European Journal of Pharmacology 741 (2014) 171–177

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Cardiovascular pharmacology

The ability of asymmetric dimethylarginine (ADMA) or monomethylarginine (L-NMMA) to block endothelium-dependent, nitric oxide-mediated relaxation in rat aorta is inversely related to the efficacy of the relaxant stimulus

Mohammed J. Al-Zobaidy, William Martin *

College of Medical, Veterinary and Life Sciences, West Medical Building, University of Glasgow, Glasgow, G12 8QQ Scotland, UK

ARTICLE INFO

Article history:
Received 30 June 2014
Received in revised form
25 July 2014
Accepted 8 August 2014
Available online 19 August 2014

Keywords:
Acetylcholine
Asymmetric N^G,N^G-dimethyl-L-arginine
Endothelium
Nitric oxide
N^G-monomethyl-L-arginine
Phenoxybenzamine

Chemical compounds studied in this article:
Dimethylarginine (ADMA) (PubChem CID: 24278027)
Monomethylarginine (L-NMMA) (PubChem CID: 24278043)

ABSTRACT

Previous work on rat aorta has shown that L-NMMA and ADMA each enhance vasoconstrictor-induced tone, consistent with blockade of basal nitric oxide activity, whereas they exert little inhibitory effect on acetylcholine-induced relaxation when tone is matched carefully to that of control tissues. The aim of this study was to determine if the ability of L-NMMA or ADMA to inhibit nitric oxide-mediated relaxation was critically determined by the efficacy of the relaxant stimulus. The effects of L-NMMA or ADMA were examined on relaxation to a range of agonists producing different maximal responses, namely, acetylcholine, the muscarinic partial agonist, butyrylcholine, and calcitonin gene-related peptide-1 (CGRP-1). The effects of L-NMMA or ADMA were also examined on relaxation to acetylcholine when its apparent efficacy at the M_3 muscarinic receptor was reduced using the irreversible receptor blocking agent, phenoxybenzamine. Maximal relaxation induced by butyrylcholine or CGRP-1 was lower than to acetylcholine. While acetylcholine-induced relaxation was largely resistant to blockade by L-NMMA or ADMA (0.1 or 1 mM), relaxation to butyrylcholine or CGRP-1 was powerfully suppressed. Phenoxybenzamine (0.1–10 μM for 30 min) concentration-dependently reduced maximal acetylcholine-induced relaxation. When the efficacy of acetylcholine was reduced by phenoxybenzamine, its residual relaxant effect was powerfully inhibited by L-NMMA or ADMA (0.1 or 1 mM). Thus, in rat aorta, the ability of L-NMMA or ADMA to block agonist-induced nitric oxide activity is critically determined by the efficacy of the relaxant stimulus.

1. Introduction

The synthesis of nitric oxide can generally be blocked by a variety of guanidino-substituted analogues of L-arginine, such as N^G-monomethyl-L-arginine (L-NMMA), N^G-nitro-L-arginine (L-NOARG) and N^G-nitro-L-arginine methyl ester (L-NAME), that act as competitive, reversible inhibitors of NOS (Palmer et al., 1988; Rees et al., 1989, 1990; Moore et al., 1990; Hobbs et al., 1999). Strikingly, a number of studies have suggested that of these three agents, L-NMMA can exhibit properties that deviate markedly from classical competitive behaviour on all three isoforms of NOS. For example, L-NMMA has been shown to act as an alternative substrate and mechanism-based suicide inhibitor of iNOS in murine macrophages (Olken and Marletta, 1993). Other studies have reported that L-NMMA, unlike L-NOARG and L-NAME, does not inhibit nNOS-related, nitrergic nerve-mediated relaxation in the bovine penile and ciliary arteries and retractor penis muscle (Liu et al., 1991; Martin et al., 1993; Overend and Martin, 2007). In addition, although L-NMMA blocks the eNOS-dependent basal nitric oxide activity that exerts a tonic vasodepressor action in rat aorta, it has little effect on acetylcholine-induced relaxation in this vessel when tone is matched carefully to that of control tissues (Frew et al., 1993). More recent reports show that a pathophysiologically-important endogenous inhibitor of NOS, asymmetric N^G,N^G-dimethyl-L-arginine (ADMA, Vallance et al., 1992), shares the ability of L-NMMA to block preferentially basal over acetylcholine-stimulated activity of nitric oxide in rat aorta (AL-Zobaidy et al., 2010, 2011).

A possible explanation for the differential effects of L-NMMA and ADMA on basal versus acetylcholine-stimulated activity of nitric oxide in rat aorta may relate to the extent to which eNOS is stimulated under the two conditions. Specifically, the low-level activation of eNOS which underpins basal activity of nitric oxide may, theoretically, be easier to block than the powerful enzyme activation resulting from agonist stimulation. This hypothesis is

* Corresponding author. Tel: +44 141 330 4489; fax: +44 141 330 5481.
E-mail address: william.martin@glasgow.ac.uk (W. Martin).

http://dx.doi.org/10.1016/j.ejphar.2014.08.002
0014-2999/

supported by previous work showing that the relative resistance of acetylcholine-induced relaxation to blockade by L-NAME in rabbit jugular vein when compared to rat aorta results from a higher efficacy of the relaxant in the former tissue because of a greater M_3 muscarinic receptor reserve (Martin et al., 1992). As a consequence, the aim of this study was to determine if in rat aorta the ability of the methylarginines, L-NMMA and ADMA, to block nitric oxide-mediated relaxation is critically determined by the efficacy of the relaxant stimulus.

