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SPECIAL REPORT

Evidence for a non-adrenoceptor, imidazoline-mediated contractile response to oxymetazoline in the porcine isolated rectal artery

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Imidazoline derivatives are known to elicit responses through both α_2-adrenoceptor and non-adrenoceptor, imidazoline sites, though as yet there are no examples of the latter on vascular smooth muscle. In the presence of 0.3 μM prazosin, neither UK-14304 (0.01–3 μM) nor oxymetazoline (0.01–30 μM) caused a significant contraction of the porcine isolated rectal artery, a preparation with a low density of α_2-adrenoceptors. In the presence of a combination of U46619 and forskolin, however, both agonists produced concentration-dependent contractions. Pretreatment with phenoxybenzamine (3 μM) abolished responses to UK-14304, but left those elicited by oxymetazoline largely unaffected. The putative I_3 imidazoline antagonist 2-(2,3 dihydro-2-benzofuranyl)-2-imidazole (KU-14R, 10 μM) caused a 6 fold rightward displacement of the phenoxybenzamine-insensitive concentration–response curve to oxymetazoline. Our data indicates that non-adrenoceptor, imidazoline sites, pharmacologically similar to the I_3 imidazoline site on islet cells, mediate vasoconstriction in the porcine isolated rectal artery.
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Abbreviations: 5-HT, 5-hydroxytryptamine; KU 14R, (2-(2,3 dihydro-2-benzofuranyl)-2-imidazole; RX-811059, 2-(2-ethoxy-1,4-benzodioxan-2-yl)-2-imidazoline; UK-14304, 5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline; U46619, (1,5)-**S**-hydroxy-11a,9a-(epoxymethano)-prosta-5Z,13E-dienoic acid

Introduction Imidazoline derivatives such as clonidine and oxymetazoline are best known for their ability to interact with α_2-adrenoceptor binding sites and, in many instances, to activate the receptor (Bylund *et al*., 1994). During the past decade, however, there has been increasing awareness that many of these agents also recognize a variety of non-adrenoceptor sites that appear to be involved in the central control of blood pressure (Eglen *et al*., 1998) and to influence insulin secretion (Morgan *et al*., 1995; Hirose *et al*., 1997) and sympathetic neurotransmission in the vasculature (Göhert *et al*., 1995). In most instances, functional demonstration of these non-adrenoceptor responses is critically-dependent upon the use of either selective α_2-adrenoceptor antagonists or non-receptor interventions, e.g., pertussis toxin treatment to inhibit receptors coupled to G_i protein, to eliminate the α_2-adrenoceptor component.

For the putative I_3 imidazoline receptors controlling insulin secretion in islets cells, an additional requirement for demonstrating the pro-secretory effect of imidazoline derivatives is the presence of glucose (Morgan *et al*., 1995). Interestingly, the obligate requirement of an ancillary agent for I_3 imidazoline responses is qualitatively similar to that for the expression of functional α_2-adrenoceptors in preparations with low receptor density, e.g. platelets (Grant & Scrutton, 1979) and vascular smooth receptor (Roberts *et al*., 1998; 1999). Although I_2 imidazoline receptors have been reported on cultured vascular smoth muscle cells (Regunathan *et al*., 1999), the possibility of non-adrenoceptor, imidazoline receptors affecting vascular tone has not been addressed.

Thus, we have compared the effects of two imidazoline derivatives, UK-14304, a selective α_2-adrenoceptor agonist (Cambridge, 1981), and oxymetazoline, a partial agonist at α_2-adrenoceptors (Trendelenburg *et al*., 1996) that also possesses non-adrenoceptor actions (Hirose *et al*., 1997), in a preparation with a very low density of α_2-adrenoceptors, the porcine isolated rectal artery (Blaylock *et al*., 2000).

Methods Porcine mesenteric tissue, from the descending colon to the anus, was obtained from a local abattoir and transported to the laboratory in Krebs-Henseleit solution at 4°C. Five to six centimetre segments (2–3 mm internal diameter) of the rectal artery were dissected and refrigerated overnight at 4°C in modified Krebs-Henseleit solution. The solution had been previously gassed with 95% O_2/5% CO_2 and contained 2% wv^{-1} ficoll to prevent osmotic swelling of the vessel. This storage procedure has been shown to have negligible effect on constrictor and dilator function in isolated blood vessels (Lot & Wilson, 1994).

