Amersham International (Little Chalfont, Bucks., U.K.). 1400W was obtained from Alexis Corporation (Nottingham, U.K.) and A771726 was a gift from Dr. R. R. Bartlett, Hoescht (Germany). The COX-2 selective antibody was bought from Cayman Chemical Company (Ann Arbor, MI, U.S.A.) and the iNOS selective antibody was a gift from Dr. Clare Bryant (University of Cambridge, U.K.). All other compounds were purchased from Sigma Chemical Company (Poole, Dorset, U.K.).

### *Data analysis*

Significant differences were determined by one-way or two-way ANOVA followed by Dunnett's or Bonferroni test. Normalized data was compared by a one-sample test. $P<0.05$ was considered to be statistically significant.

## Results

### *Effects of LPS on basal plasma concentrations of $NO_2$/$NO_3$ and 6 keto-$PGF_{1\alpha}$*

Infusion of LPS to rats caused a marked time-dependent increase in the plasma concentrations of $NO_2/NO_3$ and a smaller, less marked increase in the circulating levels of 6 keto-$PGF_{1\alpha}$ compared to controls (Figure 1, $P<0.05$, two-way ANOVA). These increases were associated with a general induction of iNOS and COX-2 throughout the vasculature as demonstrated by immunohistochemistry (data not shown).

### *Effects of supplying substrate at t=6 h on plasma concentrations of $NO_2/NO_3$ and 6 keto-$PGF_{1\alpha}$*

Administration of a bolus of arachidonic acid (3 mg $kg^{-1}$, i.v.) to vehicle treated rats at $t=6$ h caused (after 1 min) the

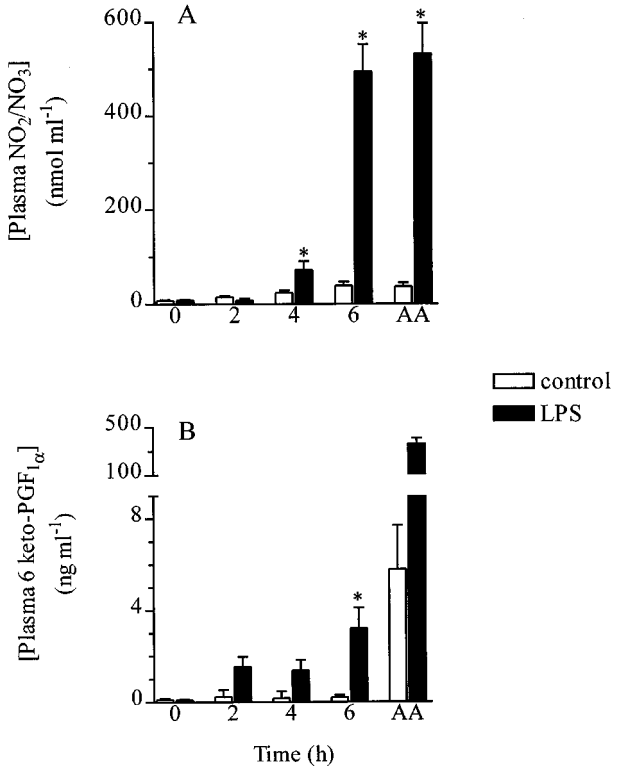


**Figure 1** The effect of LPS infusion (0.2 mg $kg^{-1}$ $h^{-1}$, n=20) to rats for 6 h followed by a bolus injection of arachidonic acid (3 mg $kg^{-1}$, i.v.) on the plasma concentrations of (A) $NO_2/NO_3$, (B) 6 keto-$PGF_{1\alpha}$. Results are expressed as mean±s.e.mean. *Denotes significant difference from $t=0$ h ($P<0.05$, paired ANOVA plus Dunnett's test).

plasma concentration of 6 keto-$PGF_{1\alpha}$ to rise to $6.1 \pm 2.4$ ng $ml^{-1}$ ($n=15$). Infusion of rats with LPS for 6 h magnified this rise in 6 keto-$PGF_{1\alpha}$ by more than 50 fold (Figure 1). Similarly, the increase in plasma 6 keto-$PGF_{1\alpha}$ concentration following 1 min after injection of bradykinin (100 $\mu$mol $kg^{-1}$, i.v.) was also greatly magnified following infusion of LPS ($t=6$ h: control $3.1 \pm 1.0$ ng $ml^{-1}$ 6 keto-$PGF_{1\alpha}$.; LPS-treated, $76 \pm 9.6$ ng $ml^{-1}$ 6 keto-$PGF_{1\alpha}$, $n=4$). The injection of arachidonic acid and bradykinin were without affect on the plasma levels of $NO_2/NO_3$ in any study groups (Figure 1, and data not shown). Bolus injection of L-arginine (10 mg $kg^{-1}$, i.v.) was also without effect on either plasma 6 keto-$PGF_{1\alpha}$ or $NO_2/NO_3$ (data not shown). Because the increases in circulating levels of 6 keto-$PGF_{1\alpha}$ caused by LPS varied between batches of rats subsequent analyses of the effects of NOS inhibitors and NSAIDs used data normalized to time- and batch-matched animals receiving LPS plus vehicle.

