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α-Adrenoceptor subtypes in dog saphenous vein that mediate contraction and inositol phosphate production

[1]Peter E. Hicks, Martine Barras, *Geneviève Herman, *Philippe Mauduit, J. Michael Armstrong & *Bernard Rossignol

Department of Pharmacology, Recherche Syntex France, 91310 Leuville-sur-Orge and *Laboratoire de Biochimie des transports cellulaires (CNRS VRA 1116), Université de Paris-Sud, 91405 Orsay, France

1 Studies have been made of the contractile responses to the α-adrenoceptor agonists phenylephrine (Phen), cirazoline (Cir) or BHT-920 (BHT) in dog isolated saphenous vein (DSV) rings, using the antagonists yohimbine (Yoh), idazoxan (Idaz), prazosin (Praz), WB-4101 (WB) and nitrendipine or zero Ca^{2+} medium.

2 Contractile concentration-response curves to Phen or BHT were displaced to the right of controls by Yoh (0.01–3 μM) with mean apparent antagonist dissociation constants (pK_Bs) of 7.9 and 8.6 respectively. Yoh did not show simple competitive antagonism against either agonist, since the Schild plot slopes were significantly less than unity. Neither the antagonist affinity of Yoh against Phen, nor the slope of the Schild plot was modified in the presence of catecholamine uptake inhibitors, nor in the presence of α,β-methylene ATP, which desensitizes P_2-purinoceptors, suggesting that Phen does not release ATP, or noradrenaline to cause contraction in DSV. In the presence of Praz (0.3 μM) the antagonist potency of Yoh (mean pK_B 7.4) against Phen was slightly decreased. Yoh had low potency against responses induced by Cir (pK_B 6.3).

3 WB (0.001–1.0 μM) was a very potent antagonist of Phen-induced contractions, however, the biphasic Schild plot against Phen could be separated into two affinity sites, a high pK_B of 9.3 (equivalent to that obtained using Cir as the agonist; pK_B 9.6) and a lower affinity (pK_B 8.6). WB showed an even lower antagonist affinity (pK_B 7.4) against BHT-induced contractions, suggesting that these effects might be mediated by α_{2A}-adrenoceptors. Praz also appeared to identify two sites using Phen-induced contractions, a high pK_B of 8.4 was equivalent to that obtained with Cir (pK_B 8.2) and a lower affinity site (pK_B 7.7; pA_2 7.6; slope 1.1) at which Praz showed competitive antagonism. Higher concentrations of Praz were required to antagonize contractions to BHT (pK_B 5.9).

4 Idaz was a weak partial agonist in this tissue with threshold contractile effects at concentrations in excess of 3 μM. Idaz (0.1–1 μM) competitively antagonized the contractile effects of BHT, but showed low antagonist affinity against Phen at these concentrations.

5 Contractions to Phen were slightly antagonized by nitrendipine (1 μM), with a 36% decrease in E_{max}. Contractions to Phen and Cir were also markedly attenuated in zero calcium medium (with EGTA), but maximum responses of 4.2 ± 0.1 and 3.6 ± 0.1 g, could be obtained with these agonists respectively. Only part of the contractile effects to Phen or Cir are therefore due to calcium influx (but L-type channels are not totally implicated), while the contractile effects of BHT were abolished in zero Ca^{2+} medium. Yoh (0.1 μM) retained its antagonist effects on Phen-induced responses in zero Ca^{2+} medium.

6 The formation of inositol phosphates (InsPs) in the presence of lithium (10 mM) was measured after incubation of intact DSV strips with myo-2-[^{3}H]-inositol. Phen (1–1000 μM) and Cir (0.01–10 μM) induced concentration-dependent increases in total labelled $InsP_{1-3}$, but BHT showed minimal InsP stimulation. InsPs were recovered after Phen (100 μM) stimulation (10 min) as labelled $InsP_1$ (71%), $InsP_2$ (25%) and $InsP_3$ (4%). Phen (100 μM)-stimulated $InsP_{1-3}$ formation was significantly antagonized by Praz (10 nM), but was not fully inhibited even after Praz 1 μM. Yoh and Praz (0.1 and 1.0 μM) were equipotent inhibitors of this response, while Idaz (0.3 μM) showed no effects.

7 The receptors in DSV which are stimulated by Phen to cause contraction show characteristics of the α_{1A}-adrenoceptor (high pM antagonist affinity for WB-4101 and extracellular calcium sensitivity) and the α_{1B}-adrenoceptor (contraction in calcium-free medium, increase in InsP and low nM antagonist affinity of WB). The paradoxical results obtained with Yoh (potent antagonist effects on Phen-stimulated PI and pK_B 7.9 on contraction) and Praz (low affinity competitive antagonist of Phen-induced contraction, pK_B 7.7 and failure to inhibit completely the PI response at 1 μM), cannot fully exclude an α_{2B}-subtype characterization of these responses. These pharmacological differences suggest that the adrenoceptor involved in the contractile and in particular the second messenger effects of Phen in DSV is not typically an α_{1B}-adrenoceptor.

Introduction

The dog saphenous vein (DSV) is known to contain a mixed population of postsynaptic α_1- and α_2-adrenoceptors (Sullivan & Drew, 1980; De Mey & Vanhoutte, 1981; Shepperson & Langer, 1981; Constantine *et al.*, 1982; Fowler *et al.*, 1984; Alabaster *et al.*, 1985; Flavahan & Vanhoutte, 1986a; Guimarães *et al.*, 1987; Eskinder *et al.*, 1988) and as such is a widely used preparation for the study of α-adrenoceptors. In addition, the α-adrenoceptor pharmacology in DSV shows striking similarities to human saphenous vein (Beckeringh *et al.*, 1987; Eskinder *et al.*, 1988).