2. Materials and methods

2.1. Preparation of rat aortic rings and tension recording

All animal care and experimental procedures complied with UK Home Office regulations. The preparation of rat aortic rings for tension recording was essentially similar to previous studies (Frew et al., 1993; AL-Zobaidy et al., 2010, 2011). Briefly, female Wistar rats weighing 150–200 g were killed by CO_2 overdose. The aorta was removed, cleared of adhering fat and connective tissue and cut into 2.5 mm wide transverse rings using a device with parallel razor blades. The aortic rings were mounted under 10 mN resting tension on stainless steel hooks in 10 ml tissue baths and bathed at 37 °C in Krebs solution containing: NaCl 118 mM, KCl 4.8 mM, $CaCl_2$ 2.5 mM, $MgSO_4$ 1.2 mM, KH_2PO_4 1.2 mM, $NaHCO_3$ 24 mM, glucose 11 mM, and gassed with 95% O_2 and 5% CO_2. Tension was recorded isometrically with Grass FTO3C transducers and displayed on a PowerLab (ADInstruments, Hastings, UK).

2.2. Experimental protocols

Experiments were conducted to assess the effects of L-NMMA or ADMA (0.1 and 1 mM) on endothelium-dependent, nitric oxide-mediated relaxation induced by a range of agonists producing different maximal responses. Specifically, the agents used were the full agonist, acetylcholine, the partial agonist, butyrylcholine (Martin et al., 1992), and the weak relaxant, CGRP-1 (Gray and Marshall, 1992). In these experiments, the NOS inhibitors were added for 1 h prior to contracting the tissues with phenylephrine or, where indicated, prostaglandin $F_{2\alpha}$. This latter vasoconstrictor was required in experiments in which the irreversible alkylating agent, phenoxybenzamine (Martin et al., 1992), was used to lower the apparent efficacy of acetylcholine or butyrylcholine at the M_3 muscarinic receptor (see below), since in these, phenylephrine lost its ability to induce tone because of additional blockade of α_1-adrenoceptors. In all experiments, care was taken to induce comparable levels of tone (~50% of max) in control and drug-treated tissues. In order to achieve this, lower concentrations of phenylephrine or prostaglandin $F_{2\alpha}$ are used in the presence of L-NMMA or ADMA because NOS inhibitors enhance vasoconstrictor tone through removal of basal nitric oxide activity which exerts a tonic vasodilator action (Martin et al., 1986; Rees et al., 1989, 1990. After stabilisation of tone in control and NOS inhibitor-treated tissues, cumulative concentration–response curves were constructed to acetylcholine (1 nM to 10 μM), butyrylcholine (100 nM to 300 μM), or calcitonin gene-related peptide-1 (0.1 nM to 1 μM) and relaxation assessed.

As indicated above, a series of experiments was carried out to reduce the apparent efficacy of the full agonist, acetylcholine, and the partial agonist, butyrylcholine, using the irreversible alkylating agent, phenoxybenzamine (Martin et al., 1992). In these experiments, some tissues were treated with phenoxybenzamine (0.1–10 μM for 30 min followed by extensive washout) and others were left as controls. The tissues were then pre-contracted to 50% of maximal prostaglandin $F_{2\alpha}$-induced tone before constructing cumulative concentration–response curves to acetylcholine or butyrylcholine. Further experiments were conducted to study the effects of L-NMMA or ADMA (both at 0.1 and 1 mM) on relaxation induced by acetylcholine after its efficacy had been reduced by treatment with phenoxybenzamine (0.3, 1 and 3 μM).

2.3. Data analysis

Relaxant responses to agonists were expressed as a percentage of the complete relaxation induced by a supramaximal concentration of papaverine (300 μM) added at the end of each experiment. Data are expressed as the mean ± S.E.M. of n separate observations, each from a different tissue. Concentration–response curves were drawn and statistical analysis was performed using one-way analysis of variance followed by Bonferroni's post-test, or by Student's t-test, as appropriate, with the aid of Prism (Graph Pad, San Diego, USA). Values were considered to be statistically different when P was ≤ 0.05.

2.4. Drugs and chemicals

Acetylcholine chloride, asymmetric N^G,N^G-dimethyl-L-arginine dihydrochloride (ADMA), butyrylcholine chloride, N^G-monomethyl-L-arginine acetate (L-NMMA), papaverine hydrochloride, phenoxybenzamine hydrochloride, phenylephrine hydrochloride and prostaglandin $F_{2\alpha}$ were all obtained from Sigma, UK. Calcitonin gene-related peptide-1 (CGRP-1, human) was obtained from Calbiochem. All drugs were dissolved and diluted in 0.9% saline except phenoxybenzamine and prostaglandin $F_{2\alpha}$ which were dissolved in 100% ethanol and calcitonin gene-related peptide-1 which was dissolved in 5% acetic acid (all stocks 10 mM).

3. Results

3.1. Relaxation to acetylcholine, butyrylcholine and calcitonin gene-related peptide-1

Following induction of ~50% maximal tone using phenylephrine (20–100 nM) in rat endothelium-containing aortic rings, acetylcholine (1 nM to 10 μM; Fig. 1A and B), butyrylcholine (0.1–300 μM; Fig. 1C and D), and CGRP-1 (0.1 nM to 1 μM; Fig. 2) each induced concentration-dependent relaxation. The maximal relaxation induced by acetylcholine was 93.3 ± 2.8%. Consistent with its actions as an M_3 muscarinic partial agonist, butyrylcholine induced significantly lower maximal relaxation (72.6 ± 5.4%; $P < 0.05$) than acetylcholine. CGRP-1 also induced significantly lower maximal relaxation (57.6 ± 3.2%; $P < 0.001$) than acetylcholine.

