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α_2-Adrenoceptor and NPY receptor-mediated contractions of porcine isolated blood vessels: evidence for involvement of the vascular endothelium

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1 Enhanced contractions to the α_2-adrenoceptor agonist UK14304 and neuropeptide Y (NPY) in the porcine ear artery can be uncovered by pharmacological manipulation. The aim of this study was to determine whether similar pharmacological manipulation can uncover enhanced contractions in the porcine splenic artery, and to determine whether the endothelium modulates these responses.

2 UK14304 (0.3 μM) and NPY (0.1 μM) produced small contractions of the porcine splenic artery. After pre-contraction of the tissue with U46619, followed by relaxation with forskolin, the responses to both UK14304 and NPY were enhanced. Enhanced contractions to both UK14304 and NPY were also obtained after relaxation with SNP. These results demonstrate that, as in the porcine ear artery, α_2-adrenoceptors and NPY receptors are able to produce enhanced contractile responses through both adenylyl cyclase-dependent and -independent signal transduction pathways.

3 Removal of the endothelium had no significant effect on responses to UK14304 either alone or in the presence of U46619 and forskolin in the porcine splenic artery. On the other hand, responses to UK14304 after relaxation with SNP were reduced after endothelium-denudation in both the porcine splenic artery and ear artery. Similar results were obtained with NPY in the porcine ear artery.

4 In conclusion, enhanced contractile responses to UK14304 and NPY in the porcine splenic artery can be uncovered using methods similar to those employed in the porcine ear artery. Under certain conditions the responses to both agents are modulated by the endothelium. These data highlight further the similarities in the signal transduction pathways used by both α_2-adrenoceptors and NPY receptors to induce vasoconstriction.

Keywords: Neuropeptide Y; porcine splenic artery; porcine ear artery; vasoconstriction; cyclic AMP; sodium nitroprusside; UK14304; α_2-adrenoceptors; endothelium

Abbreviations: ANOVA, analysis of the variance; NPY, neuropeptide Y; SNP, sodium nitroprusside; UK14304, 5-bromo-6-[2-imidazolin-2-ylamine]-quinoxaline bitartrate

Introduction

Neuropeptide Y (NPY) and α_2-adrenoceptor agonists are potent pressor agents *in vivo* (Faber, 1988; Malmstrom *et al*., 1997; Zukowska-Grojec *et al*., 1987). However, vascular responses to these agents are difficult to study in isolated arteries. This is because either the receptors exist on arteriolar blood vessels which are difficult to study *in vitro* (Nielsen *et al*., 1989; Faber, 1998), or because activation of the receptors produces small, or non-existent responses (Abel & Han, 1989; Han & Abel, 1987; Wahlestedt *et al*., 1985; Xia *et al*., 1992). However, both agents appear to interact with other vasoconstrictors, possibly at the second messenger level. These interactions lead to either potentiation of the response induced by the vasoconstrictor (Abel & Han, 1989; Han & Abel, 1987; Wahlestedt *et al*., 1985; Xia *et al*., 1992; Cressier *et al*., 1995), or enhancement of the NPY receptor or α_2-adrenoceptor-mediated response (Sulpizio & Hieble, 1987; Dunn *et al*., 1989; Templeton *et al*., 1989; Aidulis *et al*., 1991; Grundemar & Hogestatt, 1992).

Previous studies of the porcine splenic artery have demonstrated the presence of α_2-adrenoceptor binding sites, albeit in relatively low density (Wright *et al*., 1995). α_2-Adrenoceptor agonists produce only a small contractile response suggesting that the α_2-adrenoceptors in the porcine splenic artery are functionally insignificant (Wright *et al*., 1995; Barbieri *et al*., 1998). Furthermore, pre-contraction of the tissue with a low concentration of phenylephrine failed to enhance contractions to the selective α_2-adrenoceptor agonist B-HT 920 (Barbieri *et al*., 1998), suggesting that, unlike other tissues, there is no interaction between α_2-adrenoceptor agonists and other vasoconstrictors. Similarly, the presence of a vasoconstrictor alone also failed to enhance the α_2-adrenoceptor-mediated vasoconstriction in the porcine isolated ear artery (Roberts *et al*., 1998). On the other hand, pre-contraction of the ear artery with the thromboxane-mimetic U46619, followed by relaxation back to baseline with the adenylyl cyclase activating agent forskolin, revealed a large α_2-adrenoceptor-mediated response (Roberts *et al*., 1998). Similar pharmacological manipulation also enhances responses to NPY in the porcine ear artery (Roberts *et al*., 1999). The enhanced responses under these conditions are believed to be due to a disinhibition of the U46619 contraction by a reduction of cyclic AMP levels i.e. an adenylyl cyclase-dependent pathway (Roberts *et al*., 1998; 1999). There is, however, evidence for a second intracellular signalling pathway in the porcine ear artery. Relaxation of the tissues with either sodium nitroprusside (SNP) or dibutyryl cyclic AMP after pre-contraction with U46619 also leads to enhanced contractile responses to both NPY and UK14304. The enhanced responses obtained under these conditions appear to be mediated through a pathway independent of an inhibition of adenylyl cyclase (Roberts *et al*., 1998; 1999). It

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is not clear whether these two different pathways are important for α_2-adrenoceptor or NPY receptor-mediated vasoconstriction in general, or are an anomaly of the porcine ear artery.