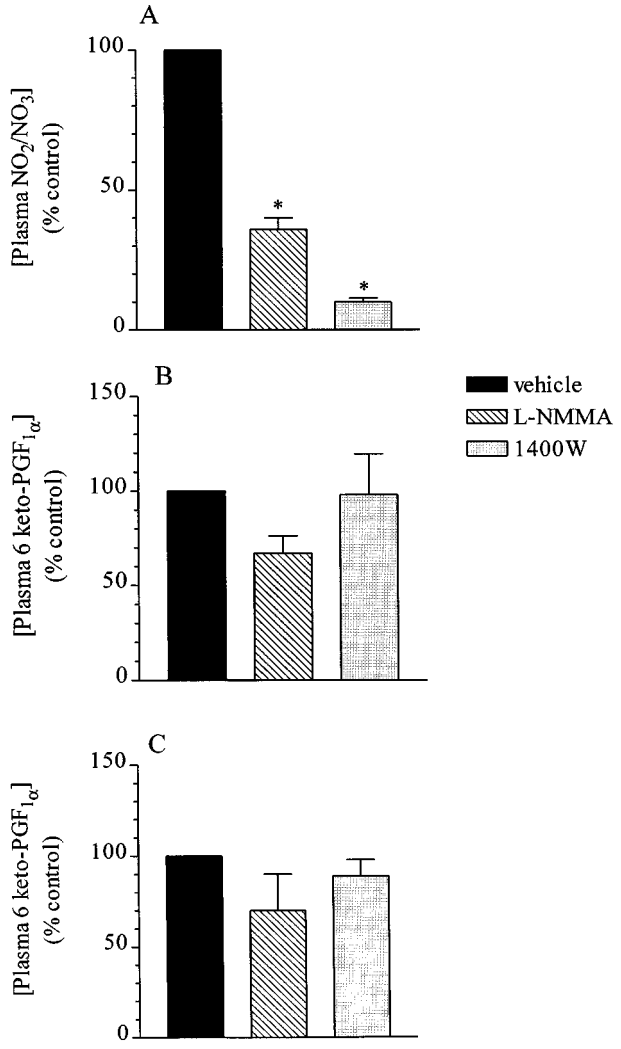


**Figure 2** Effect in LPS-treated rats of the NOS inhibitors 1400W ($n=8$) and L-NMMA ($n=8$), or vehicle ($n=8$), on the plasma concentrations of (A) $NO_2/NO_3$, (B) 6 keto-$PGF_{1\alpha}$, and (C) 6 keto-$PGF_{1\alpha}$ 1 min following bolus injection of arachidonic acid (3 mg $kg^{-1}$, i.v.). Results are normalized to batch- and time-matched controls receiving LPS plus drug-vehicle and are expressed as mean $\pm$ s.e.mean. *Denotes significant difference from control ($P<0.05$, one-sample test).

### *Effects of NOS inhibitors*

Treatment of the rats with L-NMMA or 1400W inhibited the rise in plasma $NO_2/NO_3$ by, respectively, $>60\%$ and $>85\%$ (Figure 2A) without having any significant effect on the circulating levels of 6 keto-$PGF_{1\alpha}$ (Figure 2B). Similarly, neither L-NMMA nor 1400W affected the increase in 6 keto-$PGF_{1\alpha}$ that followed bolus injection of arachidonic acid (Figure 2C). To ensure that pooling of results had not masked any relationship between plasma $NO_2/NO_3$ and 6 keto-$PGF_{1\alpha}$ during the 6 h experimental procedure, data was compared for individual rats using a scattergram (Figure 3). This analysis revealed no relationship between 6 keto-$PGF_{1\alpha}$ formation and $NO_2/NO_3$ in rats receiving LPS plus vehicle, LPS plus L-NMMA or LPS plus 1400W (Figure 3).