3.2. Effects of L-NMMA or ADMA on relaxation to acetylcholine, butyrylcholine and calcitonin gene-related peptide-1

As previously reported (Frew et al., 1993; AL-Zobaidy et al., 2010, 2011), L-NMMA and ADMA (0.1 and 1 mM) had little effect on acetylcholine-induced relaxation when experiments were conducted at the same level of phenylephrine-induced tone as control tissues (Fig. 1A and B); there was a maximal ~2-fold reduction in sensitivity to acetylcholine but no change in maximal relaxation. In contrast to these modest effects on relaxation to acetylcholine, L-NMMA and ADMA each produced powerful blockade of maximal relaxation to butyrylcholine (Fig. 1C and D) and to an even greater extent, CGRP-1 (Fig. 2).

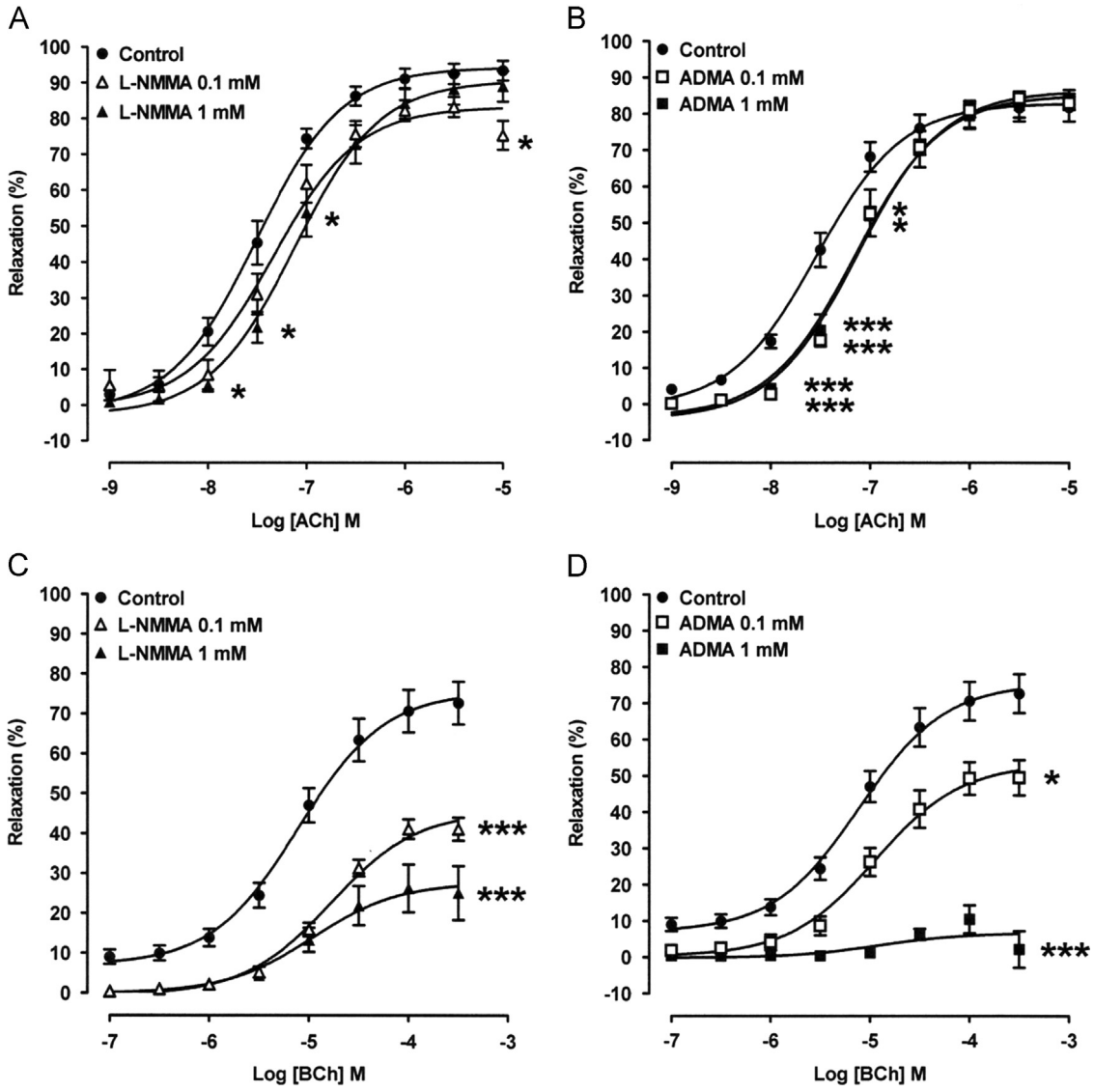

Fig. 1. Cumulative concentration–effect curves showing relaxation to acetylcholine (A, B) and butyrylcholine (C, D) in rat endothelium-containing aortic rings submaximally contracted with phenylephrine in the absence and presence of 0.1 or 1 mM L-NMMA (A and C) or ADMA (B, D). L-NMMA and ADMA each produced a small ~2-fold decrease in tissue sensitivity to acetylcholine without affecting the maximal relaxation. Each point represents the mean ± S.E.M. of 5–12 observations. $^{*}P < 0.05$ and $^{***}P < 0.001$ indicate significant differences in relaxation; significant differences are shown for each concentration of acetylcholine, but for clarity they are shown only for the highest concentration of butyrylcholine.

3.3. Effects of L-NMMA or ADMA on acetylcholine-induced relaxation in tissues precontracted with prostaglandin $F_{2\alpha}$

Since the above experiments showed that L-NMMA and ADMA were able to block relaxation to agents that had a lower maximum response than acetylcholine, we wished to explore their effects when the efficacy of the muscarinic agonist was reduced using the irreversible receptor alkylating agent, phenoxybenzamine (Martin et al., 1992). As a prelude to these experiments, prostaglandin $F_{2\alpha}$ was chosen to replace phenylephrine as the contractile agent, since the latter's action is also blocked by phenoxybenzamine.