The majority of evidence for an α-adrenoceptor heterogeneity in DSV comes from the use of selective α_1 and α_2-adrenoceptor agonists and antagonists. However, contractions evoked by phenylephrine (Phen) (usually considered as α_1; McGrath, 1982) are only weakly antagonized by prazosin (Praz) in DSV (Shoji *et al.*, 1983; Alabaster *et al.*, 1985; Akers

[1] Author for correspondence.

et al., 1987; Hicks et al., 1987, Guimarães et al., 1987; Eskinder et al., 1988). Furthermore Guimarães et al. (1987) have concluded that Phen (and also methoxamine) can stimulate α_2-adrenoceptors to cause contraction in DSV, since after partial receptor occlusion with the irreversible antagonist phenoxybenzamine, the contractions to these agonists were potently antagonized by yohimbine (Yoh), at concentrations which also blocked the contractions induced by the α_2-selective agonist UK-14304 (Cambridge, 1981). Stimulation of α_2-adrenoceptors by Phen has recently been implicated in human resistance vessels (Hair et al., 1988). However, a number of observations are not consistent with Phen acting as an α_2-adrenoceptor agonist in DSV. Rauwolscine (pA_2 8.7 vs BHT-920 or xylazine; Flavahan et al., 1984, or UK-14304; pA_2 8.5; Alabaster et al., 1985) was shown to be less potent against Phen-induced contractions in this tissue (pA_2 6.9; Alabaster et al., 1985).

It is known that Phen increases $^{45}Ca^{2+}$ uptake in DSV (Janssens & Verhaeghe, 1984), which is resistant to blockade by nifedipine (Jim et al., 1985). Furthermore calcium antagonists are less effective as antagonists of Phen-induced responses in this tissue relative to their marked inhibitory effects against responses induced by α_2-adrenoceptor agonists such as UK-14304 (Flavahan & Vanhoutte, 1986a; Guimarães et al., 1987), BHT-920 (Cooke et al., 1985; Hicks et al., 1988) or the aminotetralin M7 (Cavero et al., 1983). Phen-induced contractions in DSV are therefore at least partly dependent on calcium entry, although not entirely through dihydropyridine-sensitive calcium channels. In contrast to the α_2-adrenoceptor agonist BHT-920, Phen can also stimulate intracellular calcium release as shown by $^{45}Ca^{2+}$ efflux (Janssens & Verhaeghe, 1984; Jim & Matthews, 1985).

At least two second messenger systems are known to be involved in agonist-mediated intracellular Ca^{2+} release. G protein(s) functionally couple receptors to phospholipase C, which subsequently hydrolyses membrane bound phosphatidylinositol-4,5-bisphosphate to form inositol 1,4,5-trisphosphate (Ins[1,4,5]P_3) which then releases calcium from the endoplasmic reticulum (Berridge & Irvine, 1984; 1989; Abdel-Latif, 1986). A second pathway involves the formation of diacylglycerol which activates protein kinase C and may be responsible for a sustained response and/or promotion of calcium entry. A number of investigations on phosphoinositide (PI) hydrolysis stimulated by α-adrenoceptor agonists, including Phen, have been reported in blood vessels (Zeleznikar et al., 1983; Campbell et al., 1985; Fox et al., 1985; Heagerty et al., 1986; Chiu et al., 1987; Ollerenshaw et al., 1988; Eid & De Champlain, 1988; Tsujimoto et al., 1989); however, it is difficult to ascertain the absolute affinity of Praz in antagonizing these effects, since many workers have only used high concentrations of the antagonist to block the PI-response.

In the course of our studies on DSV we have routinely observed Yoh to be a potent antagonist of Phen-mediated contractions in this tissue, consistent with reported literature (see above), but with higher antagonist affinity than usually associated with Yoh at classical α_1-adrenoceptors in other tissues (Weitzell et al., 1979; Drew, 1985), with the possible exception of rat aorta (Ruffolo et al., 1981). Phen can readily contract DSV in calcium-free medium (Janssens & Verhaeghe, 1984) suggesting intracellular calcium release. These observations, coupled with the recent proposal that phenylethylamine agonists such as Phen or methoxamine can apparently stimulate α_2-adrenoceptors (Guimarães et al., 1987) in DSV particularly under conditions of low receptor reserve, has therefore prompted further analysis of the contractile and second messenger (InsP formation) effects of this agonist with the aim of clarifying the identity of the α-adrenoceptor subtype involved in these effects.

Part of this work has been presented in abstract form to the British Pharmacology Society (Barras et al., 1989) and the 7th meeting on Adrenergic mechanisms, PORTO, Oct. 1989, (Hicks et al., 1989).

Methods

Preparation

Saphenous veins were obtained from mongrel dogs of either sex under pentobarbitone (35 mg kg^{-1}, i.v.) anaesthesia. Tissues were either used fresh or after 24 h at 4°C storage in oxygenated Krebs bicarbonate. The contractions induced by either KCl (80 mM), or Phen (0.1–100 μM) were not significantly different between fresh or 24 h-stored tissues as previously reported (Eskinder & Gross, 1986). Veins cleared of connective tissue were cut into rings of approximately 5 mm length and were denuded of endothelium by carefully rubbing with forceps. The success of this technique was shown in selected tissues by demonstrating the failure of acetylcholine (ACh) to relax a Phen-mediated contraction. Although ACh-induced endothelium-dependent relaxations are smaller in DSV than in other arteries, they can nevertheless be demonstrated in the presence of an intact endothelium.

