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Responsiveness to noradrenaline in aorta from wild-type, nitric oxide synthase-2, nitric oxide synthase-3 and $\alpha_{2A/D}$-adrenoceptor knockout mice

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Abstract

We have investigated the responsiveness of mouse aorta to noradrenaline (10 μM). In wild-type mice, noradrenaline produced an initial peak contraction (3.35 ± 0.28 mN) and a significantly smaller plateau response (2.15 ± 0.41 mN). The contractions were similar in aorta from nitric oxide synthase-2 (NOS-2) knockout mice. In vessels from NOS-3 knockout mice, noradrenaline contractions consisted of an early steeply rising phase with a later shallow rising phase to a maximum (10.21 ± 0.84 mN), which was significantly greater than in wild-type and NOS-2 knockout mice, and resembled the contraction to phenylephrine (10 μM) in wild-type. In $\alpha_{2A/D}$-adrenoceptor knockout mice, the noradrenaline maximum was significantly smaller than in NOS-3 knockout but significantly larger than in wild-type. Following N^G-nitro-L-arginine methyl ester (L-NAME, 10 μM), responses in wild-type and $\alpha_{2A/D}$-adrenoceptor knockout were as in NOS-3 knockout mice. The α_{2D}-adrenoceptor antagonist BRL 44408 (2-((4,5-dihydro-1*H*-imidazole-2-yl)methyl)-2,3-di-hydro-1-methyl-1*H*-isoindole maleate; 1 μM) increased noradrenaline-induced contractions and the α_2-adrenoceptor agonist xylazine reduced Prostaglandin $F_{2\alpha}$-induced contractions, in wild-type but not NOS-3 knockout. Contractions to noradrenaline in mouse aorta are modulated by NOS-3 and part of the effect involves activation of $\alpha_{2A/D}$-adrenoceptors.

Keywords: Nitric oxide (NO); NOS-2 knockout; NOS-3 knockout; $\alpha_{2A/D}$-Adrenoceptor knockout; L-NAME (N^G-nitro-L-arginine methylester)

1. Introduction

Contractions of blood vessels to vasoconstrictors such as noradrenaline are influenced by endothelial-derived modulators such as nitric oxide (NO: Furchgott and Zawadzki, 1980; Palmer et al., 1987; Taylor and Weston, 1988), prostacyclin (Moncada and Vane, 1979) and endothelium-derived hyperpolarizing factor (EDHF: Taylor and Weston, 1988; Komori et al., 1988; Edwards et al., 1998). EDHF has a major role in resistance arteries, but NO predominates in large arteries (Garland and McPherson, 1992; Garland et al., 1995). Of the three isoforms of nitric oxide synthase, neuronal (NOS-1), inducible (NOS-2) and endothelial (NOS-3) (Alderton et al., 2001), only NOS-2 and NOS-3 are likely to be of importance in blood vessels (Briones et al., 2000).

Although removal of the endothelium or addition of nitric oxide synthase inhibitors (Martin et al., 1986; Rees et al., 1990) have been shown to increase vasoconstrictor responses, presumably by inhibition of basal or stimulated release of NO, knockout technology may give a more useful strategy to investigate the roles of NOS enzymes in modulation of contractions.

Noradrenaline acts on three subtypes of α_1-adrenoceptor (α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors; Heible et al., 1995) and three subtypes of α_2-adrenoceptor (α_{2A}-, α_{2B}- and α_{2C}-adrenoceptors: the rat α_{2D}-adrenoceptor is a species orthologue of the human α_{2A}-adrenoceptor; see Docherty, 1998; Guimaraes and Moura, 2001). Noradrenaline is known to modulate vasoconstriction by acting on endothelial α_2-adrenoceptors to produce the release of endothelium-derived relaxant factor (EDRF: Feres et al., 1998; Ohgushi et al., 1993; Thorin et al., 1997). Bockman et al. (1996) suggested

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the involvement of the $\alpha_{2A/D}$-adrenoceptor subtype in this effect. In addition, other α_1- and α_2-adrenoceptor subtypes are localized either on smooth muscle cells, endothelium or adventitia and could be part of these actions of noradrenaline (Docherty, 1998; Faber et al., 2001). Indeed, we have recently found in investigations of endothelium-denuded aorta from wild-type and $\alpha_{2A/D}$-adrenoceptor knockout mice that an $\alpha_{2A/D}$-adrenoceptor exerts an inhibitory modulation of contraction in wild-type mouse (Vandeputte and Docherty, 2002a). Therefore, the endothelium may not be the only site of modulation of contractions to noradrenaline.