On the following day the rectal artery was cleaned of connective tissue and divided into 5 mm ring segments. Stainless steel wire (0.2 mm thick) supports were then inserted into the lumen and each segment suspended in a 5 ml isolated organ bath containing modified Krebs-Henseleit solution maintained at 37°C and gassed with 95% O_2/5% CO_2. The composition of the modified Krebs-Henseleit solution was (mM) NaCl 118.4, KCl 4.7, $CaCl_2$ 1.25, $MgSO_4$ 1.2, $NaHCO_3$ 24.9, KH_2PO_4 1.2, glucose 11.1. The lower support was fixed, and the upper support connected to a Grass FT-03 transducer

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which in turn was linked to an AD Instruments Quad Bridge pre-amplifier unit coupled to a Maclab 4e unit running Chart 3.5.4. The results were displayed on a Macintosh LC II computer. After 30 min equilibration, an initial resting tension of 6 g wt was slowly applied to the segments which was then allowed to relax. Sixty minutes later the resting tension was finally re-adjusted to 2–3 g wt.

In some experiments preparations were exposed to 3 μM phenoxybenzamine for 60 min to inactivate both α_1- and α_2-adrenoceptors, followed by repeated washing over a 40 min period. All preparations were then exposed to 60 mM KCl and the response allowed to reach maximum. This was repeated on two further occasions until the responses were reproducible and, where necessary, the resting tension readjusted to 2–3 g wt. Following an equilibration period of 30 min, each preparation was stimulated with 5–20 nM U-46619 (the thromboxane-mimetic, (1,5), S-hydroxy-11a,9a-(epoxymethano)-prosta-5Z,13E-dienoic acid) to produce a contraction equivalent to 50–70% of the response to 60 mM KCl and then to increasing concentrations of forskolin (0.1–0.7 μM) to lower the residual vasoconstrictor tone to less than 5% of the final contraction to 60 mM KCl. For those preparations not exposed to either phenoxybenzamine or noradrenaline, 0.3 μM prazosin was added to inhibit α_1-adrenoceptors. Once a stable contraction was established the preparations were exposed to cumulatively increasing concentrations of either UK-14304, oxymetazoline, noradrenaline or 5-HT. In some experiments 1 μM RX-811059 (2-(2-ethoxy-1,4-benzodioxan-2-yl)-2-imidazoline), a selective α_2-adrenoceptor antagonist, was added at the end of the concentration response curve.

Unless indicated otherwise, the effect of the agonists have been calculated as a percentage of the final contraction to 60 mM KCl, and have been expressed as the mean $\pm$ s.e.mean of observations in tissues from different animals. The potency of UK-14304 and noradrenaline have been determined as the negative logarithm of the concentration required to produce 50% of the maximum response (pD_2), while for oxymetazoline, which failed to elicit a true maximum, potency has been estimated as the negative logarithm of the concentration producing a response equivalent to 20% of the response to 60 mM KCl (pEC_{20}). Where necessary, a Student's *t*-test has been used to assess whether drug-induced effects were statistically significant ($P<0.05$).

The drugs used were: U46619 (Upjohn); oxymetazoline HCl (Sigma); prazosin HCl (Pfizer); RX-811059 (2-(2-ethoxy-1,4-benzodioxan-2-yl)-2-imidazoline, Reckitt and Coleman); forskolin (Sigma); UK-14304 (5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline bitartrate, Pfizer); Ficoll 70,000 (Sigma); noradrenaline bitartrate (Sigma); 5-hydroxytryptamine (5-HT) creatinine sulphate (Sigma); phenoxybenzamine HCl (Smith Kline Beecham); KU 14R (2-(2,3 dihydro-2-benzofuranyl)-2-imidazole HCl was synthesized in the Department of Chemistry, University of Keele, U.K.). Prazosin (1 mM) was dissolved in 0.1 M lactic acid and dilutions made in distilled water, while phenoxybenzamine (1 mM) was dissolved in absolute alcohol plus a drop of 0.1 N HCl. The drugs were added to organ baths in a volume of 20 μl or less and the concentration of the vehicle never exceeded 0.3% vv^{-1}.