Functional responses

Preparations were mounted in 10 ml organ baths on steel hooks, under 2 g resting tension in Krebs bicarbonate solution (PSS) at 37°C bubbled with 95% O_2 and 5% CO_2. The PSS was of the following composition (mM): NaCl 117, KCl 4.7, $NaHCO_3$ 25, $MgSO_4$ 1.2, KH_2PO_4 1.0, $CaCl_2$ 2.5, glucose 11 and contained propranolol (1 μM). Except for a specific study, inhibitors of neuronal or extraneuronal uptake were not routinely included since the contractile effects of Phen or BHT-920 were shown not to be modified in DSV by desipramine or corticosterone. Isometric contractile concentration-response curves were obtained in separate preparations using Phen, BHT-920 (BHT) or cirazoline (Cir) and responses displayed on a Gould BS-274 chart recorder. Two consecutive concentration-response curves were obtained to either BHT or Phen before incubating tissues with the antagonist. Antagonists were evaluated at varying concentrations, only one concentration being used in each preparation ($n = 4$–7 tissues/group). Appropriate concentrations of antagonists were added to the bath for a period of 30 min before repeating a second or third agonist-response curve. As the effects of cirazoline washed out slowly, only one concentration-response curve per preparation was obtained to this agonist in vehicle or antagonist-treated DSV rings after an initial contraction to KCl (80 mM) which served as a 100% reference response.

For studies in Ca^{2+}-free medium, preparations were first contracted with KCl (80 mM) in Krebs containing [Ca^{2+}] 2.5 mM, followed by 1 h incubation in zero Ca^{2+}-Krebs in the presence of EGTA (2 mM). Phen, Cir and BHT were then studied under these conditions. In a further series of experiments, concentration-response curves to Phen were constructed in separate preparations ($n = 6$/group) incubated for 30 min with or without Yoh (0.1 μM).

Incorporation of [^{3}H]-inositol into phospholipids

DSV rings were prepared as described above and then cut open into rectangular strips (weighing about 10–17 mg). These were incubated at 37°C for 3 h in PSS buffer supplemented with 5.5 mM glucose and propranolol (1 μM), containing myo-2-[^{3}H]-inositol (60 μCi ml^{-1}) and equilibrated in 95% O_2; 5% CO_2.

A preliminary study on the time course of incorporation of [^{3}H]-inositol into membrane phospholipids, shows that equilibrium between intracellular medium and phospholipids is reached after 3 h of incubation.

After this time DSV strips were washed three times with unlabelled PSS buffer containing 10 mM LiCl and further incubated in PSS for 10 min. Results from this study show that a significant increase of the label (about 30%) into total phosphatidylinositol phosphates (PtdInsPs) occurred after stimulation with Phen (100 μM; for 10 min; Table 1).

Table 1 Incorporation of [^{3}H]-inositol into membrane phospholipids and the recovery of labelled $InsP_1$, $InsP_2$, $InsP_3$ and total $InsP_{1-3}$ in control (non-stimulated) or phenylephrine (Phen 100 μM, for 10 min)-stimulated dog saphenous vein strips in the presence of 10 mM LiCl

	Total PtdIns	*$InsP_{1-3}$*	*$InsP_1$* (c.p.m. mg^{-1} tissue)	*$InsP_2$*	*$InsP_3$*
Control (n = 10)	6422 ± 1518	270 ± 85 (100%)	183 ± 27 (67%)	58 ± 11 (21.5%)	29 ± 6 (11%)
Phen(100 μM)(n = 11)	8638 ± 2397	1760 ± 203* (100%)	1257 ± 154* (71%)	432 ± 65* (25%)	72 ± 17 (4%)
Stim. over basal	29%	552%	587%	645%	148%

Values shown in parentheses are the percentage recovery of individual InsPs with respect to total $InsP_{1-3}$. Stimulation over basal is the % increase with respect to non stimulated controls. Total PtdInsPs are PtdIns, PtdInsP and $PtdInsP_2$.
* Significantly greater than control (P less than 0.05)

Measurement of labelled inositol phosphate production

Separate groups of tissues were then treated for an additional 30 min with either vehicle or antagonists under study. In these experiments, preparations were stimulated with either Phen (1–1000 μM), Cir (0.1–10 μM) or BHT (0.1–10 μM) for 10 min periods. Total recovery of [^{3}H]-InsPs comprised $InsP_1$ (71%), $InsP_2$ (25%) and $InsP_3$ (4%) after stimulation with Phen (100 μM; for 10 min; Table 1). No measurement of InsP isomers was attempted. Each incubation was stopped by filtration and plunging each tissue (in 1 ml of ice-cold H_2O) into liquid N_2. Strips were then lyophilised and weighed. After addition of TCA (7.5% wt/vol), acid extraction of water-soluble inositol metabolites was performed and each tube further frozen at −20°C for 30 min. Subsequent thawing of frozen tissue was sufficient to liberate all the InsPs. This simple manoeuvre obviated the tissue homogenization which is extremely difficult in blood vessels, due to the large proportion of elastic/fibrous components.

After centrifugation for 15 min at 20,000 g at 0°C, the inositol phosphate-containing supernatants were treated (5 × 4 ml) with water-saturated diethylether to remove TCA and then neutralized at pH between 6 and 7 with 100 mM Tris base. The supernatants were applied to anion-exchange columns containing 800 mg of Bio-Rad AG 1-X8, (200–400 mesh, formate form, Richmond, Ca). Inositol and the different inositol phosphates were eluted stepwise according to Berridge (1983) by the successive addition of solutions containing increasing amounts of ammonium formate. An aliquot of each fraction was counted for radioactivity after addition of an equal amount of scintillant (Ready gel, Beckmann). The 20,000 g pellets containing the [^{3}H]-inositol-rich phospholipids were dissolved in 2 ml of 1N NaOH, and the radioactivity content was determined by liquid scintillation.

Calculation of results

Functional responses Concentration-contractile response curves to the various agonists were obtained in the presence of various concentrations of antagonists and the results represented graphically.