### *Effects of NSAIDs and celecoxib*

Treatment of rats with diclofenac (10 mg $kg^{-1}$, i.p., $n=5$), A771726 (50 mg $kg^{-1}$, i.p., $n=5$), or celecoxib (3 mg $kg^{-1}$, i.p., $n=6$) inhibited both the rise in circulating levels of 6 keto-$PGF_{1\alpha}$ caused by LPS and the marked production of 6 keto-$PGF_{1\alpha}$ that followed bolus injection of arachidonic acid (Figure 4B and C). However, none of these agents affected the rise in plasma $NO_2/NO_3$ (Figure 4A).

### *Effects of dexamethasone*

Treatment of the rats with dexamethasone (3 mg $kg^{-1}$, i.p., $n=6$) reduced both the rise in plasma $NO_2/NO_3$ and the basal and arachidonic acid stimulated increases in 6 keto-$PGF_{1\alpha}$ (Figure 4).

### *Cardiovascular effects*

In all groups of animals there was a significant fall in blood pressure over the 6 h time course of the experiment ($P<0.05$, paired ANOVA plus Dunnett's test). Notably in LPS-treated animals blood pressure fell significantly between 2 and 6 h ($P<0.05$, paired ANOVA plus Bonferroni test) except in rats also treated with L-NMMA or 1400W (Table 1).

## Discussion

Here using *in vivo* highly selective inhibitors of iNOS and COX-2 we have found no evidence for cross talk between the NO and COX systems. Most notably we found that despite reducing the circulating levels of $NO_2/NO_3$ to levels found in

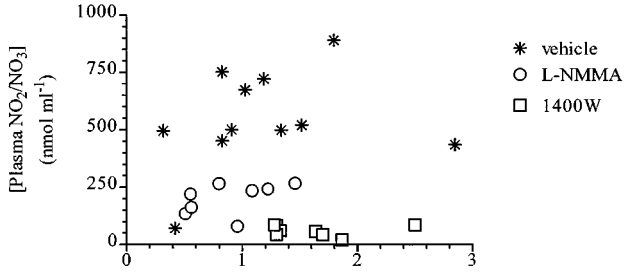


**Figure 3** Relationship at $t=6$ h between plasma concentrations of $NO_2/NO_3$ and 6 keto-$PGF_{1\alpha}$ in rats treated with LPS plus L-NMMA or LPS plus 1400W, and in batch and time-matched control rats receiving LPS plus vehicle.

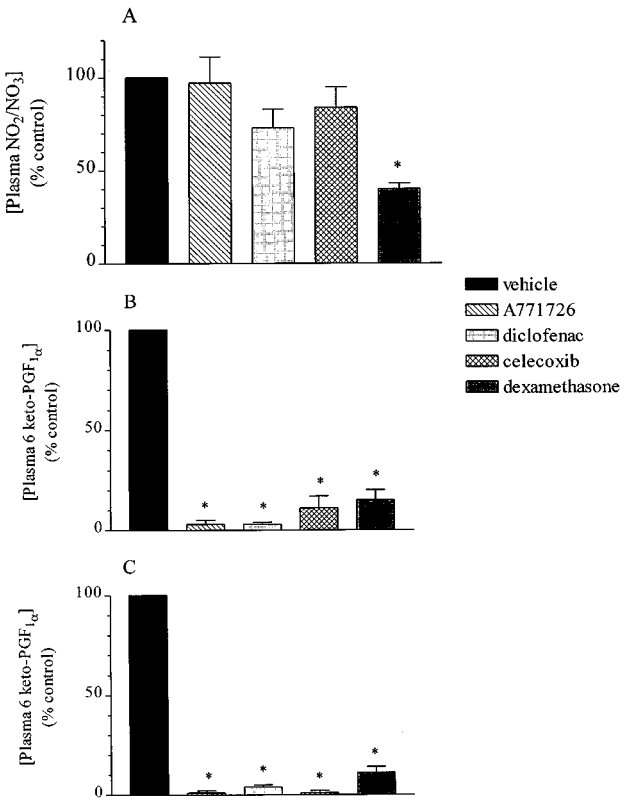


**Figure 4** Effect in LPS-treated rats of the COX inhibitors A771726 ($n=5$), diclofenac ($n=4$) and celecoxib ($n=6$), dexamethasone ($n=6$), or vehicle ($n=15$), on the plasma concentrations of (A) $NO_2/NO_3$, (B) 6 keto-$PGF_{1\alpha}$, and (C) 6 keto-$PGF_{1\alpha}$ 1 min following bolus injection of arachidonic acid (3 mg $kg^{-1}$, i.v.). Results are normalized to batch- and time-matched controls receiving LPS plus vehicle and are expressed as mean ± s.e.mean. *Denotes significant difference from control ($P<0.05$, one-sample test).