Another reason why α_2-adrenoceptor-mediated contractions are not easily observed *in vitro* is that the vascular endothelium has an inhibitory influence on the underlying smooth muscle. In some vessels removal of the vascular endothelium enhances the α_2-adrenoceptor-mediated vasoconstriction (MacLean *et al*., 1993; Miller *et al*., 1991). These blood vessels are believed to contain α_2-adrenoceptors on both the endothelium and the smooth muscle cells. The endothelial α_2-adrenoceptors mediate relaxation, possibly through the release of relaxing factors (Bullock *et al*., 1986; Bockman *et al*., 1993), whereas the smooth muscle α_2-adrenoceptors mediate constriction. In the presence of the endothelium there is a balance between the two sets of receptors such that there is only a small, or non-existent contraction. Denudation of the endothelium removes the relaxation response, resulting in a larger contraction. Therefore it is possible that the endothelium has a modulatory role in the porcine splenic artery and ear artery, such that its removal may further enhance α_2-adrenergic and NPY responses.

The aims of this study were to determine whether enhanced contractile responses to the α_2-adrenoceptor agonist UK14304 or NPY could be uncovered in the porcine splenic artery and to determine the involvement of the vascular endothelium in these responses.

Methods

Functional studies

Isometric tension recordings Porcine spleens and ears were obtained from a local abattoir and transported to the laboratory on ice. Spleens were transported in ice-cold Krebs-Henseleit buffer. Splenic arteries and ear arteries were dissected out and placed in Krebs-Henseleit buffer containing 2% ficoll, which had been pre-gassed with 95% O_2/5% CO_2, and stored overnight at 4°C (see Wright *et al*., 1995). The following day splenic arteries and ear arteries were carefully cleaned of fat and connective tissue, dissected into 5 mm ring segments, and suspended in 5 ml isolated organ baths containing Krebs-Henseleit buffer maintained at 37°C and constantly gassed with 95% O_2/5% CO_2. The lower support was fixed and the upper support was connected to a force transducer (World Precision Instruments, Sarasota, Florida, U.S.A.) linked to a MacLab data acquisition system (AD Instruments Ltd., Hastings, U.K.) *via* an amplifier. After a 20 min equilibration period, tension was applied to the tissue which was allowed to relax to a final resting tension of between 2–3 g wt^{-1} (splenic artery) or 1–1.5 g wt^{-1} (ear artery). Before each experiment the tissues were contracted 2–3 times with 60 mM KCl, until the final two responses differed by less than 10%. Between each response, tissues were washed three times with Krebs-Henseleit buffer and allowed to recover for 20 min. Control tissues were segments from the same piece of artery and were treated with UK14304 or NPY alone.

In some experiments the endothelium was removed from the blood vessel segments by rubbing the lumenal surface with a fine pair of forceps. The segments were set up in the organ bath as described above. Removal of the endothelium was confirmed by the inability of the tissue to relax to 0.1 μM substance P. Responses in endothelium-denuded segments were compared with responses in endothelium-intact segments from the same piece of artery.

Responses to α_2-adrenoceptor agonists or NPY in porcine isolated splenic artery Porcine isolated splenic arteries were pre-contracted with the thromboxane-mimetic U46619 (10 nM to 0.1 μM) to produce a contraction which was approximately 70–80% of the 60 mM KCl response. Tissues were then relaxed back to baseline (<10% of 60 mM KCl response) with either forskolin (1–3 μM), or sodium nitroprusside (SNP) (1–10 μM). Control tissues were then washed three times with Krebs-Henseleit buffer, and allowed to recover for 20 min. Tissues were then exposed to either the α_2-adrenoceptor agonist UK14304 or NPY. Increases in the tone induced by NPY or UK14304 were measured from the pre-NPY or pre-UK14304 baseline.

Effect of removal of the endothelium on responses to UK14304 in porcine isolated splenic artery, and porcine isolated ear artery Concentration-response curves to UK14304 were obtained in endothelium-intact and endothelium-denuded porcine splenic arteries under one of the following conditions: (1) UK14304 alone; (2) after pre-contraction with U46619 and relaxation with forskolin; (3) after pre-contraction with U46619 and relaxation with SNP. Responses to single concentrations of UK14304 (0.3 μM) or NPY (0.1 μM) were also obtained in endothelium-intact and endothelium-denuded porcine isolated ear arteries. Responses were obtained either alone or after pre-contraction with 0.1 μM U46619 (approximately 80% of the KCl response) and relaxation (to <10% of the 60 mM KCl response) with forskolin (1–2 μM), or SNP (100–200 μM). Responses from endothelium-denuded vessels were compared with responses from control tissues in which the endothelium remained intact.

Effect of antagonism of endothelin receptors, or inhibition of prostanoid synthesis on responses to UK14304 or NPY Splenic arteries or ear arteries with endothelium intact were prepared as described above. Tissues were pre-treated with either an endothelin antagonist (1 μM BQ-123), or the cyclo-oxygenase inhibitor flurbiprofen (1 μM), at least 1 h before the addition of agonist. Control tissues were exposed to vehicle only (Krebs-Henseleit buffer (BQ-123), or 0.1% ethanol (flurbiprofen)). Responses to UK14304 (splenic artery and ear artery) or NPY (ear artery only) were then obtained after pre-contraction with U46619 and relaxation with SNP.