Following induction of ~50% maximal tone using prostaglandin $F_{2\alpha}$ (3–10 μM), acetylcholine (1 nM to 10 μM) induced concentration-dependent relaxation (Figs. 4A and 5A). As when phenylephrine was the constrictor agent, treatment with L-NMMA or ADMA (0.1 and 1 mM) had only modest effects on acetylcholine-induced relaxation; there was a maximal ~2-fold reduction in sensitivity to acetylcholine with a tendency to reduce the maximum response which was statistically significant only with L-NMMA at 1 mM.

3.4. Effects of phenoxybenzamine on relaxation induced by acetylcholine or butyrylcholine

Aortic rings were treated with the irreversible receptor alkylating agent, phenoxybenzamine (0.1–10 μM) and subsequently contracted with prostaglandin $F_{2\alpha}$ to similar levels of tone as control tissues. Under these conditions, phenoxybenzamine produced a concentration-dependent depression of maximal relaxation to acetylcholine (1 nM to 10 μM; Fig. 3A). Maximal relaxation to butyrylcholine (100 nM to 300 μM) was even more powerfully depressed by phenoxybenzamine (Fig. 3B).

3.5. Effects of L-NMMA or ADMA on relaxation when the efficacy of acetylcholine is reduced using phenoxybenzamine

In contrast to their modest effects on control tissues (Figs. 4A and 5A), on tissues treated with phenoxybenzamine (0.3–3 μM for 30 min), L-NMMA (Fig. 4B, C and D) and ADMA (Fig. 5B, C and D) at 0.1 and 1 mM each produced powerful, concentration-dependent

blockade of residual acetylcholine (1 nM to 10 μM)-induced relaxation. The magnitude of the blockade by L-NMMA and ADMA was related to the degree to which phenoxybenzamine had reduced the maximal relaxation to acetylcholine.

4. Discussion

The main new findings in this study are that while L-NMMA and ADMA have little effect on relaxation of rat aorta induced by the full agonist, acetylcholine, they powerfully block relaxation to the weak agonists, butyrylcholine and CGRP-1, and to acetylcholine when its efficacy is reduced by lowering the available M_3 muscarinic receptor pool. These findings suggest that blockade of nitric oxide-mediated relaxation in rat aorta by L-NMMA or ADMA is critically dependent upon the efficacy of the relaxant agonist.

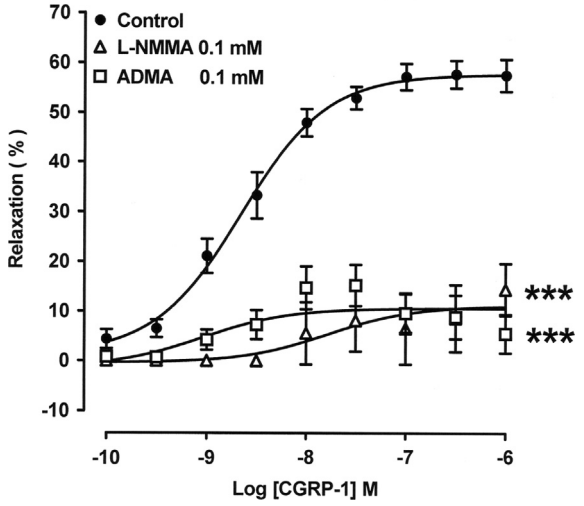

Fig. 2. Cumulative concentration–effect curves showing relaxation to calcitonin gene-related peptide-1 (CGRP-1) in rat endothelium-containing aortic rings submaximally contracted with phenylephrine in the absence and presence of L-NMMA or ADMA (both at 100 μM). Relaxation was powerfully blocked by L-NMMA or ADMA. Each point represents the mean ± S.E.M. of 5–6 observations. $^{***}P < 0.001$ indicates a significant difference in relaxation from control at the highest concentration of CGRP-1.

There is general agreement that the NOS inhibitors, L-NMMA and ADMA, block the basal nitric oxide activity that exerts a tonic vasodilator action opposing vasoconstriction in endothelium-containing rings of rat aorta (Martin et al., 1986; Rees et al., 1990; Frew et al., 1993; AL-Zobaidy et al., 2010, 2011). There is less agreement in the literature, however, regarding the ability of L-NMMA and ADMA to block the endothelium-dependent relaxation induced by agonists, such as acetylcholine, in rat aorta. Some studies do indeed report blockade of acetylcholine-induced relaxation in rat aorta by these methylarginines (Rees et al., 1990; Vallance et al., 1992; Jin and D'Alecy, 1996; Feng et al., 1998). However, much of this apparent blockade may actually be due to physiological antagonism, because the level of tone is greatly enhanced in NOS-treated compared to control tissues through loss of the vasodepressant action of basal nitric oxide activity. It is clear, however, that if levels of tone are carefully matched in control and L-NMMA- or ADMA-treated tissues that little blockade of acetylcholine-induced relaxation is seen (Frew, 1993; AL-Zobaidy et al., 2010); there is generally a ~2-fold maximal reduction in sensitivity to acetylcholine, without any great effect on maximal relaxation.