In this study, we have compared aorta from wild-type, NOS-2, NOS-3 and $\alpha_{2A/D}$-adrenoceptor knockout mice in contractions to noradrenaline. Some of these results have been published in abstract form (Vandeputte and Docherty, 2002b).

2. Methods

2.1. Animals

Male wild-type and NOS-2, NOS-3 and homozygous $\alpha_{2A/D}$-adrenoceptor knockout C57 black mice (18–28 g) were obtained from Jackson Laboratories (Bar Harbor, ME, USA) and the aorta was used as outlined below. The studies conform to the Declaration of Helsinki and have been approved by the Department of Health and by the RCSI Research Ethics Committee.

2.2. Mouse aorta

Thoracic aortic rings, 2–3 mm in length, were mounted in a small vessel myograph with 40 μm tungsten wires (Mulvany and Warshaw, 1977), for the recording of isometric contractions. Data were recorded on a dual channel electronic display recorder (Myo-Interface Model 400A) and analog acquisition system (MacPacq. MP100, Biopac Systems). Vessels were allowed to equilibrate at 37 °C in Krebs–Henseleit solution (95% O_2/5% CO_2) of the following composition (mM): NaCl 119, $NaHCO_3$ 25, glucose 11.1, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.0, EDTA 0.03 and ascorbic acid 0.28. Propanolol (3 μM) was also present. The vessel was set to a tension generated at 0.9 times the diameter of the vessel at 100 mm Hg transmural pressure (Mulvany and Warshaw, 1977). Arteries were allowed to equilibrate for 30 min under this passive tension.

In preliminary experiments, it was observed that contractile responses to phenylephrine (10 μM) and noradrenaline (10 μM) were markedly different in aorta from wild-type mice. The response to phenylephrine (10 μM) was a well-maintained contraction, but the response to noradrenaline (10 μM) consisted of an initial transient peak contraction falling to a smaller plateau. This difference caused us to choose noradrenaline (10 μM) for further investigations.

In the first set of experiments, tissues were challenged with phenylephrine (1 nM–10 μM). At the plateau of contraction induced by phenylephrine (10 μM), acetylcholine (10 μM) was added to the bath in order to test the presence of functional endothelium. Tissues from all groups except NOS-3 knockout mice relaxed to acetylcholine (data not shown). Tissues were then washed with Krebs–Henseleit solution for 60 min during which the bath was changed every 15 min. Tissues were then contracted with noradrenaline (10 μM).

In a second set of experiments, a similar protocol to the above was used. However, the NOS inhibitor N^G-nitro-L-arginine methylester (L-NAME, 10 μM), the α_{2D}-adrenoceptor selective antagonist BRL 44408 (1 μM) or vehicle was added 15 min before contracting with noradrenaline. Two segments from the same vessel were studied simultaneously; one was treated with the drug and the other received the vehicle.

In a third set of experiments, a similar protocol was used except that after arteries were mounted and allowed to equilibrate for 30 min, they were stimulated with phenylephrine (10 μM) and acetylcholine (10 μM) was added at the plateau of contraction to test the endothelial function. Segments were then washed for another 60 min and stimulated with noradrenaline (10 μM) or prostaglandin $F_{2\alpha}$ (10 μM). At the plateau of contraction, cumulative concentrations of the α_2-adrenoceptor agonist xylazine (10 nM to 1 μM) (prostaglandin $F_{2\alpha}$ contraction), or the nonselective α_2-adrenoceptor antagonist yohimbine (noradrenaline contraction) or vehicle were added to the bath.

In a fourth set of experiments, vessels from wild-type mice only were used and the same protocol as the first series was applied. After the noradrenaline (10 μM) stimulation, segments were washed for another 60 min. The calcium channel inhibitor nifedipine (10 μM), or L-NAME (10 μM) and nifedipine (10 μM), or vehicle were added to the bath 15 min before further stimulation with noradrenaline (10 μM).

2.3. Drugs

Acetylcholine chloride (Sigma, Poole, UK), L-NAME (Research Biochemicals, Natick, USA), nifedipine, noradrenaline bitartrate, phenylephrine hydrochloride, propranolol hydrocholoride, prostaglandin $F_{2\alpha}$, xylazine and yohimbine were purchased from Sigma, Dublin, Ireland. BRL 44408 (2-((4,5-dihydro-1*H*-imidazole-2-yl)methyl)-2,3-di-hydro-1-methyl-1*H*-isoindole maleate: Tocris, Avonmouth, UK).