Drugs

Neuropeptide Y (NPY) (Bachem (U.K.) Ltd.); (5Z, 9a, 11a, 13E, 15 (S))-15-hydroxy-9 (11) methanoepoxyprosta-5,13-dien-1oic acid (U46619) (Cascade Biochem Ltd); Forskolin (Sigma); Sodium Nitroprusside (David Bull Labs); Substance P (Bachem (U.K.) Ltd.); 5-bromo-6-[2-imidazolin-2-ylamine]-quinoxaline bitartrate (UK14304), (Pfizer); 2-fluoro-α-methyl-4-biphenyl-acetic acid (flurbiprofen), (Sigma); 2-(2-ethoxy-1,4-benzodioxan-2-yl)-2-imidazoline, (RX-811059) (Reckitt and Coleman). All other compounds were obtained from Sigma, Poole, U.K.

Statistics

For single comparisons, an *F*-test for equal variances was performed on all the data to test for normality. Normally distributed data were then subjected to a Student's two-tailed, unpaired *t*-test. Data that were not normally distributed were subjected to a non-parametric two-tailed, Mann-Whitney *U*-test. Paired data were subject to either a Student's paired *t*-test, or a non-parametric Wilcoxon Signed Rank test. Multiple

comparisons were performed using analysis of the variance (ANOVA) followed by a Bonferroni test. Statistical significance was assumed when $P<0.05$. Contractile responses were expressed as a percentage of the response to 60 mM KCl. Results were expressed as mean ± s.e.mean.

Results

Responses to UK14304 in porcine isolated splenic artery

A submaximal concentration of UK14304 (0.3 μM (see concentration-response curves in Figure 3)) produced only a small contraction in porcine splenic artery segments (Figure 1a). However, if the tissue was pre-contracted with U46619 to give a response which was 70–80% of the response to 60 mM KCl, and then relaxed back to baseline with forskolin (1–3 μM), then the subsequent response to UK14304 was greatly enhanced (Figure 1a). Similarly, pre-contraction of the tissue with U46619, and then relaxation with SNP (1–10 μM) prior to addition of UK14304 also resulted in an enhanced contraction (Figure 1b).

In a separate set of experiments, the selective α_2-adrenoceptor antagonist RX-811059 (1 μM; Mallard *et al.*, 1992) was added to tissues contracted with either UK14304 alone, or after pre-contraction with U46619 and relaxation with SNP. This resulted in 90–100% inhibition of the responses to UK14304 (data not shown), confirming that UK14304 was acting *via* α_2-adrenoceptors.

Responses to NPY in porcine isolated splenic artery

NPY (1 nM to 0.1 μM) produced only a small contraction in porcine splenic artery segments (Figure 2). As with the α_2-adrenoceptor agonist, pre-contraction with U46619 followed by relaxation back to baseline with forskolin, or SNP enhanced responses to NPY (Figure 2). The response to 0.1 μM NPY after relaxation with forskolin was significantly greater than the response to the same concentration of NPY after relaxation with SNP (Figure 2). In this series of experiments, the combination of U46619 and SNP was often associated with the development of large transient contractions (>60% of the response to 60 mM KCl). Only those preparations that responded to NPY with a sustained, discernible contraction were included in this study (around 50% of segments examined). These transient contractions precluded further detailed examination of the NPY responses under these conditions.

Responses to UK14304 in endothelium-intact and endothelium-denuded porcine isolated splenic artery

Substance P (0.1 μM) relaxed endothelium-intact splenic arteries pre-contracted with 3 μM phenylephrine (36.0 ± 1.3% relaxation, $n=23$), but had no effect on tissues in which endothelium had been removed. The magnitude of the