In addition, when care is taken to match levels of tone in control and L-NMMA- or ADMA-treated rings of rat aorta, relaxation to ATP or calcium ionophore A23187 is, like that to acetylcholine, largely resistant to blockade (Frew, 1993; AL-Zobaidy et al., 2011). Thus, a pattern has emerged suggesting that the methylarginines, L-NMMA and ADMA, preferentially block basal over agonist-stimulated activity of nitric oxide in rat aorta. A striking property common to acetylcholine, ATP and A23187, is that they are all strong agonists, producing maximal relaxation in excess of 90% of vasoconstrictor tone. We therefore considered the possibility that the differential effects of L-NMMA or ADMA on basal and agonist-stimulated activity of nitric oxide might relate to the degree of eNOS activation under the two conditions, i.e. that these NOS inhibitors were better able to oppose the low-level enzyme activation responsible for basal activity than the high-level activation stimulated by agonist. Such a possibility was consistent with an earlier report showing that the relative resistance of acetylcholine-induced relaxation to blockade by L-NAME in rabbit jugular vein when compared to rat

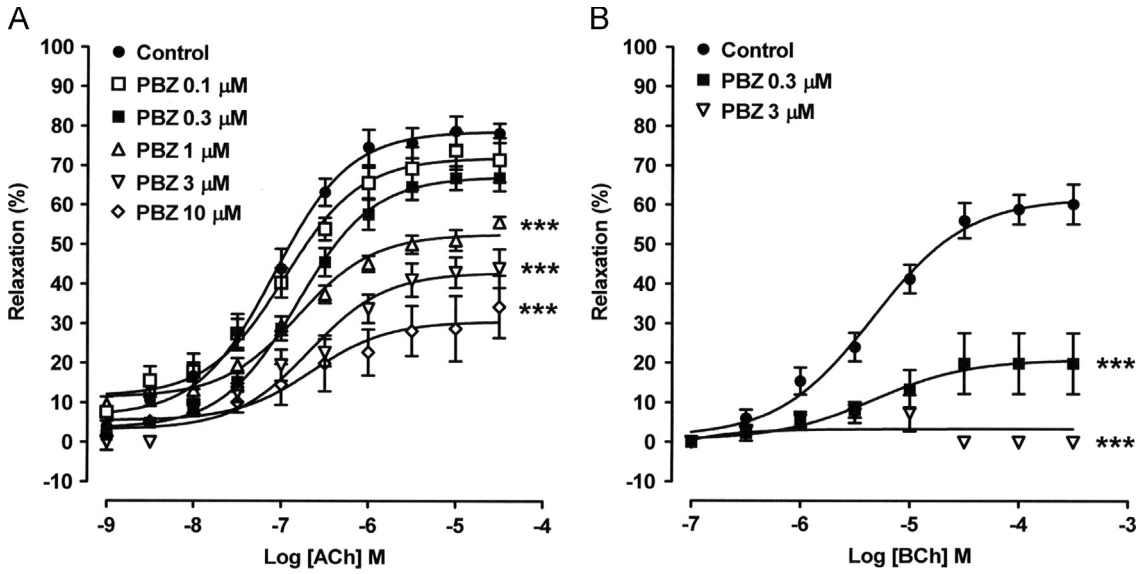

Fig. 3. Cumulative concentration–effect curves showing relaxation to acetylcholine (A) and butyrylcholine (B) in rat endothelium-containing aortic rings submaximally contracted with prostaglandin $F_{2\alpha}$. Also shown is that phenoxybenzamine produces a concentration-dependent depression in maximal relaxation to acetylcholine or butyrylcholine. $^{***}P < 0.001$ indicates a significant difference in relaxation from control at the highest concentration of acetylcholine or butyrylcholine.