Drugs were dissolved in distilled water except nifedipine and prostaglandin $F_{2\alpha}$, which were first dissolved in 100% ethanol to give a stock solution of 10 mM. Final percentage of ethanol in the organ bath was 0.1.

2.4. Statistics

Values are mean ± S.E.M. from *n* experiments. Vehicle data from L-NAME, BRL 44408 and nifedipine experiments

were combined in Fig. 2. Contractions were compared between groups by analysis of variance for multiple comparisons. Intergroup data were compared with the Student's *t*-test for unpaired data and intragroup data were compared by the Student's *t*-test for paired data. Statistical analysis was carried out using Instat for Macintosh.

3. Results

Noradrenaline (10 μM)-induced biphasic contractions, which were similar in shape and magnitude in wild-type and NOS-2 knockout mice, consisting of an early peak contraction at 13 ± 1 and 16 ± 2 s (nonsignificant) in wild-type and NOS-2 knockout mice, respectively (3.35 ± 0.28 mN, $n = 19$ and 3.20 ± 0.53 mN, $n = 8$, respectively) followed by a decrease in tension stabilizing at a plateau (Figs. 1 and 2). Noradrenaline-induced contractions of aorta from NOS-3 and $\alpha_{2A/D}$-adrenoceptor knockout mice were much more monophasic consisting of an early steep rise in tension followed by a later slow rise, although there was a clear early peak contraction at 18 ± 1 s ($P < 0.05$ vs. wild-type) in tissues from $\alpha_{2A/D}$-adrenoceptor knockout, which was absent from NOS-3 knockout (Fig. 1). The magnitude of this early peak contraction in tissues from $\alpha_{2A/D}$-adrenoceptor knockout mice (and the response at 16 s time point in tissues from NOS-3 knockout animals) was not significantly different from the peak contraction in wild-type or NOS-2 knockout mice (Figs. 1 and 2).

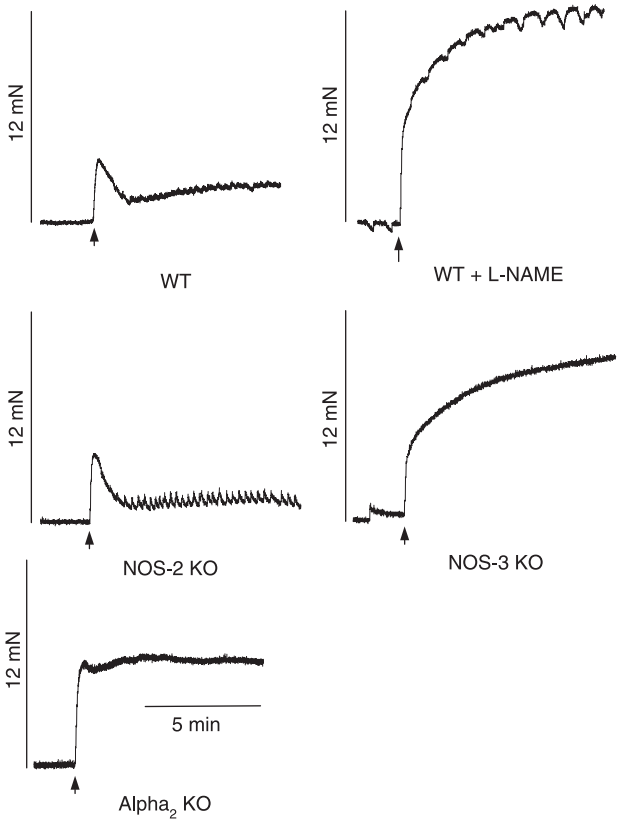

Fig. 1. Typical original recordings showing the effects of noradrenaline (10 μM) on isometric tension in aorta from wild-type (in absence or presence of L-NAME; WT and WT + L-NAME), NOS-2 knockout (NOS-2 KO), NOS-3 knockout (NOS-3 KO) and $\alpha_{2A/D}$-adrenoceptor knockout (α_2-KO) mice. Tension and time calibrations are shown.