Results KCl (60 mM) caused a sustained contraction of the porcine isolated rectal artery (12.3 $\pm$ 1.2 g wt., $n=10$), while noradrenaline (0.03–30 μM) produced concentration dependent contractions (pD_2 5.37 $\pm$ 0.16, $n=10$) with a maximum response equivalent to 166.8 $\pm$ 27.5% of the KCl-induced response. In contrast, neither 5-HT (10 μM, $n=6$), UK-14304 (0.01–3 μM) nor oxymetazoline (0.03–30 μM) produced a significant contraction (<5% of the response to 60 mM KCl) (Figure 1). U46619 (10–60 nM) caused a sustained contraction (60–70% of the response to 60 mM KCl) and the addition of 0.1–0.7 μM forskolin caused a slow relaxation towards baseline tone. In the presence of the combination of U46619 and forskolin, UK-14304 (pD_2 6.85 $\pm$ 0.22, $n=6$) and oxymetazoline elicited concentration-dependent contractions (Figure 1), while 5-HT (0.1–10 μM) was devoid of activity ($n=8$). The maximum response to UK-14304 (40.5 $\pm$ 11.5%, $n=6$) was smaller ($P<0.05$) than that produced by the highest concentration of oxymetazoline (30 μM) examined (62.2 $\pm$ 6.9%, $n=6$). The addition of 1 μM RX-811059 at the end of the concentration response curve abolished the response to 3 μM UK-14304 ($n=6$) but produced only a small reduction (10.9 $\pm$ 2.3%, $n=8$) of the response to 30 μM oxymetazoline.

Prior exposure of the rectal artery to 3 μM phenoxybenzamine abolished contractions to noradrenaline ($n=6$) and reduced the maximum response to UK-14304 in the presence of forskolin and U46619 from 36.6 $\pm$ 5.0% to 5.1 $\pm$ 2.2% ($n=8$) (Figure 2a). In contrast, phenoxybenzamine-pretreat-

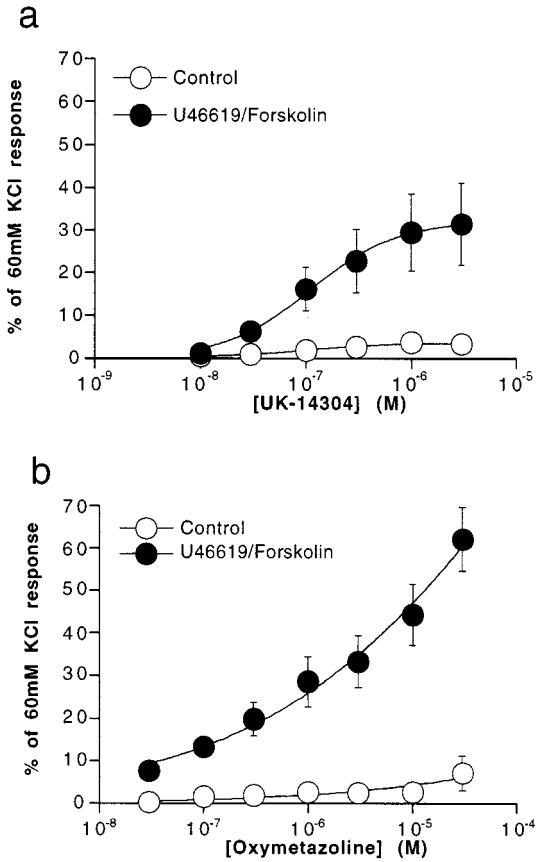

Figure 1 The effect of (a) UK-14304 and (b) oxymetazoline on the porcine isolated rectal artery in the absence (control) and the presence of a combination of U46619 and forskolin. Responses have been expressed as a percentage of the contraction to 60 mM KCl and are shown as the mean $\pm$ s.e.mean of 6–10 observations.