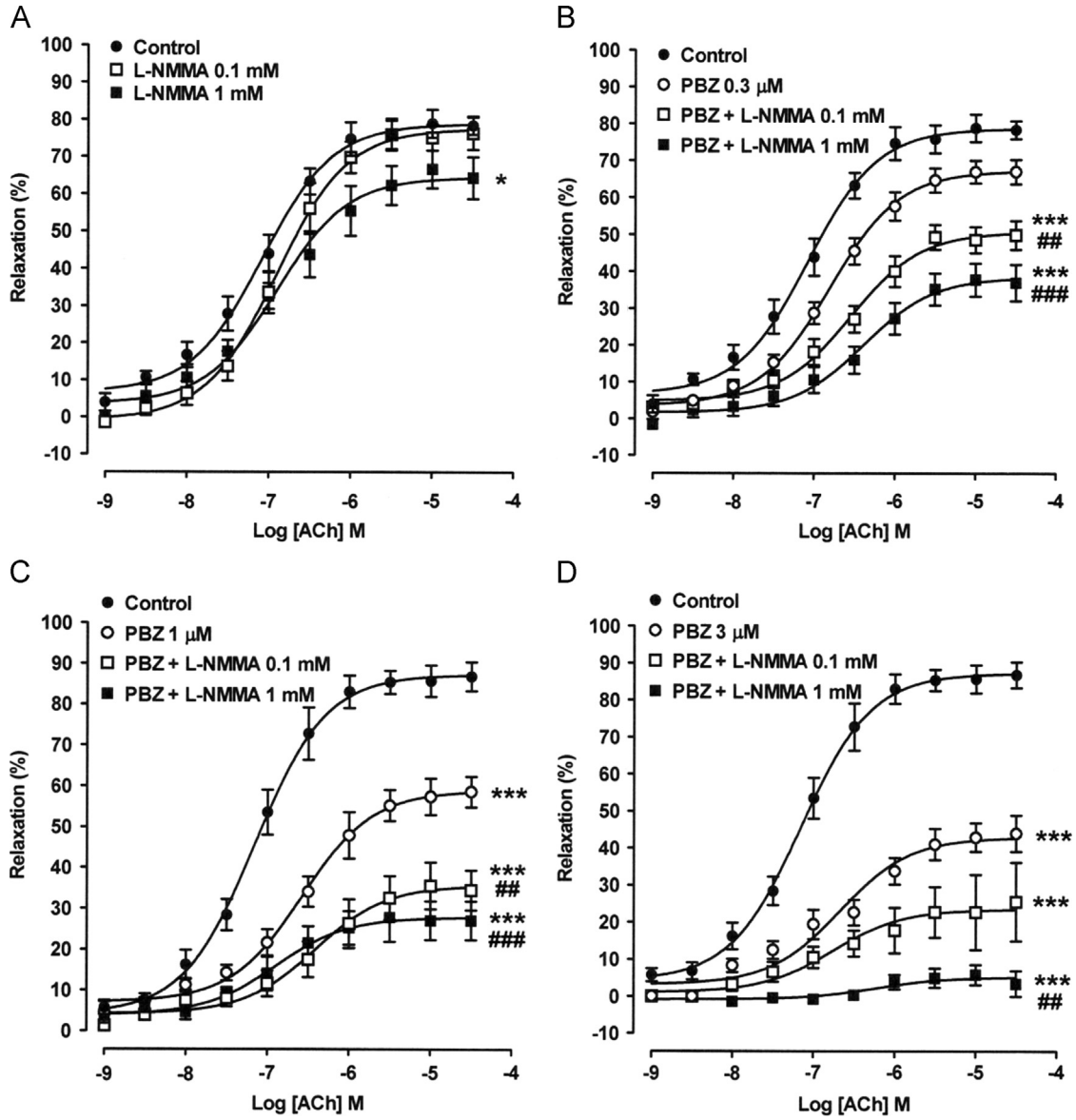

Fig. 4. Cumulative concentration–effect curves showing relaxation to acetylcholine in rat endothelium-containing aortic rings submaximally contracted with prostaglandin $F_{2\alpha}$. Responses show the effects of L-NMMA (0.1 and 1 mM) on control tissues (A), and on tissues pretreated with phenoxybenzamine (PBZ) at concentrations of 0.3 μM (B), 1 μM (C) or 3 μM (D). Following treatment with phenoxybenzamine, L-NMMA produced powerful blockade of relaxation. Each point represents the mean ± S.E.M. of 6–12 observations. $^{*}P < 0.05$ and $^{***}P < 0.001$ indicate a significant difference in relaxation from control at the highest concentration of acetylcholine; $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ indicate a significant difference in relaxation from tissues treated only with phenoxybenzamine.

aorta was due to the agonist having a higher efficacy on the former tissue because of a greater M_3 muscarinic receptor reserve (Martin et al., 1992). We therefore wished to test the hypothesis that the ability of L-NMMA or ADMA to block nitric oxide-mediated relaxation is critically determined by the efficacy of the relaxant agonist. We pursued this by adopting a two-component strategy similar to Martin et al. (1992), i.e. by examining the effects of L-NMMA or ADMA on relaxation induced by a range of agonists with different efficacies and by reducing the apparent efficacy of acetylcholine through the use of the irreversible receptor alkylating agent, phenoxybenzamine.

The three agonists we chose to examine were the full agonist, acetylcholine, the M_3 partial agonist, butyrylcholine (Martin et al., 1992) and the low-efficacy agonist, CGRP-1 (Gray and Marshall, 1992). As expected, we found that acetylcholine induced the greatest maximal relaxation in rat aortic rings. Consistent with its actions as a partial agonist, maximal relaxation induced by butyrylcholine was lower than for acetylcholine, and CGRP-1 was the weakest relaxant of the three. When the ability of L-NMMA or ADMA to block relaxation of rat aortic rings by these three agonists was examined, we found, in keeping with previous reports (Frew et al., 1993; AL-Zobaidy et al., 2010, 2011), that relaxation to acetylcholine was affected very little. In stark contrast, relaxation to butyrylcholine was powerfully inhibited and that to CGRP-1 was almost abolished. Thus, with little effect on the relaxant actions of the strong agonists acetylcholine, ATP and calcium ionophore A23187 (Frew et al., 1993; AL-Zobaidy et al., 2010, 2011), but with powerful blockade of those of the weaker agonists butyrylcholine and CGRP-1, these findings suggested there was an inverse relationship between the ability of L-NMMA or ADMA to block nitric oxide-mediated relaxation and the efficacy of the relaxant agonist.

We next wished to conduct experiments to lower the efficacy of acetylcholine by reducing the M_3 muscarinic receptor pool with the irreversible alkylating agent, phenoxybenzamine (Martin et al., 1992), to see how this affected the ability of L-NMMA or ADMA to