**Table 1** Mean arterial blood pressures rats treated with LPS inhibitors or vehicle

| | | *Time of infusion (h)* | | | |
|---|---|---|---|---|---|
| *Treatment* | n | *0* | *2* | *4* | *6* |
| Control | 19 | 97±4 | 89±3 | 91±4 | 87±4* |
| LPS+ vehicle | 20 | 108±4 | 92±3 | 93±3 | 88±3* |
| LPS+ L-NMMA | 8 | 109±4 | 99±4 | 101±4 | 95±5 |
| LPS+ 1400W | 8 | 122±4 | 100±5 | 106±5 | 103±4 |
| LPS+ A771726 | 5 | 92±4 | 91±7 | 89±8 | 71±7* |
| LPS+ diclofenac | 4 | 99±6 | 96±5 | 85±11 | 72±4* |
| LPS+ celecoxib | 6 | 116±6 | 91±3 | 85±8 | 74±7* |
| LPS+ dexamethasone | 6 | 129±5 | 106±3 | 102±2 | 85±9* |

*($P<0.05$, paired ANOVA plus Bonferroni test) between $t=2$ h and $t=6$ h.

non-LPS-treated animals 1400W was without effect on the circulating levels of 6 keto-$PGF_{1\alpha}$. Similarly, treatment with 1400W did not affect the great increase in 6 keto-$PGF_{1\alpha}$ following injection of arachidonic acid bolus. These data clearly indicate that NO produced by iNOS *in vivo* is neither an activator of prostanoid production, nor a co-factor and/or co-stimulator of COX activity. We also found that celecoxib, despite causing reductions of more than 95% in the circulating levels of 6 keto-$PGF_{1\alpha}$ and the release of 6 keto-$PGF_{1\alpha}$ following injection of arachidonic acid had no effect on the plasma levels of $NO_2/NO_3$.

How then can we explain the lack of effect that we found in our studies compared to earlier reports of iNOS and COX-2 cross talk? Clearly, the most important difference is in the degree of selectivity of the inhibitors used. Previously used 'selective' inhibitors such as aminoguanidine and L-$N^6$-iminoethyl-lysine have been characterised in *in vitro* assays to be 10 to 50 fold selective for iNOS compared to eNOS (Stenger *et al.*, 1995; Misko *et al.*, 1993). Under similar conditions 1400W is more than 5000-fold selective for iNOS over eNOS (Garvey *et al.*, 1997). Indeed, the lesser selectivity of aminoguanidine *in vitro* may well explain why it has been found to inhibit constitutive NOS *in vivo*, where selectivities as low as 2 fold have been reported (Laszlo *et al.*, 1995). 1400W, on the other hand, is more than 50 fold selective for iNOS *in vivo* (Garvey *et al.*, 1997). Clearly, therefore, 1400W is a much more useful tool than compounds such as aminoguanidine in discriminating the roles of iNOS and eNOS *in vivo*. A second difference between our experiments and those performed previously lies in the doses of non-selective inhibitors used. In particular, it is only high doses of L-NMMA (60 mg $kg^{-1}$ $h^{-1}$) and L-NAME (6 mg $kg^{-1}$ $h^{-1}$) that have been reported to decrease prostanoid formation in LPS-treated rats (Salvemini *et al.*, 1995). Here, although we only investigated the effects of a ten times lower dose of L-NMMA, we did try additional experiments using L-NAME. Unfortunately in our hands rats did not tolerate high doses of L-NAME. Thus of nine animals receiving between 0.5 and 3 mg $kg^{-1}$ $h^{-1}$ L-NAME six animals died after 5 h or less (data not shown). However, it is worth restating that, despite our lack of success in repeating these earlier studies using non-selective NOS inhibitors, we clearly found 1400W to abolish the increase in plasma $NO_2/NO_3$ caused by LPS infusion without affecting the accompanying rise in plasma 6 keto-$PGF_{1\alpha}$ concentration. We could, therefore, find no correlation between inhibition of iNOS and inhibition of prostanoid production by COX-2. Indeed, even when we compared the plasma concentrations of these mediators in individual animals, to ensure that data pooling was not masking a significant effect, no relationship between plasma $NO_2/NO_3$ and 6 keto-$PGF_{1\alpha}$ was found. These results indicate that reductions in prostanoid production found by previous investigators using non-selective or less selective iNOS inhibitors were most probably iNOS-independent.