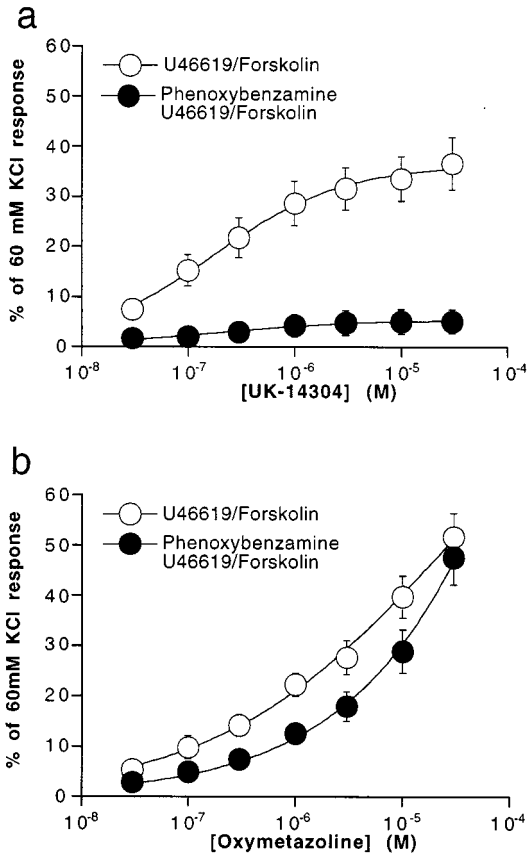

Figure 2 The effect of pretreatment with phenoxybenzamine (3 μM for 60 min followed by repeated washing) on contractions to (a) UK-14304 and (b) oxymetazoline on the porcine isolated rectal artery in the presence of a combination of U46619 and forskolin. Responses have been expressed as a percentage of the contraction to 60 mM KCl and are shown as the mean$\pm$s.e.mean of eight observations.

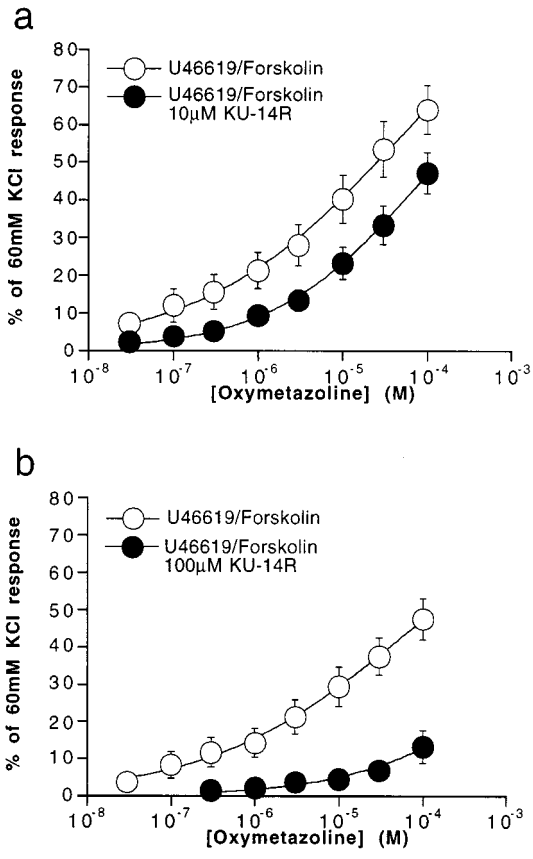

Figure 3 The effect of (a) 10 μM KU-14R and (b) 100 μM KU-14R on oxymetazoline-induced contractions of the porcine isolated rectal artery pretreated with phenoxybenzamine in the presence of U46619 and forskolin. Responses have been expressed as a percentage of the contraction to 60 mM KCl and are shown as the mean$\pm$s.e.mean of 12–14 observations.

ment failed to affect the response to 30 μM oxymetazoline in the presence of U46619 and forskolin (Figure 2b), but caused a 6 fold rightward shift in the lower portion of the concentration response curve (Control pEC_{20} -6.13 ± 0.16; Phenoxy' pEC_{20} -5.35 ± 0.14. $n=8$).