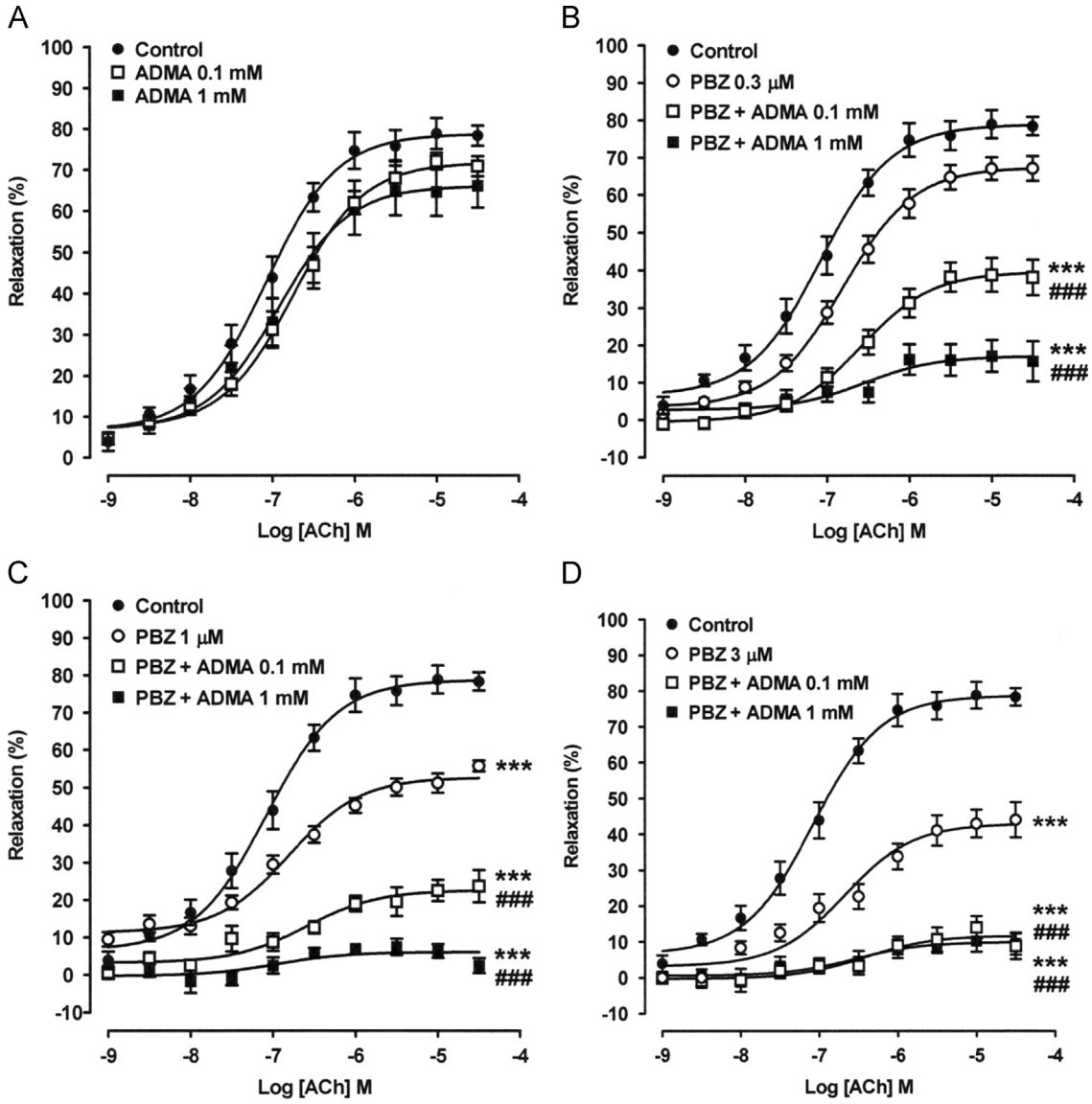

Fig. 5. Cumulative concentration–effect curves showing relaxation to acetylcholine in rat endothelium-containing aortic rings submaximally contracted with prostaglandin $F_{2\alpha}$. Responses show the effects of ADMA (0.1 and 1 mM) on control tissues (A), and on tissues pretreated with phenoxybenzamine (PBZ) at concentrations of 0.3 μM (B), 1 μM (C) or 3 μM (D). Following treatment with phenoxybenzamine, ADMA produced powerful blockade of relaxation. Each point represents the mean ± S.E.M. of 6–10 observations. $^{***}P < 0.001$ indicates a significant difference in relaxation from control at the highest concentration of acetylcholine; $^{###}P < 0.001$ indicates a significant difference in relaxation from tissues treated only with phenoxybenzamine.

block relaxation. However, before doing so we first had to select an alternative contractile agent to phenylephrine because the α_1-adrenoceptor on which it acts is also irreversibly inhibited by phenoxybenzamine (Furchgott, 1966). In the present study prostaglandin $F_{2\alpha}$ was chosen as the alternative contractile agent because the tone it induces is unaffected by phenoxybenzamine.

In keeping with receptor theory (Stephenson, 1956; Furchgott, 1966), we found that phenoxybenzamine produced a concentration-dependent depression of maximal acetylcholine-induced relaxation. Also, as expected, phenoxybenzamine had a more profound depressant effect on maximal relaxation to the partial agonist, butyrylcholine, that to the full agonist, acetylcholine, because the latter requires a smaller percentage of receptors in order to produce a response. In contrast to the modest effects of L-NMMA or ADMA seen in control preparations, these agents profoundly inhibited relaxation to acetylcholine when its efficacy had been reduced following treatment with phenoxybenzamine. Indeed, the magnitude of the inhibition by the two methylarginines was related to the degree to which phenoxybenzamine had reduced the maximal response to acetylcholine. Thus, the results of these experiments too support the hypothesis that the ability of L-NMMA or ADMA to block nitric oxide-mediated relaxation is strictly determined by the efficacy of the relaxant stimulus.

It is now well established that L-NMMA and ADMA are produced endogenously, and plasma levels of the latter in particular are known to accumulate and potentially contribute to the pathology of a number of clinical states including renal failure, cardiovascular disease and critical illness (Vallance et al., 1992; Siroen et al., 2006; Brinkmann et al., 2014). Our finding that ADMA blocks endothelium-dependent, nitric oxide-mediated relaxation in a manner inversely related to the efficacy of the relaxant stimulus will therefore have relevance in these pathological conditions.

In conclusion, our previous studies (Frew et al., 1993; AL-Zobaidy et al., 2010, 2011) revealed a seemingly anomalous ability of the methylarginines, L-NMMA and ADMA, to block basal activity of nitric oxide in rat aorta without affecting substantially relaxation to the powerful agonists, acetylcholine, ATP or calcium ionophore A23187. The present study, demonstrating the ability

of L-NMMA or ADMA to inhibit powerfully relaxation to the weak agonists, butyrylcholine or CGRP-1, or to acetylcholine when its efficacy was reduced by lowering the available pool of M_3 muscarinic receptors, provides an explanation for these earlier findings. Specifically, these new findings show there is an inverse relationship between the ability of L-NMMA or ADMA to block nitric oxide-mediated relaxation and the efficacy of the relaxing stimulus.