Treatment of the rats with either a selective COX-2 inhibitor, celecoxib, or the non-selective inhibitors A771726 or diclofenac abolished the LPS-induced increases in both circulating 6 keto-$PGF_{1\alpha}$, and 6 keto-$PGF_{1\alpha}$ production following arachidonic acid. This is in accord with the increase in circulating 6 keto-$PGF_{1\alpha}$ being dependent upon the expression of COX-2. It is of particular interest that injection of arachidonic acid revealed such a marked elevation in 6 keto-$PGF_{1\alpha}$ production (Takahashi *et al.*, 1995). This indicates that measurement of the circulating concentration of 6 keto-$PGF_{1\alpha}$ does not give a true reflection of the potential synthetic capacity of COX-2. It also implies that even if NO were a stimulator of COX-2 activity in LPS-treated rats, it would be producing a level of COX-2 activity that was only a tiny fraction of that available. As a further support to this conclusion, the production of 6 keto-$PGF_{1\alpha}$ following administration of the pro-inflammatory mediator bradykinin was also greatly enhanced by the induction of COX-2 *in vivo*, as has been shown in the inflamed kidney (Seibert *et al.*, 1996).

Thus, even in the presence of very high *in vivo* concentrations of NO, COX-2 is barely activated. These experiments accentuate the point that the production of prostanoids by COX is tightly regulated by the supply of arachidonic acid.

It is interesting to note that injection of arachidonic acid, the substrate for COX, significantly elevated prostanoid production in LPS-treated rats. Similar injection of L-arginine, the substrate for NOS, was however without effect on the $NO_2$/$NO_3$ levels. Previous reports have shown that in cells treated with LPS there is an increase in the amount of L-arginine transport into the cell (Baydoun *et al*., 1993) although it is the supply of the co-factor tetrahydrobiopterin that appears to limit the activity of NOS (Schoedon *et al*., 1993). This highlights a difference between the COX and NOS systems, with COX activity highly dependent on supply of substrate. Consequently, in our system we see no change in activity following a bolus of L-arginine.

To date, several different mechanisms have been proposed for the influence of NO on COX activity. Firstly, the vast majority of effects mediated by NO follow from its interaction with iron or iron-containing enzymes. COX enzymes are hemoproteins (Van der Ouderaa *et al*., 1979; Picot *et al*., 1994) and are therefore a potential target for NO (Salvemini *et al*., 1993). Secondly, NO is a free radical which can nitrosylate free cysteine groups (Moriguchi *et al*., 1992). The COX enzyme has several free cysteine residues, some of which are critical to activity (Kennedy *et al*., 1994). Nitrosylation of these particular cysteine groups will have a significant effect on COX activity. Finally, it is possible that NO can affect the transcription of the COX-2 enzyme by altering the availability of transcription factors (Dela Torre *et al*., 1997). From our investigation, we propose that if NO were to activate COX, the only rational explanation would be that NO stimulates the activity of phospholipase $A_2$ to release arachidonic acid (Flower, 1988). However, no such system has been established.

One other conclusion that we can draw is that at doses that inhibited both the basal and the arachidonic acid-stimulated activity of COX-2 neither classical NSAIDs nor the novel COX-2-selective inhibitor celecoxib affected the induction of iNOS. This confirms our previous *in vitro* experiments with A771726, where we found that iNOS and COX-2 induction were only affected at concentrations 100 times higher than those required to inhibit the formation of prostanoids (Hamilton *et al*., 1997b). Indeed, it may well be a common phenomenon that it is only at suprapharmacological concentrations that NSAID effects on inductive processes leading to iNOS and COX-2 expression are found (Frantz and O'Neill, 1995). This contrasts with dexamethasone, which has been demonstrated in most previous *in vitro* and *in vivo* studies to inhibit the expression of both iNOS and COX-2.