Antagonist concentration-ratios (CR) were then calculated at the EC_{50} level of the agonist in the presence of each concentration of antagonist. Apparent antagonist dissociation constants (pK_B) were calculated for each concentration of antagonist by the methods of Furchgott (1972). Where quoted, mean pK_B refers to the averaged values over a given antagonist concentration-range. For the experiments conducted with Cir, EC_{50} values calculated for control tissues were used for the calculation of K_B.

$$\text{where } K_B = \frac{\text{concentration of antagonist } [-\log \text{M}]}{\text{CR} - 1}$$

Where relevant, pA_2 values were calculated by the method of Arunlakshana & Schild (1959). Due to the biphasic nature of some of the Schild plots, differences in pK_B values were also statistically evaluated between treatment groups at different concentrations of the antagonist using a non-paired Student's t test. Significance was accepted at 5%.

Calculation of labelled inositol phosphates The amounts of each labelled inositol phosphate ($InsP_1$, $InsP_2$ and $InsP_3$) were cumulated and expressed as c.p.m. mg^{-1} lyophilised tissue. Total [^{3}H]-InsP formation in response to increasing concentrations of agonist was calculated and expressed as stimulation over basal in non-treated or antagonist treated preparations. In some experiments tissue dry weight was correlated with protein content by the method of Lowry *et al.* (1951). One mg lyophilized tissue was equivalent to 0.4 mg protein and represented about 24% of wet tissue weight.

Drugs

The following drugs were used: yohimbine HCl (Sigma); WB-4101 (*N*-[2-(2,6-dimethoxyphenoxy)-ethyl]1,4-benzodioxane-2-methylamine, Research Biochemicals Inc.); (−)-phenylephrine HCl (Sigma); BHT-920 HCl ([2-amino-6-allyl-5,6,7,8-tetrahydro-4*H*-thiazolo-(4,5-d)azepine] Boehringer Ingleheim); idazoxan HCl and prazosin HCl (synthesized by Syntex Palo Alto); (±)-propranolol HCl (Sigma), cirazoline HCl (Synthelabo); nitrendipine (synthesized by Syntex France); myo-2-[^{3}H]-inositol (Amersham); α,β-methylene adenosine triphosphate HCl (α,β-MeATP) and β,γ-methylene adenosine triphosphate HCl (β,γ-MeATP) (Sigma); desipramine HCl (Sigma); corticosterone acetate (Sigma). Corticosterone was dissolved in ethanol, all other compounds were solubilized and diluted in distilled water.

Results

Functional responses in dog saphenous vein

Concentration-dependent contractile-response curves obtained to Phen or BHT-920 were progressively displaced to the right of controls by Yoh (0.001–3 μM) with little change in the maximum responses to these agonists (Figure 1). In control tissues three subsequent response curves to these agonists were superimposible. High concentrations of Yoh (10 μM) were, however required to displace the Cir-induced contractile response curves to the right of controls (Figure 1c). The calculated mean pK_B value for all concentrations of Yoh against Phen was 7.9 ± 0.2 and against BHT was 8.6 ± 0.1. Schild analysis of these data gave apparent pA_2 values of 8.2 and 8.8 respectively, although the slope of each Schild plot was less than unity (Figure 2a; Table 2). Log(CR − 1) data calculated for either agonist in the presence of Yoh were significantly different at each concentration of antagonist tested (Figure 2a). However, strict competitive antagonism was not demonstrated by Yoh against either Phen or BHT.

Incubation of DSV rings with desipramine (1 μM) and corticosterone (40 μM) did not modify the concentration-response curve to Phen, the antagonist affinity or the slope of the Schild plot for Yoh against Phen (Table 2).

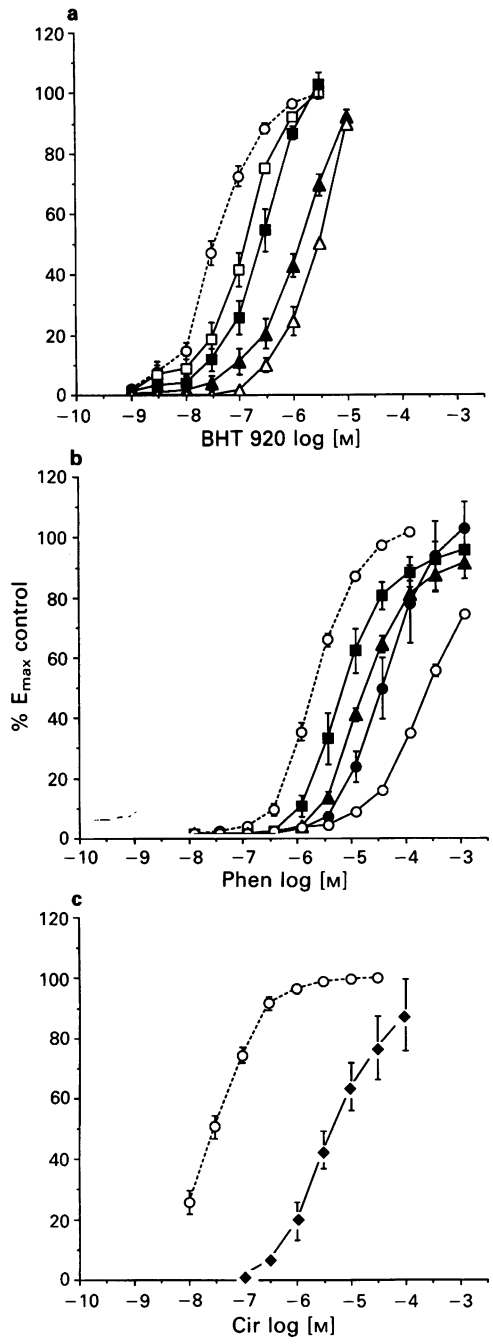

Figure 1 Contractile concentration-response curves for (a) BHT 920, (b) phenylephrine (Phen) and (c) cirazoline (Cir) in dog isolated saphenous vein rings in the absence (○- - - -○), or presence of different concentrations of yohimbine (μM): 0.001 (□- - - -□); 0.01 (■———■); 0.1 (▲———▲); 0.3 (△———△); 1 (●———●); 3 (○———○), and 10 (◆———◆). Results are expressed as % max response to each agonist with s.e. mean shown by vertical bars. n = 4–7 preparations/curve.