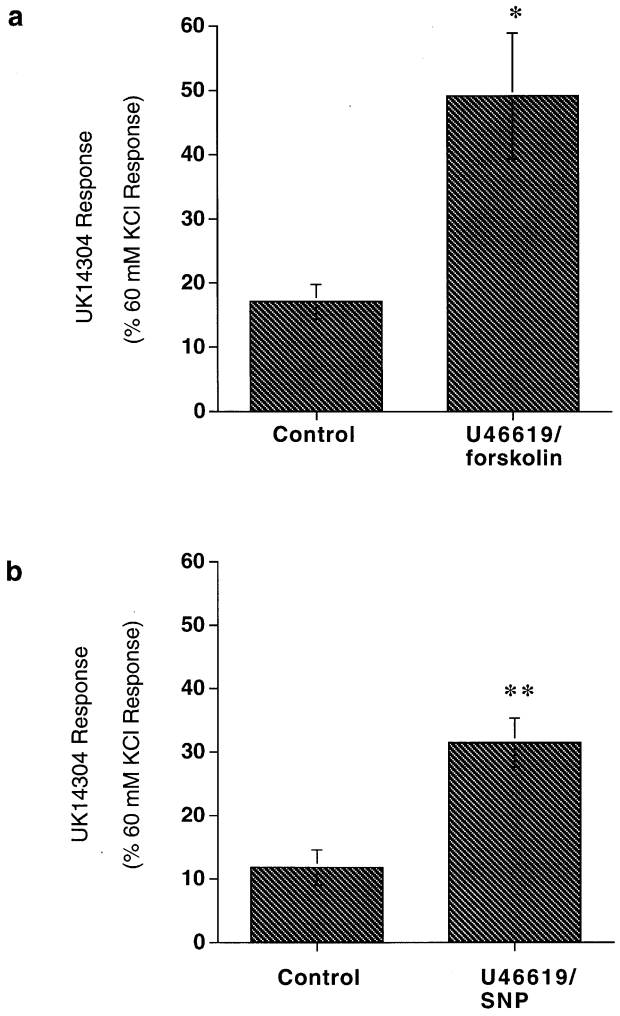

Figure 1 Responses to 0.3 μM UK14304 in porcine splenic artery ring segments expressed as per cent 60 mM KCl response. (a) responses to UK14304 alone (Control), and after pre-contraction with U46619 (to 60–80% of 60 mM KCl response) and relaxation back to baseline with forskolin (U46619/forskolin); $n=8$. (b) responses to UK14304 alone (Control), and after pre-contraction with U46619 (to 60–80% of 60 mM KCl response) and relaxation with SNP back to baseline (U46619/SNP); $n=4$. *Indicates $P<0.05$ vs Control, two-tailed, Mann-Whitney *U*-test. **Indicates $P<0.05$ vs Control, two-tailed, Student's unpaired *t*-test.

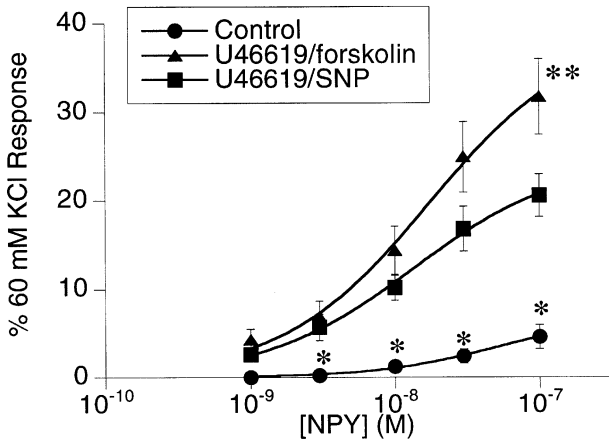

Figure 2 Concentration-response curves to NPY in porcine splenic artery ring segments expressed as per cent 60 mM KCl response. Shown are the mean responses ± s.e.mean ($n=7–9$) to NPY alone (Control), after pre-contraction with U46619 (to 60–80% of 60 mM KCl response) and relaxation back to baseline with forskolin (U46619/forskolin), and after pre-contraction with U46619 (to 60–80% of 60 mM KCl response) and relaxation with SNP back to baseline (U46619/SNP) *Indicates statistical significance control vs U46619/forskolin, and control vs U46619/SNP, ANOVA followed by Bonferroni test, $P<0.01$. **Indicates statistical significance U46619/forskolin vs U46619/SNP, ANOVA followed by Bonferroni test, $P<0.05$.

contraction to 60 mM KCl was unaffected by the removal of the endothelium (11.9 ± 0.7 g wt^{-1} endothelium intact compared to 11.7 ± 0.7 g wt^{-1} endothelium denuded, $n=23$). Removal of the endothelium had no effect on the maximum response to UK14304 alone ($20.0 \pm 3.3\%$ in the presence of the endothelium compared to $24.2 \pm 4.1\%$ in endothelium-denuded vessels, $n=8$) (Figure 3a)). Removal of the endothelium also had no significant effect on the responses to UK14304 after pre-contraction with U46619, and relaxation with forskolin (maximum response $46.3 \pm 11.0\%$ in the presence of the endothelium compared to $58.9 \pm 9.6\%$ in endothelium-denuded vessels, $n=8$ (Figure 3b)). On the other hand the maximum response to UK14304 after pre-contraction with U46619, and relaxation with SNP was significantly reduced by removal of the endothelium ($40.7 \pm 6.5\%$ in the presence of the endothelium compared to $21.9 \pm 2.8\%$ in endothelium-denuded vessels, $P<0.05$, two-tailed, unpaired t-test; $n=7$ (Figure 3c)). There was no significant effect on the pD_2 value (7.1 ± 0.1 in the presence of the endothelium compared to 7.4 ± 0.2 in endothelium-denuded vessels, $n=7$).