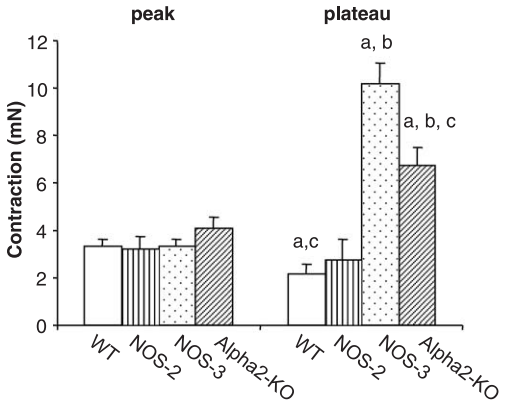

Fig. 2. Peak and plateau values of noradrenaline (10 μM)-induced increase in isometric tension in aorta from wild-type (WT, $n = 19$), NOS-2 knockout (NOS-2, $n = 8$), NOS-3 knockout (NOS-3, $n = 12$) and $\alpha_{2A/D}$-adrenoceptor knockout (α_2-KO, $n = 13$) mice. Vertical bars represent S.E. of mean. For NOS-3, in which there was no early peak contractile response, the response at the approximate time of peak contraction in wild-type was measured (see Fig. 1). Letters denote significance of difference between peak contraction and plateau contractions within groups and between wild-type and other groups (a: $P < 0.05$ vs. peak contraction in the same group, b: $P < 0.05$ vs. WT plateau, c: $P < 0.05$ vs. NOS-3 plateau).

In aorta from NOS-3 knockout and $\alpha_{2A/D}$-adrenoceptor knockout mice, the plateau contraction and indeed maximum contraction to noradrenaline was significantly increased compared to wild-type mice (Fig. 2). Maximal noradrenaline-induced maintained contractions were 2.15 ± 0.41 ($n = 19$), 10.21 ± 0.84 ($n = 12$) and 6.72 ± 0.76 mN ($n = 13$), respectively, in tissues from wild-type, NOS-3 and $\alpha_{2A/D}$-adrenoceptor knockout mice, respectively ($P < 0.05$ from wild-type). The plateau response was significantly smaller than the peak contraction in tissues from wild-type, but significantly greater than the early peak contraction (or equivalent time point) in tissues from NOS-3 and $\alpha_{2A/D}$-adrenoceptor knockout mice (Fig. 2). In NOS-2, the plateau response was not significantly different from the early peak contraction (Fig. 2).

On the contrary, the α_1-adrenoceptor selective agonist, phenylephrine (10 μM) induced a monophasic-shaped contraction in aorta from wild-type, NOS-3 knockout and $\alpha_{2A/D}$-adrenoceptor knockout mice (Fig. 3). The maximal response to phenylephrine (10 μM) was 2.86 ± 0.44 ($n = 9$), 5.78 ± 1.02 ($n = 5$, $P < 0.05$ vs. wild-type) and 3.40 ± 0.47 mN ($n = 7$, n.s. vs. wild-type) in wild-type, NOS-3 knockout and $\alpha_{2A/D}$-adrenoceptor knockout mice, respectively. Hence, NOS-3 knockout, but not $\alpha_{2A/D}$-adrenoceptor knockout, increased the maximum response to phenylephrine.

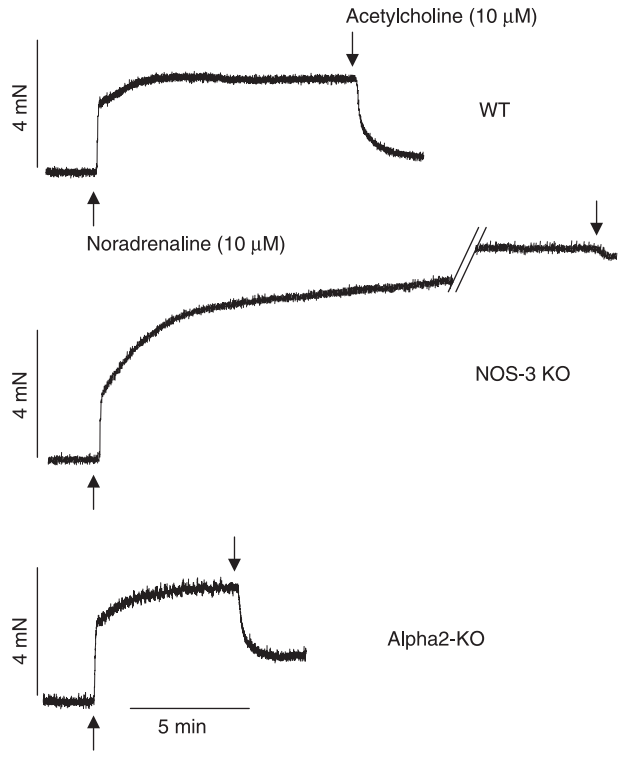

Fig. 3. Typical original recordings showing the effects of phenylephrine (10 μM) (in each case the first arrow) and acetylcholine (10 μM) (in each case the second arrow) on isometric tension in aorta from wild-type (WT), NOS-3 knockout (NOS-3 KO) and $\alpha_{2A/D}$-adrenoceptor knockout (α_2-KO) mice. Acetylcholine was added when the contraction had stabilized, which took much longer in aorta from NOS-3 KO (note break in recording timescale; acetylcholine was injected at approximately 40 min after addition of phenylephrine). Tension and time calibrations are shown.