Finally, it is interesting to note that LPS infusion induces a fall in blood pressure which can be attenuated by 1400W and L-NMMA. Dexamethasone has little effect on the hypotension caused by LPS infusion yet it appears equally effective as the NOS inhibitors at inhibiting plasma $NO_2$/$NO_3$ production. Previously, it has been shown that dexamethasone *in vivo* can interact with vasoconstrictor and vasodilator mechanisms (Gardiner *et al*., 1996). In this study, dexamethasone was given to animals two hours prior to LPS infusion. Consistent with the other groups of animals, blood pressure recordings began at $t=0$ h, the onset of LPS infusion. At the time, dexamethasone-treated animals had higher starting blood pressures, suggesting that dexamethasone had exerted a small pressor action. Indeed, in rats not receiving LPS, dexamethasone treatment was associated with blood pressures maintained above 110 mmHg for the entire 6 h infusion period (data not shown). Consequently, haemodynamic data from dexamethasone-treated rats may well be affected by non-iNOS dependent mechanisms.

In summary, our data show that NO produced by iNOS in the LPS-treated rat does not stimulate the release of prostanoids following activation of COX-2. This lack of interaction may well explain the inability of non-selective NOS inhibitors to affect prostaglandin production in models of inflammation (Paya *et al*., 1997).

LCH is the recipient of a British Heart Foundation Ph.D. studentship (FS/97013) and TDW holds a British Heart Foundation Lectureship (BS/95003). This work was also supported by a grant from Boehringer-Ingelheim, Germany. The authors would like to thank Dr. Jane A. Mitchell for helpful discussions.

## References

AMIN, A.R., ATTUR, M., PATEL, R.N., THAKKER, G.D., MARSHALL, P.J., REDISKE, J., STUCHIN, S.A., PATEL, I.R. & ABRAMSON, S.B. (1997). Superinduction of cyclooxygenase-2 activity in human osteoarthritis-affected cartilage. Influence of nitric oxide. *J. Clin. Invest.,* **99,** 1231 – 1237.

BAYDOUN, A.R., BOGLE, R.G., PEARSON, J.D. & MANN, G.E. (1993). Arginine uptake and metabolism in cultured murine macrophages. *Agents Actions,* **38,** C127 – 129.

BISHOP-BAILEY, D., LARKIN, S.W., WARNER, T.D., CHEN, G. & MITCHELL, J.A. (1997). Characterization of the induction of nitric oxide synthase and cyclo-oxygenase in rat aorta in organ culture. *Br. J. Pharmacol.,* **121,** 125 – 133.

CORBETT, J.A., KWON, G., TURK, J. & MCDANIEL, M.L. (1993). IL-1$\beta$ induces the coexpression of both nitric oxide synthase and cyclooxygenase by islets of Langerhans: activation of cyclooxygenase by nitric oxide. *Biochemistry,* **32,** 13767 – 13770.

DELA TORRE, A., SCHROEDER, R.A. & KUO, P.C. (1997). Alteration of NF-kappa B P50 binding kinetics by S-nitrosylation. *Biochem. Biophys. Res. Commum.,* **238,** 703 – 706.

FLOWER, R.J. (1988). Eleventh Gaddum memorial lecture. Lipocortin and the mechanism of action of the glucocorticoids. *Br. J. Pharmacol.,* **94,** 987 – 1015.

FRANCHI, A.M., CHAUD, M., RETTORI, V., SUBURO, A., MCCANN, S.M. & GIMENO, M. (1994). Role of nitric oxide in eicosanoid synthesis and uterine motility in estrogen-treated rat uteri. *Proc. Natl. Acad. Sci. U.S.A.,* **91,** 539 – 543.

FRANTZ, B. & O'NEILL, E.A. (1995). The effect of sodium salicylate and aspirin on NF-$\kappa$B. *Science,* **270,** 2017 – 2019.

GARDINER, S.M., KEMP, P.A., MARCH, J.E. & BENNETT, T. (1996). Effects of dexamethasone and SB 209670 on the regional haemodynamic responses to lipopolysaccharide in conscious rats. *Br. J. Pharmacol.,* **118,** 141 – 149.

GARVEY, E.P., OPLINGER, J.A., FURFINE, E.S., KIFF, R.J., LASZLO, F., WHITTLE, B.J. & KNOWLES, R.G. (1997). 1400W is a slow, tight binding, and highly selective inhibitor of inducible nitric-oxide synthase *in vitro* and *in vivo*. *J. Biol. Chem.,* **272,** 4959 – 4963.

HABIB, A., BERNARD, C., LEBRET, M., CREMINON, C., ESPOSITO, B., TEDGUI, A. & MACLOUF, J. (1997). Regulation of the expression of cyclooxygenase-2 by nitric oxide in rat peritoneal macrophages. *J. Immunol.,* **158,** 3845 – 3851.