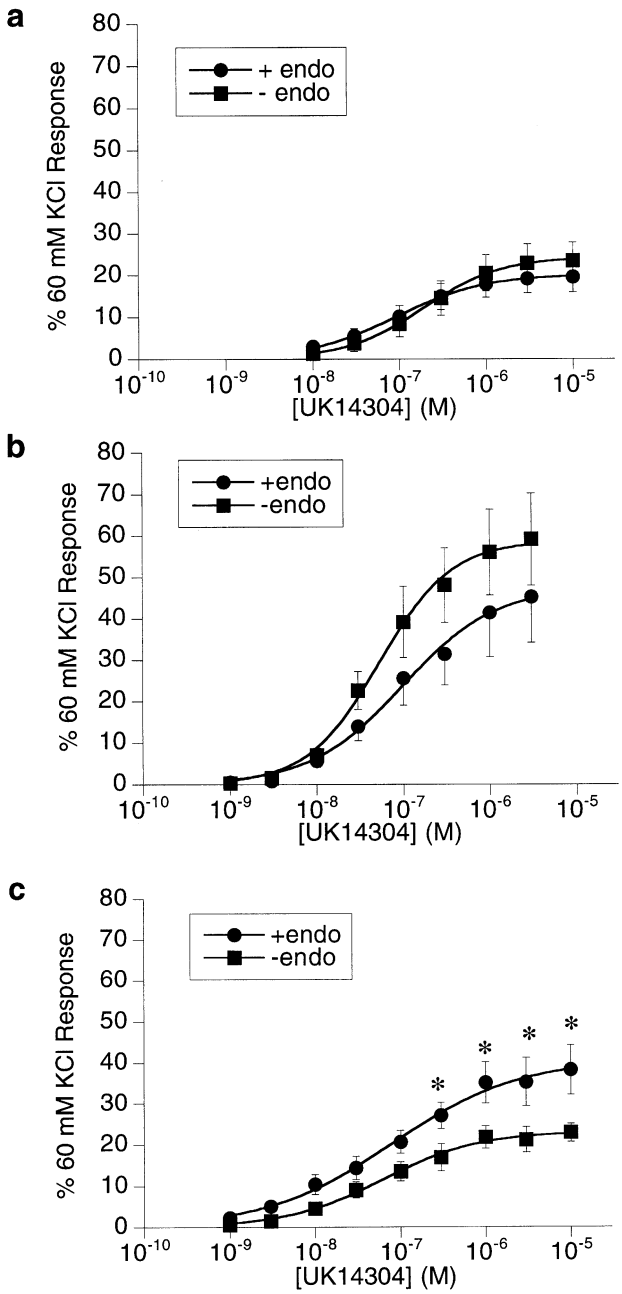

Figure 3 Concentration-response curves for UK14304 in porcine splenic artery ring segments expressed as per cent 60 mM KCl response in the same tissues. (a) Responses to UK14304 alone in the presence (+endo), or absence (−endo) of the vascular endothelium ($n=8$). (b) Responses to UK14304 after pre-contraction with U46619 and relaxation with forskolin in the presence (+endo), or absence (−endo) of the vascular endothelium ($n=8$). (c) Responses to UK14304 after pre-contraction with U46619 and relaxation with SNP in the presence (+endo), or absence (−endo) of the vascular endothelium ($n=7$). *Indicates $P<0.05$, two-tailed, unpaired t-test +endo vs −endo.

Responses to UK14304 in endothelium-intact and endothelium-denuded porcine isolated ear arteries

Substance P (0.1 μM) relaxed endothelium-intact ear arteries pre-contracted by 0.1 μM U46619 ($21.5 \pm 2.5\%$ relaxation,