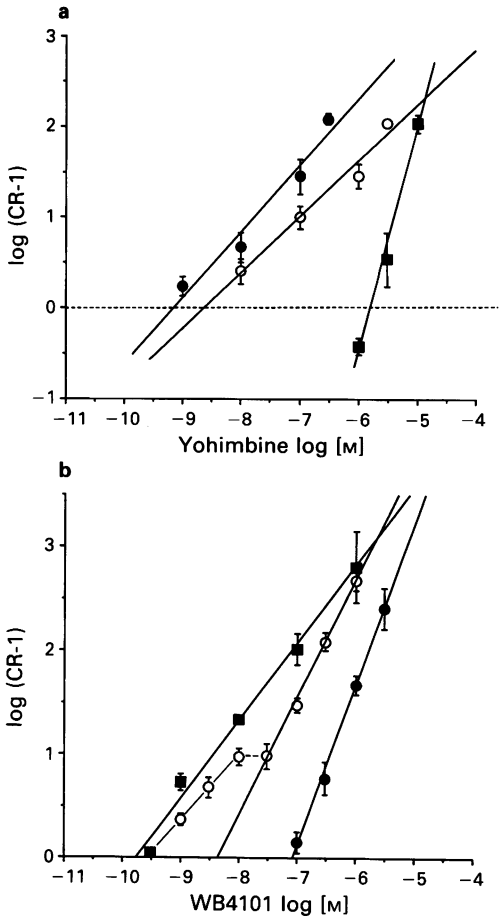

Figure 2 Schild plots (log[concentration-ratio] − 1 versus negative log[M] of antagonist) for yohimbine (a) or WB-4101 (b) against contractile responses induced by cirazoline (■), phenylephrine (○) or BHT-920 (●). Each point represents the mean of 4–7 preparations at each concentration; vertical bars show s.e.mean.

Yoh was a weak affinity antagonist (mean pK_B of 6.3 ± 0.2; Figure 2a; Table 2) against Cir-induced contractions, but a greater antagonist affinity value was obtained after Yoh (10 μM; 6.9 ± 0.1), than at 3 μM (pK_B 6.2 ± 0.1).

Praz (1 μM) failed to antagonize the contractile effects of BHT and Praz (0.01 − 1 μM) showed relatively weak antagonist effects against Phen-induced contractions. A 'high' affinity antagonist effect on Phen-induced responses was seen with Praz at 0.01 μM (pK_B 8.4) with a lower affinity antagonist effect over the concentration-range 0.1–1 μM, which was apparently competitive (pK_B 7.7, pA_2 7.6; slope 1.1; Table 2). Against responses to Cir, only one antagonist affinity value for Praz could be derived (mean pK_B 8.2 ± 0.1; pA_2 8.4, slope 0.8; Table 2).

In the presence of Praz (0.3 μM), the antagonist effects of Yoh (mean pK_B 7.4) against Phen were slightly decreased, however, the antagonist potency of Yoh against BHT-920 was not altered by Praz (1 μM; Table 2).

The α_2-adrenoceptor antagonist Idaz (0.1–1 μM) caused concentration-dependent, parallel rightward displacements of the BHT response curve (not shown). The calculated antagonist affinity for Idaz against BHT responses (mean pK_B 7.5; pA_2 7.6; slope 0.9; Table 2) was higher than against Phen (pK_B 6.2) or Cir (pK_B 5.9)-induced responses. At higher concentrations Idaz (3–100 μM) caused weak contractile effects in DSV.

WB-4101 (WB: 0.001–1 μM) progressively displaced the Phen-induced contractile response curve to the right of controls (Figure 3) and was the most potent antagonist tested. Over the range 0.001–0.03 μM WB, the response curves to Phen were not displaced proportionally to the increases in antagonist concentration. Close inspection of the Schild plot for WB showed two apparent slopes for this antagonist against Phen (Figure 2b) and allowed the calculation of a high affinity (mean pK_B 9.3; interpolated pA_2 9.4) and a lower affinity (mean pK_B 8.6; interpolated pA_2 8.4). WB also showed high antagonist affinity against responses induced by Cir and was a competitive antagonist against this agonist over the concentration-range 0.0003–1 μM (mean pK_B 9.6; pA_2 9.7; slope 0.8; Table 2).

Responses induced by BHT were antagonized by WB over the concentration-range 0.1–3 μM (Figure 2b) with a mean pK_B

Table 2 Relative antagonist affinities (pA_2 or mean pK_B) of α-adrenoceptor antagonists against contractions elicited by BHT-920, cirazoline (Cir) or phenylephrine (Phen) in dog saphenous vein