HAMILTON, L.C., MITCHELL, J.A. & WARNER, T.D. (1997a). Induction of COX-2 *in vivo* leads to greatly increased production of 6-keto-$PGF_{1\alpha}$ following administration of exogenous arachidonic acid or bradykinin. *Br. J. Pharmacol.,* **122,** 22P.

HAMILTON, L.C., TOMLINSON, A., DYE, L.J., MITCHELL, J.A. & WARNER, T.D. (1998). Use of selective enzyme inhibitors provide no evidence for cross-talk between inducible nitric oxide synthase and cyclooxygenase-2 in endotoxin-treated rats. *Br. J. Pharmacol.,* **123,** 11P.

HAMILTON, L.C., VOJNOVIC, I., BAKHLE, Y.S., WARNER, T.D. & VANE, J.R. (1997b). The anti-inflammatory drug leflunomide inhibits *in vitro* and *in vivo* the activity of COX-2 more potently than the induction of COX-2 or iNOS. *Br. J. Pharmacol.,* **120,** 49P.

HULKOWER, K.I., POLLOCK, J.S., WALSH, R.E., HUANG, R., OTIS, E.R., BROOKS, C.D. & BELL, R.L. (1996). Leukotrienes do not regulate nitric oxide production in RAW 264.7 macrophages. *Prostaglandins Leukot. Essent. Fatty Acids,* **55,** 145–149.

INOUE, T., FUKUO, K., MORIMOTO, S., KOH, E. & OGIHARA, T. (1993). Nitric oxide mediates interleukin-1-induced prostaglandin $E_2$ production by vascular smooth muscle cells. *Biochem. Biophys. Res. Commun.,* **194,** 420–424.

KANNER, J., HAREL, S. & GRANIT, R. (1992). Nitric oxide, an inhibitor of lipid oxidation by lipoxygenase, cyclooxygenase and hemoglobin. *Lipids,* **27,** 46–49.

KENNEDY, T.A., SMITH, C.J. & MARNETT, L.J. (1994). Investigations of the roles of systeines in catalysis by prostaglandin endoperoxide synthase. *J. Biol. Chem.,* **269,** 27357–27364.

LASZLO, F., EVANS, S.M. & WHITTLE, B.J. (1995). Aminoguanidine inhibits both constitutive and inducible nitric oxide synthase isoforms in rat intestinal microvasculature *in vivo*. *Eur. J. Pharmacol.,* **272,** 169–175.

MISKO, T., MOORE, P., KASTEN, W.M., NICKOLS, T.P., CORBETT, J.A., TILTON, R.G., MCDANIEL, M.L., WILLIAMSON, J.R. & CURRIE, M.G. (1993). Selective inhibition of the inducible nitric oxide synthase by aminoguanidine. *Eur. J. Pharmacol.,* **233,** 119–125.

MONCADA, S. (1997). Nitric oxide in the vasculature: physiology and pathophysiology. *Ann. N. Y. Acad. Sci.,* **811,** 60–67.

MORIGUCHI, M., MANNING, L.R. & MANNING, J.M. (1992). Nitric oxide can modify amino acid residues in proteins. *Biochem. Biophys. Res. Commun.,* **183,** 598–604.

PAYA, M., GARCIA, P.P., COLOMA, J. & ALCARAZ, M.J. (1997). Nitric oxide synthase and cyclo-oxygenase pathways in the inflammatory response induced by zymosan in the rat air pouch. *Br. J. Pharmacol.,* **120,** 1445–1452.

PENNING, T.D., TALLEY, J.J., BERTENSHAW, S.R., CARTER, J.S., COLLINS, P.W., DOCTER S, GRANETO, M.J., LEE, L.F., MALECHA, J.W., MIYASHIRO, J.M., ROGERS, R.S., ROGIER, D.J., YU, S.S., ANDERSON, G.D., BURTON, E.G., COGBURN, J.N., GREGORY, S.A., KOBOLDT, C.M., PERKINS, W.E., SEIBERT, K., VEENHUIZEN, A.W., ZHANG, Y.Y. & ISAKSON, P.C. (1997). Synthesis and biological evaluation of the 1,5-diarylpyrazole class of cyclooxygenase-2 inhibitors: identification of 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benze nesulfonamide (SC-58635, celecoxib). *J. Med. Chem.,* **40,** 1347–1365.

PICOT, D., LOLL, P.J. & GARAVITO, R.M. (1994). The x-ray crystal structure of the membrane protein prostaglandin H2 synthase-1. *Nature,* **367,** 243–249.