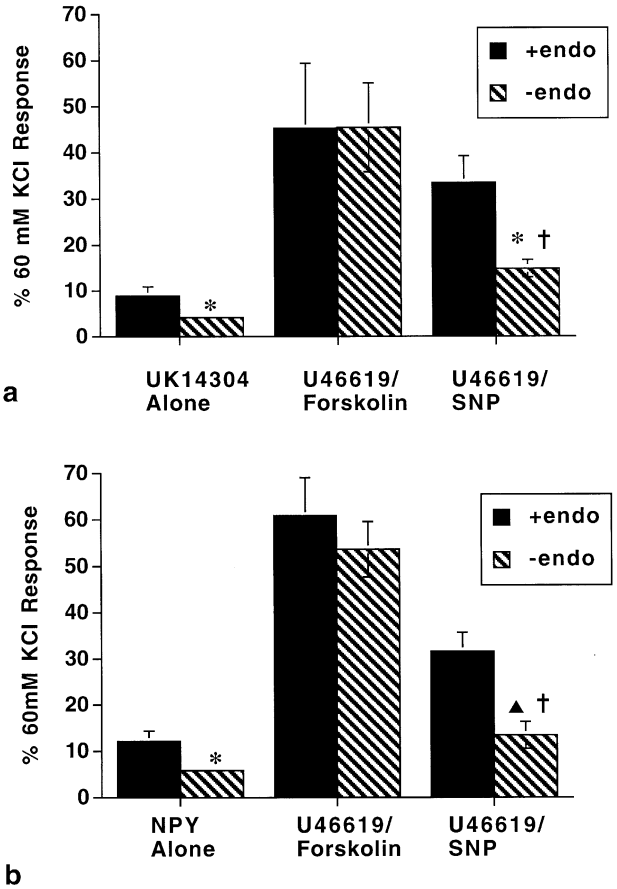

Figure 4 (a) Responses to 0.3 μM UK14304 in the porcine isolated ear artery expressed as mean ± s.e.mean in the presence (+endo) or absence (−endo) of the vascular endothelium. Shown are the responses to UK14304 alone ($n=17$), after pre-contraction with U46619 and relaxation with forskolin (U46619/forskolin; $n=5$)), and after pre-contraction with U46619 and relaxation with SNP (U46619/SNP; $n=6$). *Indicates $P<0.05$ −endo vs +endo, Wilcoxon Signed Rank test. †Indicates $P<0.05$ U46619/SNP −endo vs UK14304 alone −endo, two-tailed, Student's unpaired t-test. (b) Responses to 0.1 μM NPY in the porcine isolated ear artery expressed as mean ± s.e.mean in the presence (+endo) or absence (−endo) of the vascular endothelium. Shown are the responses to NPY alone ($n=23$), after pre-contraction with U46619 and relaxation with forskolin (U46619/forskolin; $n=6$)), and after pre-contraction with U46619 and relaxation with SNP (U46619/SNP; $n=7$). *Indicates $P<0.01$ −endo vs +endo, Wilcoxon Signed Rank test. ▲ Indicates $P<0.001$ −endo vs +endo, two-tailed, Student's paired t-test. †Indicates $P<0.05$ U46619/SNP −endo vs NPY alone −endo, two-tailed, Student's unpaired t-test.

$n=18$), but had no effect on tissues in which the endothelium had been removed. Unlike the splenic artery, the magnitude of the contraction to 60 mM KCl was significantly less in endothelium-denuded segments compared to endothelium intact segments (1.5 ± 0.1 g wt^{-1} in endothelium-denuded segments compared to 3.0 ± 0.2 g wt^{-1} in endothelium intact segments, $n=24$).

A submaximal concentration of UK14304 (0.3 μM (see Roberts *et al*., 1998)) produced a small contractile response in porcine ear artery segments. This responses was reduced by removal of the endothelium (Figure 4a). The enhanced response to 0.3 μM UK14304 after pre-contraction with 0.1 μM U46619, and relaxation back to baseline with forskolin (1–2 μM) was unaffected by removal of the endothelium (Figure 4a). However, the responses to 0.3 μM UK14304 after pre-contraction with U46619 and relaxation with SNP were significantly reduced by the removal of the endothelium (Figure 4a). Despite this reduction, the responses to 0.3 μM UK14304 after pre-contraction with U46619 and relaxation with SNP were still significantly larger than those obtained with 0.3 μM UK14304 alone in endothelium-denuded vessels ($P<0.05$, two-tailed, Student's unpaired *t*-test, see Figure 4a).

Responses to NPY in endothelium-intact and endothelium-denuded vessels

A submaximal concentration of NPY (0.1 μM (see Roberts *et al*., 1999)) produced a small contractile response in porcine isolated ear arteries (Figure 4b). This response was significantly reduced in endothelium-denuded tissues (Figure 4b). The enhanced response to 0.1 μM NPY after pre-contraction with 0.1 μM U46619 and relaxation back to the baseline with forskolin (1–2 μM) was unaffected by removal of the endothelium (Figure 4b). However, the responses to 0.1 μM NPY after pre-contraction with 0.1 μM U46619, and relaxation with SNP (100–200 μM), were significantly reduced in endothelium-denuded vessels compared to endothelium-intact vessels (Figure 4b). Although the response to 0.1 μM NPY after relaxation with SNP was reduced after removal of the endothelium, it was still significantly larger than the response to NPY alone in endothelium-denuded vessels ($P<0.05$, two-tailed, Student's unpaired *t*-test, see Figure 4b).

Effect of antagonism of endothelin receptors, or inhibition of prostanoid synthesis on responses to UK14304 or NPY

BQ-123 (1 μM), an endothelin ET_A receptor antagonist (Sumner *et al*., 1992) had no effect on responses to UK14304 in the porcine splenic artery in the presence of U46619 and SNP (42.7 ± 5.8% (maximum response in the presence of BQ-123) compared to 40.8 ± 4.6% (control; $n=6$)). Likewise, flurbiprofen (1 μM), a cyclo-oxygenase inhibitor (Nozu, 1978) also had no effect on responses to UK14304 in the porcine splenic artery in the presence of U46619 and SNP (35.8 ± 3.7% (maximum response in the presence of flurbiprofen) compared to 33.7 ± 5.3% (control; $n=6$)). Similarly, responses to 0.3 μM UK14304 in the porcine ear artery in the presence of U46619 and SNP were unaffected by pre-incubation with either flurbiprofen (21.6 ± 9.1% (in the presence of flurbiprofen) compared to 21.3 ± 7.9% (control; $n=7$)) or BQ-123 (18.8 ± 8.7% (in the presence of BQ-123) compared to 20.4 ± 6.4% (control; $n=6$)). The responses to 0.1 μM NPY in the porcine ear artery in the presence of U46619 and SNP were also unaffected by pre-incubation with either BQ-123 (26.7 ± 4.9% (BQ-123) compared to 24.9 ± 4.7% (control; $n=4$)) or flurbiprofen (16.1 ± 3.9% (flurbiprofen) compared to 17.1 ± 6.0% (control; $n=4$)).