L-NAME (10 μM) treatment in wild-type and $\alpha_{2A/D}$-adrenoceptor knockout mice had no effect on the amplitude of early peak contraction but significantly increased the maximum contraction (12.16 ± 1.61 mN, $n=6$ and 12.60 ± 0.98 mN, $n=7$, respectively, $P<0.05$ vs. vehicle, Fig. 4). L-NAME (10 μM) also altered the shape of

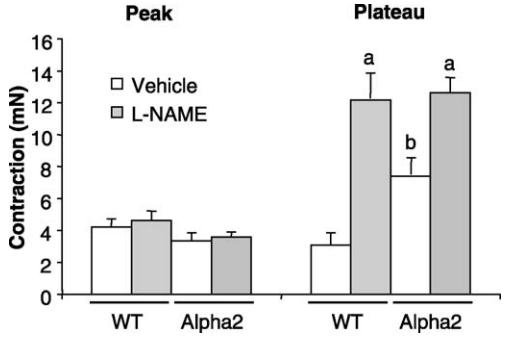

Fig. 4. Effect of L-NAME (10 μM) on peak and plateau values of noradrenaline (10 μM)-induced contraction in aorta from wild-type (WT, $n=5–6$) and $\alpha_{2A/D}$-adrenoceptor knockout (α_2, $n=7$) mice. Vertical bars represent S.E. of mean. a: $P<0.05$ vs. respective vehicle, b: $P<0.05$ vs. WT receiving the same treatment.

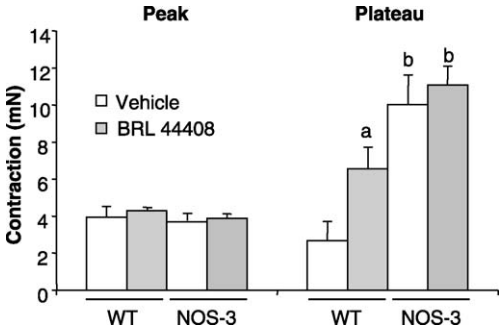

Fig. 5. Effect of the α_{2D}-adrenoceptor selective antagonist BRL 44408 (1 μM) on peak and plateau values of noradrenaline (10 μM)-induced contraction in aorta from wild-type (WT, $n=5$) and NOS-3 knockout (NOS-3, $n=5$) mice. Vertical bars represent S.E. of mean. a: $P<0.05$ vs. respective vehicle, b: $P<0.05$ vs. WT receiving the same treatment.

contraction so that it resembled that found in NOS-3 knockout mice (Fig. 1). The maximum responses after treatment with L-NAME were not significantly different from each other and from that found in NOS-3 knockout in the absence of L-NAME (10.21 ± 0.84 mN, $n=12$) (compare Figs. 2 and 4).

In wild-type mice, the $\alpha_{2A/D}$-adrenoceptor selective antagonist BRL 44408 (1 μM) significantly increased maximal contraction induced by noradrenaline (10 μM) but had no effect on the early peak of contraction (Fig. 5). On the contrary, BRL 44408 treatment in NOS-3 knockout mouse aorta had no effect on noradrenaline-induced contraction (Fig. 5). However, after BRL 44408 treatment, the maximal contraction of aorta from wild-type mice (6.54 ± 1.18 mN, $n=5$) was still significantly smaller than that from NOS-3 knockout mice in the absence or presence of BRL 44408 (10.01 ± 1.62 mN, $n=5$ and 11.07 ± 1.04 mN, $n=5$, respectively, $P<0.05$, Fig. 5).