Agonist		*Yoh*	*Praz* High	*Praz* Low	*Yoh + Praz*	*Idaz*	*WB-4101* High	*WB-4101* Low
BHT-920	pA_2	8.8	—	—	—	7.6	7.1	
	slope	0.7a	—	—	—	0.9	1.4a	
	pK_B	8.6 ± 0.1*	—	5.9 ± 0.2b	8.7 ± 0.1b	7.5 ± 0.2	7.4 ± 0.2*	
Cir	pA_2	6.1	8.4	—	—	—	9.7	—
	slope	1.4	0.8	—	—	—	0.8	—
	pK_B	6.3 ± 0.2*	8.2 ± 0.1*	—	—	5.9 ± 0.2	9.6 ± 0.2	—
Phen	pA_2	8.2		7.6	8.1	—	9.4	8.4
	slope	0.6a	—	1.1	0.6a	—	0.9	0.9
	pK_B	7.9 ± 0.2	8.4 ± 0.1e	7.7 ± 0.2	7.4 ± 0.2c	6.2 ± 0.1	9.3 ± 0.2e	8.6 ± 0.1
Phen/desipramine/corticosterone								
	pA_2	8.6						
	slope	0.5a						
	pK_B	7.9 ± 0.2		NT	NT	NT	NT	
Phen/α,β-MeATP								
	pK_B	7.9 ± 0.2d		NT	7.6 ± 0.2c	NT	NT	

Yoh = yohimbine, Praz = prazosin, Idaz = idazoxan, α,β-MeATP, α,β-methylene ATP
* Significantly different from Phen, P less than 0.05.
(a) Significantly different from unity, P less than 0.05.
(b) Prazosin at 1 μM; (c) prazosin at 0.3 μM; (d) yohimbine at 0.1 μM. (e) Significantly different from Phen low, P less than 0.05.
Slope calculated from log(CR − 1) vs −log[M] antagonist; pK_B = −log M antagonist dissociation constant.
NT, not tested.

7.4. The slope of the Schild plot was however, greater than unity (Table 2).

Incubation of DSV rings with the P_2-purinoceptor desensitizing agent α,β-MeATP (10 μM) or the P_2-receptor agonist β,γ-MeATP (100 μM), evoked contractile responses (5.0 ± 0.5 g, and 6.2 ± 0.6 g respectively; n = 5/group). These contractile effects waned to base-line tension within 10 min. Incubation of a further group of DSV (n = 5) with α,β-MeATP (10 μM) abolished the contractile effects induced by β,γ-MeATP (100 μM).

In the presence of α,β-MeATP (10 μM) and Praz (0.3 μM) the contractile response curves to Phen were unchanged from those obtained in the absence of the P_2-receptor desensitizing agent. The apparent antagonist affinity of Yoh against Phen (0.1 μM; pK_B 7.9 ± 0.2) was not significantly modified by α,β-MeATP and Praz (Table 2).

Effects of nitrendipine and zero calcium medium on responses to phenylephrine

Concentration-dependent contractile effects of Phen were displaced slightly to the right of controls by nitrendipine (1 μM) in a non-competitive manner with a 36% decrease in the maximum effect of Phen (Figure 4b). Nitrendipine (10 μM) failed to modify further the contractions induced by Phen (not shown). These concentrations of nitrendepine were far greater than those required to antagonize voltage-operated calcium channels in this tissue, since the pIC_{50} for nitrendipine to relax KCl (80 mM)-induced contractions was 8.7 ± 0.2; n = 8.

In zero calcium medium, Phen caused concentration-related contractions over the range 1 to 1000 μM and although these responses were markedly reduced with respect to control tissues, Phen was capable, under these conditions, of evoking contractions of 4.2 ± 0.8 g tension (Figure 4b). Under the same conditions of zero calcium, Cir was also capable of contracting DSV (2.8 ± 0.5g), but BHT responses were abolished (Figure 4a). Under normal incubation conditions, Yoh (0.1 μM) displaced the Phen-induced contractile response curve to the right of controls (Figure 4c). In the presence of Yoh (0.1 μM) the Phen-induced contractile response curves in zero calcium medium were also displaced to the right of control non-Yoh-treated tissues, albeit with depression of the maximum response (Figure 4d).

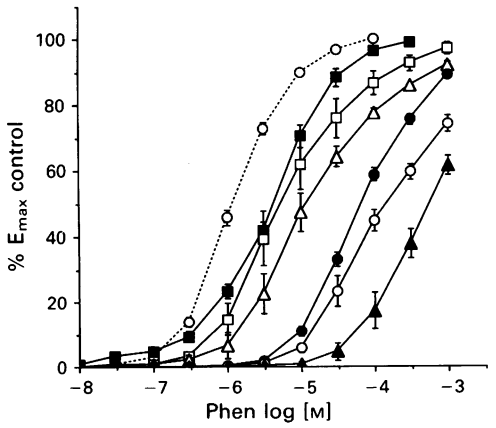

Figure 3 Contractile concentration-response curves for phenylephrine (Phen) in dog isolated saphenous vein rings in the absence (○- - - -○; n = 26), or presence of different concentrations of WB-4101. Concentrations (μM) are 0.001 (■———■); 0.003 (□———□); 0.03 (△———△); 0.1 (●———●); 0.3 (○———○); 1 (▲———▲). Results are expressed as % max response to Phen; vertical bars show s.e.mean. n = 4–7 preparations for each concentration of WB.

Measurement of labelled inositol phosphate production

Preparations of DSV incubated with [^{3}H]-inositol for 3 h readily incorporated the label into membrane polyphosphoinositides (PtdIns). A typical elution profile for labelled $InsP_1$, $InsP_2$ and $InsP_3$ is shown in two DSV strips after 10 min stimulation with 100 μM Phen or under non-stimulated conditions (Figure 5).

Small amounts of glycerophosphoinositol (GroPIns) were measured under basal conditions, which remained unchanged after Phen stimulation. $InsP_1$ and $InsP_2$ increased about 7 fold after Phen (100 μM), while $InsP_3$ increased 2.5 times at 10 min (Table 1).