Discussion

Previous studies have shown that responses to the α_2-adrenoceptor selective agonist UK14304, or NPY (*via* NPY Y_1 receptors) in the porcine isolated ear artery are small (Roberts *et al*., 1998; 1999). However, enhanced responses to both agents can be obtained by prior pharmacological manipulation of the tissue (Roberts *et al*., 1998; 1999). Like the porcine ear artery (Roberts *et al*., 1998), the porcine splenic artery produces only small contractions after exposure to UK14304, and this is associated with a low binding site density (Barbieri *et al*., 1998; Wright *et al*., 1995). This current study demonstrates that the responses to 0.3 μM UK14304 are enhanced approximately 3 fold after pre-contraction of the splenic artery with U46619, and relaxation with forskolin. This is similar to the results obtained previously in the porcine ear artery in which the responses to UK14304 were enhanced 3–4 fold under these conditions (Roberts *et al*., 1998), and indicates the presence of an adenylyl cyclase-dependent pathway (i.e. reduction in cyclic AMP levels; Roberts *et al*., 1998) in both vessels. Responses to UK14304 are also enhanced in both the porcine ear artery (Roberts *et al*., 1998) and splenic artery (this study) after pre-contraction with U46619, and relaxation with SNP. Under these conditions responses are believed to be mediated through a pathway which is independent of the inhibition of adenylyl cyclase (adenylyl cyclase-independent pathway; Roberts *et al*., 1998). Similar pharmacological manipulation in the rat tail artery also uncovers enhanced responses to UK14304 (Aidulis *et al*., 1992). Taken together, these results indicate that enhanced α_2-adrenoceptor vasoconstriction mediated through both adenylyl cyclase-dependent and -independent pathways is common to a number of blood vessels, and not confined to the porcine ear artery.

Previous studies have failed to demonstrate significant α_2-adrenoceptor-mediated contractions in the porcine splenic artery, even in the presence of a vasoconstrictor (Barbieri *et al*., 1998; Wright *et al*., 1995). This present study demonstrates that the porcine splenic artery does possess functional α_2-adrenoceptors, although uncovering the responses requires prior activation of both constrictor and relaxatory mechanisms.

Like α_2-adrenoceptor-mediated responses, NPY contractile responses are enhanced in the presence of U46619 and forskolin, or U46619 and SNP in both the porcine ear artery (Roberts *et al*., 1999) and porcine splenic artery (this study). This indicates that, like the α_2-adrenoceptor agonist UK14304, the responses to NPY in these tissues are mediated through both adenylyl cyclase-dependent, and -independent pathways. These data further demonstrate the similarities in the signal transduction pathways both between α_2-adrenoceptors and NPY receptors, and between these two isolated blood vessels. An enhanced contraction to NPY can also be obtained in the rat femoral artery after pre-contraction with phenylephrine and relaxation with SNP or histamine (Grundemar & Hogestatt, 1992). This indicates that, like α_2-adrenoceptors, enhanced responses to NPY mediated through both adenylyl cyclase-dependent and -independent pathways may be mechanisms common to a number of blood vessels. Furthermore, pharmacological manipulation with a vasoconstrictor (AII) and an adenylyl cyclase-independent relaxant (SNP) has also been found to enhance 5-HT_1-like vasoconstric-

tion (Randall *et al*., 1996), suggesting vasoconstriction mediated through both adenylyl cyclase-dependent and -independent pathways may be common to a number of G_i-coupled receptors.

Involvement of the vascular endothelium

In some blood vessels removal of the endothelium enhances α_2-adrenergic vasoconstriction (MacLean *et al*., 1993; Miller *et al*., 1991). However, removal of the endothelium did not appear to enhance responses to UK14304 alone in the porcine splenic artery. There was a slight enhancement of the UK14304 response in endothelium-denuded vessels after pre-contraction with U46619, and relaxation with forskolin, but this was not significant. These findings are similar to results obtained in rabbit lateral saphenous vein in which removal of the endothelium had no effect on the responses to UK14304 (McGrath *et al*., 1990). An unexpected finding was that the response to UK14304 in the porcine splenic artery after relaxation with SNP was significantly reduced by endothelium denudation, rather than enhanced. Similar findings were obtained in the porcine isolated ear artery, although the responses to UK14304 alone were also reduced in endothelium-denuded vessels, along with the responses after relaxation with SNP. A reduction in enhanced α_2-adrenoceptor responses after relaxation with dibutyryl cyclic AMP (an adenylyl cyclase-independent pathway (Roberts *et al*., 1998)) in endothelium-denuded porcine ear artery segments has also been demonstrated (Roberts *et al*., unpublished data).

Endothelium-denudation also reduced the responses to NPY alone in the porcine ear artery, as well as reducing the enhanced responses to NPY after relaxation with SNP, but not after relaxation with forskolin. A reduction in enhanced NPY responses after relaxation with dibutyryl cyclic AMP in endothelium-denuded porcine ear artery segments has also been demonstrated (Roberts *et al*., unpublished data). These data demonstrate further the functional similarities between α_2-adrenoceptors and NPY receptors in porcine blood vessels.

The fact that none of the responses was enhanced by endothelial denudation suggests that α_2-adrenergic and NPY receptors in the porcine splenic artery and ear artery do not stimulate the release of an endothelium-derived relaxing factor as indicated in other tissues (Bullock *et al*., 1986; Bockman *et al*., 1993). Furthermore, these data indicate that basal release of nitric oxide does not exert a major inhibitory effect on the underlying smooth muscle in the porcine splenic artery or ear artery. These blood vessels are not unusual in this respect since endothelial-denudation of human mammary (Yang *et al*., 1991), mesenteric (Martinez *et al*., 1994), and uterine arteries (Jovanovic *et al*., 1995) also failed to significantly increase the sensitivity of the vessel to vasoconstrictor agents.