Prostaglandin $F_{2\alpha}$ induced similar contraction in aorta from wild-type and NOS-3 knockout mice (12.28 ± 1.66 mN, $n=5$ and 14.52 ± 0.89 mN, $n=4$, respectively, $P>0.05$). The α_2-adrenoceptor agonist xylazine (1 μM)

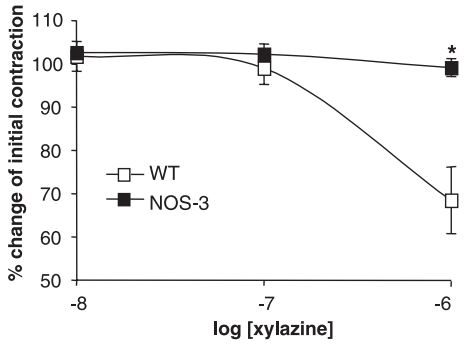

Fig. 6. Effect of the addition of increasing concentrations of the α_2-adrenoceptor agonist xylazine (10 nM to 1 μM) on $PGF_{2\alpha}$ (10 μM)-induced contraction of wild-type ($n=5$) and NOS-3 knockout ($n=4$) mice aorta. Vertical bars represent S.E. of mean. *$P<0.05$ vs. WT.

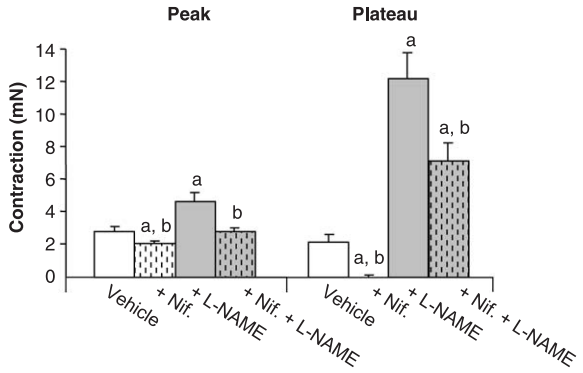

Fig. 7. Effects of vehicle (veh, $n=5$), nifedipine (10 μM) (+Nif.), L-NAME (10 μM) (+L-NAME, $n=6$) and nifedipine (10 μM) plus L-NAME (10 μM) (+Nif.+L-NAME) on peak and plateau values of the contraction induced by noradrenaline (10 μM) in aorta from wild-type mice. a: $P<0.05$ vs. vehicle, b: $P<0.05$ vs. L-NAME alone.

induced a relaxation of Prostaglandin $F_{2\alpha}$-induced tone in wild-type but not in NOS-3 knockout mouse aorta (Fig. 6). Xylazine had no effect at concentrations less than 1 μM.

The nonselective α_2-adrenoceptor antagonist yohimbine (10 nM to 1 μM) had no effect on noradrenaline-induced contractions in $\alpha_{2A/D}$-adrenoceptor knockout mouse aorta. In presence of yohimbine (1 μM), the contraction to noradrenaline (10 μM) was 104 ± 3% ($n=6$) of the initial contraction, which was not significantly different from the 106 ± 8 % ($n=6$) observed with vehicle.

Nifedipine (10 μM) significantly inhibited both the early peak and plateau of noradrenaline-induced contraction in tissues from wild-type but had a more marked effect on the plateau (Fig. 7). In wild-type in the presence of L-NAME (10 μM) and nifedipine (10 μM), noradrenaline (10 μM) produced a maximum contraction (7.13 ± 1.13 mN, $n=5$), which was significantly less than in wild-type treated with L-NAME alone (12.16 ± 1.61 mN, $n=6$) (Fig. 7).

4. Discussion

In this study, we have examined how deletion of NOS-2 or NOS-3 enzymes or $\alpha_{2A/D}$-adrenoceptors modulates responsiveness of mouse aorta to noradrenaline.

In wild-type mouse aorta, noradrenaline (10 μM), but not phenylephrine (10 μM), induced an initial peak of contraction followed by a decrease in tension stabilizing at a plateau of small amplitude whereas in NOS-3 knockout mice, noradrenaline induced an initial fast increase in tension rising further to a high amplitude plateau, which resembled the contraction to phenylephrine (10 μM) in wild-type in shape but not magnitude. Thus, we can assume that noradrenaline acts on membrane receptors on both smooth muscle cells (α_1-adrenoceptors mediating contraction) and endothelial cells (α_2-adrenoceptors and possibly α_1-adrenoceptors, mediating NO release and relaxation), with a shorter time-course for the signal transduction leading to the early peak contraction. The decrease in tension following the peak contraction is probably due to the release of NO from endothelial cells and is the result of the equilibrium between the NO release to inhibit smooth muscle contraction and the direct smooth muscle cell contraction. Noradrenaline presumably directly stimulates NOS-3 by action at α_2-adrenoceptors and/or α_1-adrenoceptors. However, the differences between the effects of NOS-3 knockout and $\alpha_{2A/D}$-adrenoceptor knockout on the responses to noradrenaline and the selective α_1-adrenoceptor agonist phenylephrine suggest two components to the stimulation of NOS-3. The first component of the stimulation of NOS-3 by noradrenaline involves $\alpha_{2A/D}$-adrenoceptors. The second component of the stimulation of NOS-3 by noradrenaline and the phenylephrine-mediated component presumably involve α_1-adrenoceptors. These two components will be discussed below.