As shown in Figure 3a, 10 μM 2-(2,3 dihydro-2-benzofuranyl)-2-imidazole (KU-14R), a putative I_3 receptor antagonist (Chan *et al*., 1998), caused a 6 fold rightward shift of the concentration response curve to oxymetazoline (phenoxybenzamine-treated and in the presence of U46619 and forskolin) (Control pEC_{20} -5.91 ± 0.28; 10 μM KU-14R pEC_{20} -5.03 ± 0.17, $n=12$). A higher concentration of KU-14R (100 μM) caused a greater reduction of responses to oxymetazoline, reducing the contraction to 100 μM oxymetazoline from $47.5\pm4.9\%$ to $13.3\pm4.3\%$ ($n=14$) (Figure 3b). However, 100 μM KU-14R significantly reduced U46619-induced contractions (to $31.7\pm9.6\%$ of control, $n=8$), while 10 μM KU-14R failed to affect the response to U46619 ($105.1\pm6.3\%$ of control, $n=8$).

Discussion This study provides the first example of a non-adrenoceptor, imidazoline-mediated response in a blood vessel that shares pharmacological similarities with the I_3 imidazoline receptor present on pancreatic islet cells (Morgan *et al*., 1995; Eglen *et al*., 1998). Identification of this response has been made possible by both the properties of the isolated rectal artery and the experimental conditions employed. Radioligand binding studies have shown that the isolated rectal artery possesses a small population of α_2-adrenoceptor binding sites (Blaylock *et al*., 2000), which probably accounts for the failure of both UK-14304 and oxymetazoline to elicit contractions in the presence of prazosin. However, the combination of a vasoconstrictor agent, U46619, counterbalanced by the inhibitory effect of forskolin, uncovered large vasoconstrictor responses to both UK-14304 and oxymetazoline. Interestingly, responses to oxymetazoline, a presumed partial agonist at α_2-adrenoceptors (Jasper *et al*., 1998), were observed over a 1000 fold range without the attainment of a true maximum. Also, the largest response to oxymetazoline was greater than that elicited by UK-14304, raising the possibility that the former may act at two different sites.

In the case of UK-14304, the contractile response appears to be mediated entirely by α_2-adrenoceptors since it is blocked by RX-811059, a selective α_2-adrenoceptor antagonist (Mallard *et al*., 1992), and phenoxybenzamine, an irreversible antagonist of both α_1- and α_2-adrenoceptors (Furchgott, 1972). Thus, the ability of U46619 and forskolin to uncover constrictor responses to UK-4304 in the isolated rectal artery is qualitatively similar to that noted in other porcine blood vessels (Roberts *et al*., 1998; 1999). In contrast

to UK-14304, several observations suggest that oxymetazoline may also act on a non-adrenoceptor site. First, the largest response to oxymetazoline (in the presence of U46619 and forskolin) was largely unaffected by RX-811059. Second, pretreatment with phenoxybenzamine, which abolished responses to UK-14304, caused only a small rightward shift of the lower portion of the concentration response curve to oxymetazoline and failed to reduce the response to the highest concentration of the agonist (see Figure 2a). Third, oxymetazoline has also been reported to activate 5-HT_1 receptors (Schoeffter & Hoyer 1991), but since 5-HT failed to elicit a response, either in the absence or in the presence of U46619 and forskolin, it seems unlikely that this receptor can account for the phenoxybenzamine-insensitive response.

KU-14R is an imidazoline derivative reported to selectively inhibit the putative imidazoline receptor (I_3) on pancreatic islet cells (Chan *et al*., 1998). This site is thought to mediate the increase in insulin secretion produced by high concentrations of a variety of imidazoline-based agents (Eglen *et al*., 1998), including oxymetazoline (Hirose *et al*., 1997). Phenoxybenzamine-resistant contractions to oxymetazoline (in the presence of U46619 and forskolin) were inhibited by KU-14R. Although caution is required in the interpretation of the observations with 100 μM KU-14R, since it reduced the submaximal contractions to U46619, the lower concentration of KU-14R exerted a selective inhibitory effect on oxymetazoline-induced contractions. Significantly the activity of KU-14R at this oxymetazoline-sensitive site was 10 fold greater than that reported in islet cells (Chan *et al*., 1998; Mourtada *et al*., 1999) and manifest over the whole agonist concentration range. It was noteworthy that previous studies have been largely based on the activity of KU-14R against a single concentration of an agonist used to stimulate insulin release.