Acknowledgement

Dr. Mohammed AL-Zobaidy was a recipient of a PhD Scholarship funded by the Iraqi Ministry of Higher Education and Scientific Research.

References

AL-Zobaidy, M.J., Craig, J., Martin, W., 2010. Differential sensitivity of basal and acetylcholine-induced activity of nitric oxide to blockade by asymmetric dimethylarginine (ADMA) in the rat aorta. Br. J. Pharmacol. 160, 1476–1483.

AL-Zobaidy, M.J., Craig, J., Brown, K., Pettifor, G., Martin, W., 2011. Stimulus-specific blockade of nitric oxide-mediated dilatation by asymmetric dimethylarginine (ADMA) and monomethylarginine (L-NMMA) in rat aorta and carotid artery. Eur. J. Pharmacol. 673, 78–84.

Brinkmann, S.J.H., de Boer, M.C., Buijs, N., van Leeuwen, P.A.M., 2014. Asymmetric dimethylarginine and critical illness. Curr. Opin. Clin. Nutr. Metab. Care 17, 90–97.

Feng, Q.P., Lu, X.G., Fortin, A.J., Pettersson, A., Hedner, T., Kline, R.L., Arnold, J.M.O., 1998. Elevation of an endogenous inhibitor of nitric oxide synthesis in experimental congestive heart failure. Cardiovasc. Res. 37, 667–675.

Frew, J.D., Paisley, K., Martin, W., 1993. Selective inhibition of basal but not agonist-stimulated activity of nitric oxide in rat aorta by NG-monomethyl-L-arginine. Br. J. Pharmacol. 110, 1003–1008.

Furchgott, R.F., 1966. The use of ß-haloalkylamines in the differentiation of receptors and determination of dissociation constants of receptor-agonist complexes. Adv. Drug Res. 3, 21–55.

Gray, D.W., Marshall, I., 1992. Nitric oxide synthesis inhibitors attenuate calcitonin gene-related peptide endothelium-dependent vasorelaxation in rat aorta. Eur. J. Pharmacol. 212, 37–42.

Hobbs, A.J., Higgs, A., Moncada, S., 1999. Inhibition of nitric oxide synthase as a potential therapeutic target. Annu. Rev. Pharmacol. Toxicol. 39, 191–220.

Jin, J.S., D'Alecy, L.G., 1996. Central and peripheral effects of asymmetric dimethylarginine, an endogenous nitric oxide synthetase inhibitor. J. Cardiovasc. Pharmacol. 28, 439–446.

Liu, X., Gillespie, J.S., Gibson, I.F., Martin, W., 1991. Effects of N^G-substituted analogues of L-arginine on NANC relaxation of the rat anococcygeus and bovine retractor penis muscles and the bovine penile artery. Br. J. Pharmacol. 104, 53–58.

Martin, G.R., Bolofo, M.L., Giles, H., 1992. Inhibition of endothelium-dependent vasorelaxation by arginine analogs – a pharmacological analysis of agonist and tissue dependence. Br. J. Pharmacol. 105, 643–652.

Martin, W., Gillespie, J.S., Gibson, I.F., 1993. Actions and interactions of N^G-substituted analogues of L-arginine on NANC neurotransmission in the bovine retractor penis and rat anococcygeus muscles. Br. J. Pharmacol. 108, 242–247.

Martin, W., Villani, G.M., Jothianandan, D., Furchgott, R.F., 1986. Depression of contractile responses in rat aorta by spontaneously released endothelium-derived relaxing factor. J. Pharmacol. Exp. Ther. 237, 529–538.

Moore, P.K., al-Swayeh, O.A., Chong, N.W.S., Evans, R.A., Gibson, A., 1990. L-N^G-nitro-arginine (L-NOARG), a novel, L-arginine-reversible inhibitor of endothelium-dependent relaxation in vitro. Br. J. Pharmacol. 99, 408–412.

Olken, N.M., Marletta, M.A., 1993. N^G-monomethyl-L-arginine functions as an alternative substrate and mechanism-based inhibitor of nitric oxide synthase. Biochemistry 32, 9677–9685.

Overend, J., Martin, W., 2007. Differential effects of nitric oxide synthase inhibitors on endothelium-dependent and nitrergic nerve-mediated vasodilatation in the bovine ciliary artery. Br. J. Pharmacol. 150, 488–493.

Palmer, R.M.J., Ashton, D.S., Moncada, S., 1988. Vascular endothelial cells synthesise nitric oxide from L-arginine. Nature 333, 664–666.

Rees, D.D., Palmer, R.M.J., Hodson, H.F., Moncada, S., 1989. A specific inhibitor of nitric oxide formation from L-arginine attenuates endothelium-dependent relaxations. Br. J. Pharmacol. 96, 418–424.

Rees, D.D., Palmer, R.M.J., Schulz, R., Hodson, H.F., Moncada, S., 1990. Characterisation of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo. Br. J. Pharmacol. 101, 746–752.

Siroen, M.P.C., Teerlink, T., Nijveldt, R.J., Prins, H.A., Richir, M.C., Leeuwen, P.A.M., 2006. The clinical significance of asymmetric dimethylarginine. Ann. Rev. Nutr. 26, 203–228.

Stephenson, R.P., 1956. Modification of receptor theory. Br. J. Pharmacol. 11, 379–393.

Vallance, P., Leone, A., Calver, A., Collier, J., Moncada, S., 1992. Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. Lancet 339, 572–575.