Endothelium-dependent relaxation in response to endothelial α_2-adrenoceptor stimulation has been demonstrated in both resistance and capacitance vessels such as cerebral, mesenteric or carotid arteries from different species (Feres et al., 1998; Ohgushi et al., 1993; Thorin et al., 1997). In rat aorta, contractions to noradrenaline and specific α_2-adrenoceptor agonists are increased in absence of endothelium or when arteries are treated with NOS inhibitors (Carrier and White, 1985; Kaneko and Sunano, 1993). Furthermore, α_2-adrenoceptor specific agonists were shown to relax phenylephrine precontracted rat aorta, this effect being antagonized by yohimbine (Nomura et al., 1995). Taken together, these results suggest the existence of an endothelial α_2-adrenoceptor involved in the release of endothelium-dependent relaxing factor (EDRF or NO). Kim et al. (1999) also suggested that α_2-adrenoceptors mediate both an endothelium-dependent, and endothelium−independent relaxation of rat aorta, through the release of NO and the opening of glibenclamide sensitive potassium channels in smooth muscle cells respectively. In mouse blood vessels, previous studies have also shown an increase in the maximal response to phenylephrine after endothelium removal but the extent of the amplification was small, a 20−25% increase (Hussain et al., 1999; Waldron et al., 1999). Contractions to noradrenaline (3 μM) were significantly increased by about 40% in mesenteric arteries from NOS-3 knockout (no mention of changes in aorta) (Chataigneau et al., 1999). In addition, we have recently shown that, in mouse aorta without endothelium, contractions to noradrenaline are increased by knockout of $\alpha_{2A/D}$-adrenoceptors or by α_2-adrenoceptor antagonists and unaffected by the potassium channel blocker glibenclamide (Vandeputte and Docherty, 2002a), and this action may also contribute to the actions of noradrenaline in the present study. In rat mesenteric arteries and pig coronary arteries, the $\alpha_{2A/D}$-adrenoceptor was shown to be involved in NO release (Bockman et al., 1996). In that study, the authors used relative affinities to various agonists and antagonists. However, the α_2-adreno-

ceptor subtype involved in such response in a large vessel, as the aorta, remained to be identified.

Hence, we decided to study endothelium-intact aorta from $\alpha_{2A/D}$-adrenoceptor knockout mice. In aorta from this group of mice, contractions to noradrenaline showed a biphasic shape with a peak contraction followed by a small decrease in tension and a plateau significantly different from both wild-type and NOS-3 knockout. These results suggest that at least part of the activation of NOS-3 involves $\alpha_{2A/D}$-adrenoceptors: these may be at least partly non-endothelial in location (Vandeputte and Docherty, 2002a). However, $\alpha_{2A/D}$-adrenoceptor knockout had a greater effect on noradrenaline contractions in aorta with intact endothelium (approximately 200% increase in response to noradrenaline 10 μM, present results) than in endothelium-denuded aorta (approximately 75% increase; Vandeputte and Docherty, 2002a). Endothelial and non-endothelial $\alpha_{2A/D}$-adrenoceptors are likely to be involved in activation of NOS-3.

As has been shown in other tissue such as the mouse vas deferens, the deletion of an α_2-adrenoceptor subtype gene could lead to the expression of another subtype (Cleary et al., 2002). Therefore, we investigated the effect of the nonselective α_2-adrenoceptor antagonist yohimbine on noradrenaline-induced contractions of endothelium intact aorta from $\alpha_{2A/D}$-adrenoceptor knockout mice. We did not observe any effect of this antagonist suggesting that the $\alpha_{2A/D}$-adrenoceptor is the only subtype involved in the α_2-adrenoceptor component of the noradrenaline signal in the mouse aorta.