SALVEMINI, D., MISKO, T.P., MASFERRER, J.L., SEIBERT, K., CURRIE, M.G. & NEEDLEMAN. (1993). Nitric oxide activates cyclooxygenase enzymes. *Proc. Natl. Acad. Sci. U.S.A.,* **90,** 7240–7244.

SALVEMINI, D., SEIBERT, K., MASFERRER, J.L., MISKO, T.P., CURRIE, M.G. & NEEDLEMAN. (1994). Endogenous nitric oxide enhances prostaglandin production in a model of renal inflammation. *J. Clin. Invest.,* **93,** 1940–1947.

SALVEMINI, D., SETTLE, S.L., MASFERRER, J.L., SEIBERT, K., CURRIE, M.G. & NEEDLEMAN. (1995). Regulation of prostaglandin production by nitric oxide; an in vivo analysis. *Br. J. Pharmacol.,* **114,** 1171–1178.

SCHMIDT, H.H., WARNER, T.D., NAKANE, M., FORSTERMANN, U. & MURAD, F. (1992). Regulation and subcellular location of nitrogen oxide synthases in RAW264.7 macrophages. *Mol. Pharmacol.,* **41,** 615–624.

SCHOEDON, G., SCHNEEMANN, M., HOFER, S., GUERRERO, L., BLAU, N. & SCHAFFNER, A. (1993). Regulation of the L-arginine-dependent and tetrahydrobiopterin-dependent biosynthesis of nitric oxide in murine macrophages. *Eur. J. Biochem.,* **213,** 833–839.

SEIBERT, K., MASFERRER, J.L., NEEDLEMAN, P. & SALVEMINI, D. (1996). Pharmacological manipulation of cyclo-oxygenase-2 in the inflamed hydronephrotic kidney. *Br. J. Pharmacol.,* **117,** 1016–1020.

STADLER, J., HARBRECHT, B.G., DI SILVIO, M., CURRAN, R.D., JORDAN, M.L., SIMMONS, R.L. & BILLIAR, T.R. (1993). Endogenous nitric oxide inhibits the synthesis of cyclooxygenase products and interleukin-6 by rat Kupffer cells. *J. Leukoc. Biol.,* **53,** 165–172.

STENGER, S., THURING, H., ROLLINGHOFF, M., MANNING, P. & BOGDAN, C. (1995). L-$N^6$-(1-iminoethyl)-lysine potently inhibits inducible nitric oxide synthase and is superior to NG-monomethyl-arginine *in vitro* and *in vivo*. *Eur. J. Pharmacol.,* **294,** 703–712.

SWIERKOSZ, T.A., MITCHELL, J.A., WARNER, T.D., BOTTING, R.M. & VANE, J.R. (1995). Co-induction of nitric oxide synthase and cyclo-oxygenase: interactions between nitric oxide and prostanoids. *Br. J. Pharmacol.,* **114,** 1335–1342.

TAKAHASHI, S., FUTAKI, N., YOKOYAMA, M., YAMAKAWA, Y., ARAI, I., HIGUCHI, S. & OTOMO, S. (1995). Expression of prostaglandin H synthase-2 in endotoxic shock induced in rats. *Arch. Int. Pharmacodyn. Ther.,* **330,** 102–115.

TETSUKA, T., DAPHNA-IKEN, D., MILLER, B.W., GUAN, Z., BAIER, L.D. and MORRISON, A.R. (1996). Nitric oxide amplifies interleukin 1-induced cyclooxygenase-2 expression in rat mesangial cells. *J. Clin. Invest.,* **97,** 2051–2056.

VAN DER OUERAA, F.J., BUYTENHEK, M., SLIKKERVEER, F.J. and VAN DORP, D.A. (1979). On the haemoprotein character of prostaglandin endoperoxide synthetase. *Biochim. Biophys. Acta.,* **572,** 29–42.

VANE, J.R., MITCHELL, J.A., APPLETON, I., TOMLINSON, A., BISHOP-BAILEY, D., CROXTALL, J. and WILLOUGHBY, D.A. (1994). Inducible isoforms of cyclooxygenase and nitric-oxide synthase in inflammation. *Proc. Natl. Acad. Sci. U.S.A.,* **91,** 2046–2050.

WRAY, G.M., MILLAR, C.G., HINDS, C.J. and THIEMERMANN, C. (1998). Selective inhibition of the activity of inducible nitric oxide synthase prevents the circulatory failure, but not the organ injury/dysfunction, caused by endotoxin. *Shock,* in press.

*(Received June 4, 1998*
*Revised June 19, 1998*
*Accepted June 23, 1998)*