Further studies with other imidazoline derivatives are clearly required to confirm the involvement of imidazoline I_3 receptors in the non-adrenoceptor contraction to oxymetazoline. Of particular interest, will be the effect of phentolamine, since high concentrations of this α-adrenoceptor antagonist has been reported to stimulate insulin secretion in pancreatic islet cells (Schultz & Hasselblatt, 1989), and enhance spontaneous contractions in both the guinea-pig isolated bladder (Satake *et al*., 1984) and rat isolated portal vein (Schwietert *et al*., 1992), by activating a non-adrenoceptor mechanism. The latter reports raises the possibility that this non-adrenoceptor, imidazoline receptor may be present throughout the vasculature, but hitherto has escaped detection due to (i) the selectivity of compounds for α_1- and α_2-adrenoceptors receptors that are widely distributed in the blood vessels and (ii) the obligate requirement of a vasoconstrictor (U46619) and relaxant (forskolin) for full expression of the response.

In summary we have demonstrated that under the appropriate conditions imidazoline derivatives elicit vasoconstrictor responses *via* α_2-adrenoceptors and a non-adrenoceptor site in the porcine isolated rectal artery. The latter response, which shares pharmacological similarities with the putative imidazoline I_3 receptor (Eglen *et al*., 1998), may shed further light on the therapeutic potential of non-adrenoceptor actions of imidazoline-based compounds.

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References

BLAYLOCK, N.A., SHAH, A. & WILSON, V.G. (2000). Pharmacological evidence for pre- and post-junctional α_2-adrenoceptors in the porcine isolated rectal artery. *Br. J. Pharmacol.,* **131,** 80P.

BYLUND, D.B., EIKENBURG, D.C., HIEBLE, J.P., LANGER, S.Z., LEFKOWITZ, R.J., MINNEMAN, K.P., MOLINOFF, P.B., RUFFOLO, R.R. & TRENDELENBURG. U. (1994). International Union of Pharmacology nomenclature on adrenoceptors. *Pharmacol. Rev.,* **46,** 121 – 136.

CAMBRIDGE, D. (1981). UK-14304, a potent and selective α_2-agonist for the characterisation of α-adrenoceptor subtypes. *Eur. J. Pharmacol.,* **72,** 413 – 415.

CHAN, S.L.F., PALLETT, A.L., CLEWS, J., RAMSDEN, C.A., CHAPMAN, C., KANE., DUNNE, M.J. & MORGAN, N.G. (1998). Characterization of new efaroxan derivatives for use in purification of imidazoline-binding sites. *Eur. J. Pharmacol.,* **355,** 67 – 76.

EGLEN, R.M., HUDSON, A.L., KENDALL, D.A, NUTT, D.J., MORGAN, N., WILSON, V.G. & DILLON, M.P. (1998). 'Seeing through a glass darkly': casting light on imidazoline 'I' sites. *Trends in Pharmacol Sci.,* **19,** 381 – 390.

FURCHGOTT, R.F. (1972). The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In *Handbook of Experimental Pharmacology*, Vol 33. ed. Blaschko, H. & Muscholl, E. pp 283 – 335. Berlin: Springer-Verlag.

GÖHERT, M., MOLDERINGS, G.J., FINK, K & SCHLICKER, E. (1995). α_2-Adrenoceptor-independent inhibition by imidazolines and guanidines of noradrenaline release from peripheral, but not central noradrenergic neurons. *Ann. New York Acad. Sci.,* **763,** 405 – 419.

GRANT, J.A. & SCRUTTON, M.C. (1979). Novel α_2-adrenoceptors primarily responsible for inducing human platelet aggregation. *Nature,* **277,** 659 – 661.