To confirm the role of NOS-3 in modulation of contractions to noradrenaline involving $\alpha_{2A/D}$-adrenoceptors, we also assessed the actions of the nonselective NOS inhibitor, L-NAME. Following L-NAME treatment, the contractile response to noradrenaline in wild-type and $\alpha_{2A/D}$-adrenoceptor knockout mice most closely resembled that of the NOS-3 knockout mice. Furthermore, the α_{2D}-adrenoceptor antagonist BRL 44408 had no effect on noradrenaline-induced contractions of NOS-3 knockout mice, although, in wild-type mice, this drug produced an increase in the magnitude of the contraction, which was then similar to the amplitude of noradrenaline-induced contraction observed in $\alpha_{2A/D}$-adrenoceptor knockout mice. The absence of xylazine-induced relaxation of prostaglandin $F_{2\alpha}$ precontracted arteries in NOS-3 knockout mice also confirm the activation of NOS-3 enzyme by one of the α_2-adrenoceptor subtypes. These data also demonstrate that all the actions of noradrenaline at α_2-adrenoceptors to modulate contraction seem to involve NOS-3. Indeed, the fact that the contraction induced by the α_1-adrenoceptor selective agonist phenylephrine (10 μM) was similar in shape, but smaller in amplitude, to that observed to noradrenaline in NOS-3 knockout mice (see Figs. 1 and 3), also suggests that α_2-adrenoceptor-mediated activation of NOS is the most prominent.

However, α_2-adrenoceptors are not the sole mediator of NO release evoked by noradrenaline in mouse aorta. The additional effect of noradrenaline on NOS may involve α_1-adrenoceptors rather than tonic release of NO due to the contraction, given that the contraction to prostaglandin $F_{2\alpha}$ was not modified by NOS-3 knockout. Phenylephrine produced monophasic contractions which were unaffected by $\alpha_{2A/D}$-adrenoceptor knockout, demonstrating the α_1-adrenoceptor selectivity of the agonist. However, NOS-3 knockout significantly increased the maximum to phenylephrine suggesting that an α_1-adrenoceptor is also involved in release of NO. Since contractions to noradrenaline and phenylephrine in mouse aorta involve mainly α_1-adrenoceptors, it may be difficult to investigate α_1-adrenoceptor subtype-mediated actions on NOS. Certainly, contractions to α_1-adrenoceptor agonists involve mainly α_{1D}-adrenoceptors in both rat (Hussain and Marshall, 1997) and mouse (Yamamoto and Koike, 2001) thoracic aorta.

In wild-type mouse aorta, nifedipine inhibited the peak of noradrenaline-induced contraction and virtually abolished the plateau. This is in accordance with previous studies (Heaslip and Rahwan, 1983; Scarborough and Carrier, 1984) showing that the early and fast component of the noradrenaline-induced contraction in rat aorta is mainly dependent on intracellular Ca^{2+} release, whereas the slow and tonic contraction is highly dependent on extracellular Ca^{2+} entry. However, L-NAME made the plateau response to NA much more resistant to nifedipine, so that the plateau response behaved more like the peak contraction: small inhibition by nifedipine. These results may simply demonstrate that NO largely cancels out the effects of mobilisation of intracellular Ca^{2+}, leaving a response largely dependent on extracellular Ca^{2+}, or that NO affects Ca^{2+} stores or Ca^{2+}-independent mechanisms of the contraction.

Deletion of NOS-3, but not NOS-2, results in a significant increase in blood pressure (Shesely et al., 1996; Bian et al., 2001). This result is in accordance with the absence of significant effect of the deletion of NOS-2 gene on noradrenaline-induced contraction in the present study, compared to the increase in response in NOS-3 knockout mouse aorta.

The location of the NOS may be mainly endothelial in the case of NOS-3, but smooth muscle and even adventitial locations of NOS-3 and NOS-2 must be considered (Kleschyov et al., 2000). Evidence for constitutive expression of NOS-2 in vascular tissue is sparse, although it is constitutively expressed in some other tissues (Jablonka-Shariff and Olson, 1997; Starkey et al., 2001). Since we found no evidence for any effect of NOS-2 deletion in our studies, it seems that NOS-2 is not induced in vitro under our experimental conditions. However, some of the $\alpha_{2A/D}$-adrenoceptor-mediated actions to release NO, presumably involving NOS-3, are non-endothelial (Vandeputte and Docherty, 2002a,b). Both α_1- and $\alpha_{2A/D}$-adrenoceptors-mediated activation of NOS-3 appear to be specific actions and not an indirect consequence of the smooth muscle contraction (see above).

It is concluded that contractions to noradrenaline in mouse aorta are modified by NOS-3 and that part of the

effect may involve the activation of $\alpha_{2A/D}$-adrenoceptors, with a possible lesser role for α_1-adrenoceptors.

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