Phen (1 to 1000 μM) and Cir (0.1–10 μM) caused concentration-dependent increases above base-line in total [^{3}H]-$InsP_{1-3}$ formation (10 min stimulation), while BHT showed only weak InsP stimulating effects, which were not concentration-related (Figure 6b). The comparative contractile and InsP stimulating effects of Phen, Cir and BHT are also shown in Figure 6. Cir and BHT were more potent contractile agonists than Phen in DSV, while Cir showed greater intrinsic contractile effects than the other agonists.

A single concentration of Phen (100 μM) was used to stimulate total [^{3}H]-$InsP_{1-3}$ formation in antagonist studies. Praz (0.01 μM) inhibited by 34% the Phen-stimulated PI response,

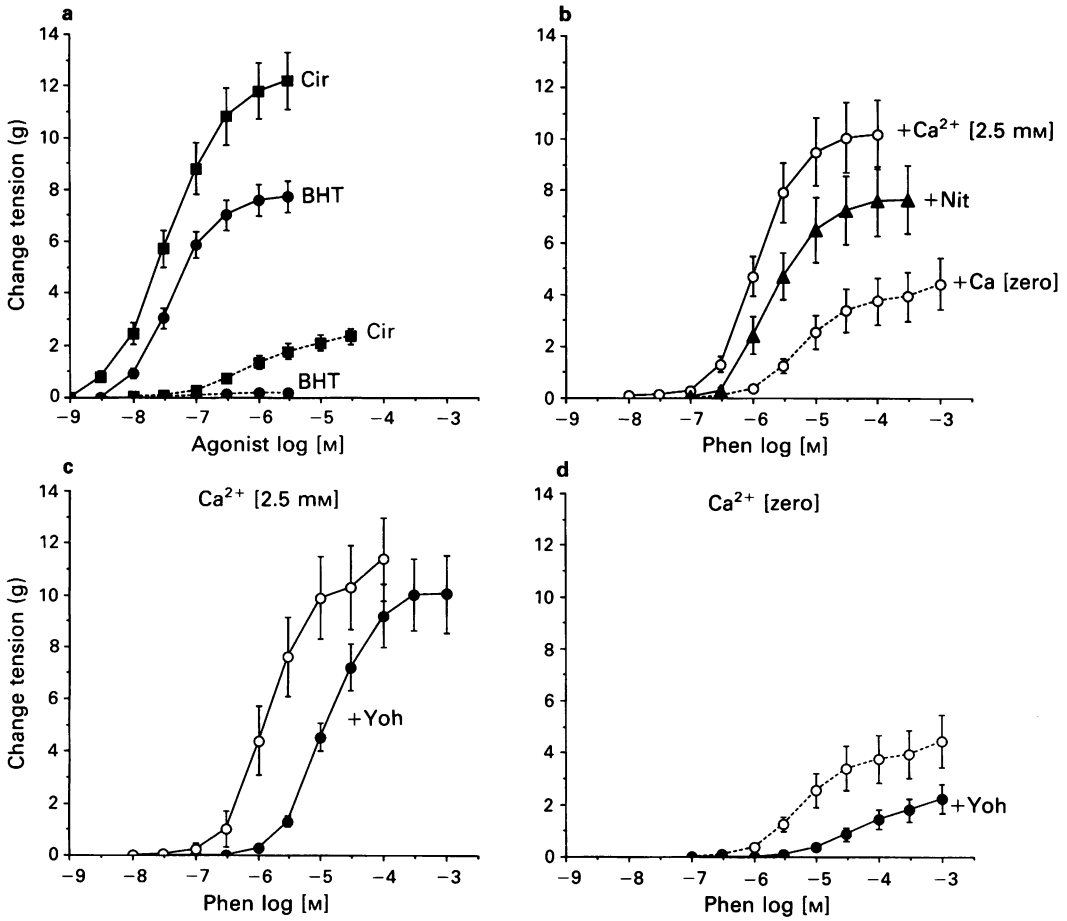

Figure 4 (a) Concentration effect response curves (change tension, g) for cirazoline (Cir, ■) or BHT-920 (●) in dog saphenous vein in calcium 2.5 mM Krebs (———) or calcium-free medium containing EGTA (- - - -). (b) Contractile concentration-response curves (change tension, g) for phenylephrine (Phen) in dog isolated saphenous vein rings in calcium [2.5 mM] in the absence (O———O) or presence of nitrendipine (Nit 1.0 μM; ▲———▲), or in zero calcium medium containing EGTA (Ca[zero]: O- - - -O). (c) Concentration-response curves (change tension, g) for Phen in calcium 2.5 mM Krebs, before (O———O) and after 30 min treatment with yohimbine (Yoh; 0.1 μM; ●———●). (d) Concentration-response curves (change tension, g) for Phen in zero calcium medium (Ca^{2+} [zero]), before (O- - - -O) or after 30 min treatment with yohimbine (Yoh; 0.1 μM; ●———●). Vertical bars indicate s.e.mean.

but even the highest concentration of Praz (1 μM), failed to inhibit completely these effects of Phen (Figure 7). Yoh (0.01 μM), showed no inhibitory effects on Phen-stimulated InsP formation; however, at 0.1 μM and 1.0 μM both Praz and Yoh showed equivalent antagonist effects on this response (Figure 7). Although Idaz showed contractile effects in DSV at concentrations above 3 μM, Idaz showed no effects on basal InsP formation at 0.3 μM and at this concentration failed to inhibit Phen-stimulated InsP formation (Figure 7).