HIROSE, H., SETO, Y., MARUYAMA, H., DAN, K., NAKAMURA, K. & SARUTA, T. (1997). Effects of α_2-adrenergic agonism, imidazolines, and G-protein on insulin secretion in β cells. *Metabolism.,* **46,** 1146 – 1149.

JASPER, J.R., LESNICK, J.D., CHANG, L.K., YAMANISHI, S.S., CHANG, T.K., HSU, S.A.O., DAUNT, D.A., BONHAUS, D.W. & EGLEN, R.M. (1998). Ligand efficacy and potency at recombinant α_2-adrenergic receptors. *Biochem. Pharmacol.,* **55,** 1035 – 1043.

LOT, T.Y. & WILSON, V.G. (1994). Overnight storage of the porcine isolated splenic artery enhances endothelium-dependent contractions to N^G-nitro-L-arginine methyl ester without impairing endothelium-dependent dilator function. *Naunyn Schmiedeberg's Arch. Pharmacol.,* **349,** 95 – 100.

MALLARD, N.J., HUDSON, A.L. & NUTT, D.J. (1992). Characterization and autoradiographical localization of non-adrenoceptor, idazoxan binding sites in the rat brain. *Br. J. Pharmacol.,* **106,** 1019 – 1027.

MORGAN, N.G., CHAN, S.L.F., BROWN, C.A. & TSOLI, E. (1995). Characterization of the imidazoline binding site involved in regulation of insulin secretion. *Ann. New York Acad. Sci.,* **763,** 361 – 373.

MOURTADA, M., CHAN, S.L.F., SMITH, S.A. & MORGAN, N.L. (1999). Multiple effector pathways regulate the insulin secretory response to the imidazoline RX 871024 in isolated rat pancreatic islets. *Br. J. Pharmacol.,* **127,** 1279 – 1287.

ROBERTS, R., TOMLINSON, A.E., KENDALL, D.A. & WILSON, V.G. (1998). α_2-Adrenoceptor-mediated contractions of the porcine isolated ear artery: evidence for a cyclic AMP dependent and cyclic AMP-independent mechanism. *Br. J. Pharmacol.,* **124,** 1107–1114.

ROBERTS, R. KENDALL, D.A. & WILSON, V.G. (1999). NPY-mediated contractions of the porcine isolated ear artery: evidence for a cyclic AMP dependent and cyclic AMP-independent mechanism. *Br. J. Pharmacol.,* **127,** 284–290.

REGUNATHAN, S., FEINSTEIN, D.L. & REIS, D.J. (1999). Anti-proliferative and anti-inflammatory actions of imidazoline agents. Are imidazoline agents involved? *Ann. New York Acad. Sci.,* **881,** 410–419.

SATAKE, N., SHIBATA, S. & UEDA, S. (1984). Phentolamine-induced rhythmic contractions in bladder detrusor muscle of guinea-pig. *Br. J. Pharmacol.,* **83,** 965–971.

SCHOEFFTER, P. & HOYER, D. (1991). Interaction of the α-adrenoceptor agonist oxymetazoline with serotonin 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, and 5-HT_{1D}, receptors. *Eur. J. Pharmacol.,* **196,** 213–216.

SCHULTZ, A. & HASSELBLATT, A. (1989). An insulin-releasing property of imidazoline derivatives is not limited to compounds that block α-adrenoceptors. *Naunyn Schmiedeberg's Arch. Pharmacol.,* **340,** 321–327.

SCHWIETERT, R., WILHELM, D., WILLFERT, B. & VAN ZWIETEN, P.A. (1992). The effect of some α-adrenoceptor antagonists on spontaneous myogenic activity in the rat portal vein and putative involvement of ATP-sensitive K+ channels. *Eur. J. Pharmacol.,* **211,** 87–95.

TRENDELENBURG, A-U., LIMBERGER, N. & STARKE, K. (1996). The presynaptic alpha-2 autoreceptors in pig brain cortex are alpha-2A. *Naunyn Schmiedeberg's Arch. Pharmacol.,* **278,** 462–467.

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