Like the porcine ear artery and splenic artery (after relaxation with SNP), the contractile responses to α_2-adrenoceptor agonists or NPY in some blood vessels actually appear to be dependent on an intact endothelium (Fabi *et al*., 1998; Hieble *et al*., 1989; MacLean & McGrath, 1990; Thorin, 1998). For example, in bovine retinal arteries NPY produces a direct vasoconstrictor response, and also enhances the response to noradrenaline (Prieto *et al*., 1995). Removal of the endothelium, however, reduces the direct vasoconstrictor response and abolishes the potentiating response. One explanation for the dependence of the α_2-adrenoceptor or NPY receptor responses on the endothelium could be that stimulation of the receptor results in the release of an endothelium-derived contractile factor (Fabi *et al*., 1998; Thorin, 1998). Several studies have highlighted the potential for both NPY and α_2-adrenoceptor agonists to activate endothelial cells and release vasoactive substances (Bockman *et al*., 1993; Kawamura *et al*., 1991; Millar *et al*., 1992). Significantly, Barber & Miller (1997) noted that the dilator effect of UK14304 on the porcine coronary artery was enhanced following exposure to indomethacin, a cyclo-oxygenase inhibitor, indicating that α_2-adrenoceptors also have the potential to release an endothelium-derived vasoconstrictor prostanoid. In rabbit cerebral arteries, activation of endothelial α_2-adrenoceptors triggers the release of endothelin-1 which mediates a contraction (Thorin, 1998). These responses were inhibited by BQ-123, an ET_A receptor antagonist (Sumner *et al*., 1992). However, BQ-123 failed to inhibit UK14304-mediated contractions in the porcine splenic artery after relaxation with SNP. Similarly, responses to UK14304 or NPY in the presence of U46619 and SNP in the porcine ear artery were unaffected by BQ-123. Cyclo-oxygenase products have been implicated in NPY-mediated contractions in the dog coronary vasculature (Martin *et al*., 1992), and the human saphenous vein (Fabi *et al*., 1998). There is also evidence that α_2-adrenoceptors can stimulate the release of a vasoconstrictor prostanoid (Barber & Miller, 1997). Cyclo-oxygenase products do not appear to be involved in the α_2-adrenergic responses in the porcine splenic artery after relaxation with SNP as flurbiprofen, a prostaglandin synthesis inhibitor (Nozu, 1978), failed to inhibit the contractile responses. Similarly, responses to UK14304 or NPY in the porcine ear artery after pre-contraction with U46619 and relaxation with SNP were insensitive to flurbiprofen. The release of an endothelial-derived contractile factor would explain the partial dependency of the α_2-adrenergic and NPY responses on the endothelium. However, it is not clear why only some of the responses to UK14304 or NPY are sensitive to endothelial denudation, and not others. For example, the responses after relaxation with forskolin do not appear to be dependent on the endothelium. We know of no other example in which the influence of the endothelium changes depending on which ancillary agent is present. Further investigation is required to determine whether the different vasodilator agents have different effects on the release of the putative endothelium-derived vasoconstrictor. However, this first requires the identification of the putative endothelium-derived vasoconstrictor, which we have been unable to do in this present study.

Interestingly, the responses to 60 mM KCl were also reduced in endothelium-denuded ear arteries, but not splenic arteries. This suggests that removal of the endothelium in the porcine ear artery may cause a general decrease in vasoconstriction. However, as the results are expressed as a percentage of the KCl response, the changes in α_2-adrenergic and NPY contractions in this blood vessel are specific to these agents. A reduction in the KCl response after endothelial denudation is not unusual. For example, reduced responses to KCl have also been demonstrated in the porcine testicular artery after removal of the endothelium (Costa *et al*., 1996). In this tissue KCl is thought to stimulate the release from the endothelium of the cyclo-oxygenase-derived contractile factor. An alternative reason for the reduced KCl responses could be that in removing the endothelium we have damaged the smooth muscle. Although great care was taken to prevent damage to the smooth muscle, a certain amount of rubbing of the lumen is required to remove the endothelium (measured by the abolishment of the relaxation to substance P). A similar method was used to remove the endothelium from the porcine splenic artery. In this tissue removal of the endothelium had no effect on the KCl responses. However, changes in the responses

to UK14304 and NPY, similar to those seen in the porcine ear artery, were observed.

In conclusion, enhanced contractions in response to the α_2-adrenoceptor agonist UK14304, and NPY can be obtained in the porcine splenic artery by prior pharmacological manipulation. These results are similar to those obtained in the porcine isolated ear artery in which enhanced responses can be obtained through adenylyl cyclase-dependent and -independent mechanisms. This current study indicates that similar signal transduction pathways mediate α_2-adrenoceptor and NPY receptor contractile responses in the porcine splenic artery, demonstrating further the importance of these signal transduction pathways in vasoconstriction. Furthermore, the endothelium also appeared to be involved in these enhanced responses under certain conditions, although the nature of this involvement has yet to be determined.

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