Discussion

The results of this study confirm previously published data that Phen-induced contractions of the DSV are potently antagonized by Yoh (Sullivan & Drew, 1980; Guimarães *et al.*, 1987). However, the rather weak antagonist effects of Praz previously reported against this agonist in DSV (Alabaster *et al.*, 1985, Akers *et al.*, 1987; Eskinder *et al.*, 1988; Shi *et al.*, 1989), comprise high and low affinity sites. In contrast to Yoh, idazoxan was a weak antagonist of Phen-induced contractions. WB-4101 was a very potent antagonist of Phen and Cir-induced contractile responses in DSV and demonstrated two apparent affinity values against Phen, which were both higher than the affinity against BHT-induced contractile responses. Contractions to Phen were only partly dependent on extracellular Ca^{2+}, since responses (although much reduced) were still obtained in zero calcium medium. Nitrendipine showed weak inhibitory effects on Phen-induced contractions, suggesting a minimal influence of L-type calcium channels in these responses. Phen and Cir (at high concentrations), but not BHT, increased InsP production in a concentration-dependent manner in DSV and the effects of Phen were potently antagonized by Yoh, but not by Idaz.

Antagonism by Yoh of both Phen-induced contractions and PtdIns hydrolysis, might imply that these effects are mediated through α_2-adrenoceptors, as suggested by Guimarães *et al.* (1987). However, preliminary results in DSV which show rauwolscine to be inactive on Phe-stimulated InsP formation (Rees & Matthews, 1986) and weakly active on contraction (Alabaster *et al.*, 1985) and failure of Idaz to block either Phen (present study) or NA-stimulated InsP production in rat femoral vein (Stubbs *et al.*, 1988) strongly argue against an α_2-adrenoceptor involvement in PtdIns hydrolysis in this or other tissues.

Can the Phen-induced contractile effects in DSV be considered an α_2-response? The current nomenclature identifies at least two subtypes of α_2-adrenoceptors (Bylund, 1988). α_{2A}-Sites are labelled with high affinity in various tissues with antagonists which include Yoh, rauwolscine and Idaz. Human α_2-platelet clone (α_2-C10; Regan *et al.*, 1988) is defined as α_{2A}. Affinity values for Praz obtained at α_{2A}-adrenoceptors are usually in the high nM to low micromolar range, consistent with pA_2 determinations in functional tests. The contractile responses of DSV to agonists such as BHT (and UK-14304; Alabaster *et al.*, 1985) are, therefore, likely to be α_{2A}-adrenoceptor mediated.

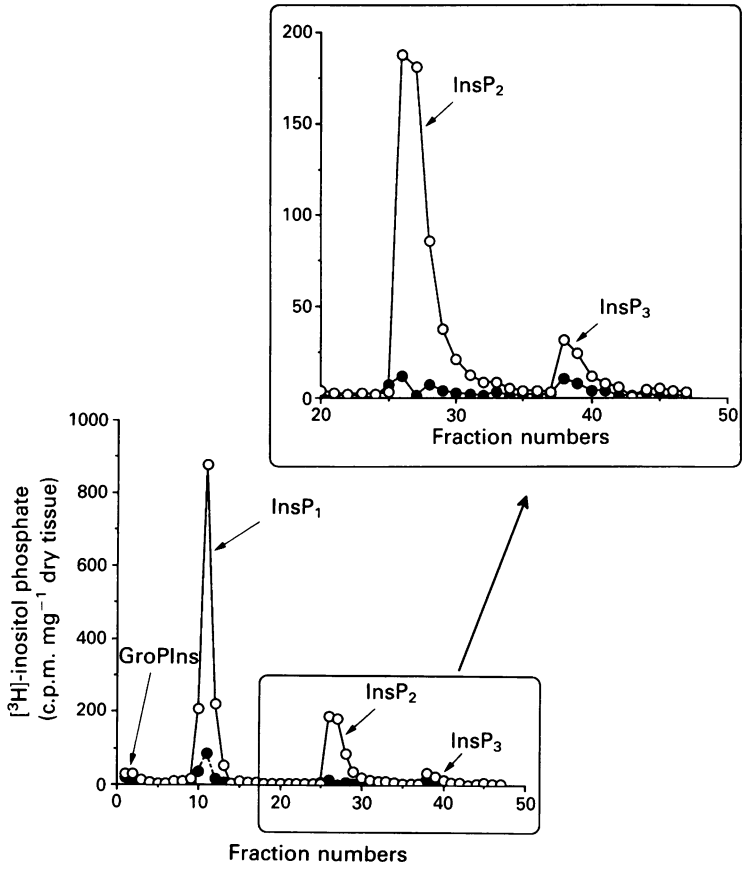

Figure 5 Typical elution profile of [^{3}H]-inositolphosphates (glycerophosphoinositol; GroPIns; $InsP_1$; $InsP_2$ and $InsP_3$; c.p.m. mg^{-1} dry tissue) under basal conditions (●) or after 10 min stimulation with phenylephrine (○, 100 μM) in dog isolated saphenous vein strips.

The failure of Praz to block contractions to BHT (pK_B 5.9 present study) except at high concentrations, is consistent with the potency of Praz at presynaptic α_2-sites in functional studies (see Drew, 1985 and refs therein) and on [^{3}H]-rauwolscine or yohimibine binding in cerebral cortex and other tissues (Cheung *et al.*, 1982; Bylund *et al.*, 1988; Bylund, 1988; Michel *et al.*, 1989b), or human α_2-platelet clone (α_2-C10; Regan *et al.*, 1988). While Yoh showed higher antagonist potency against contractile responses to BHT in DSV, than normally associated with its presynaptic α_2-adrenoceptor antagonist effects (Ruffolo *et al.*, 1981), Idaz showed considerable selectivity for BHT-induced contractile responses in DSV (pK_B 7.5) compared with Phen (pK_B 6.2). The adrenoceptors which are stimulated by Phen to cause contraction and InsP formation in DSV do not therefore appear to be α_{2A}-adrenoceptors.

α_{2B}-Adrenoceptors have been characterized by use of [^{3}H]-yohimbine (or rauwolscine) in kidney from several animal species (Summers, 1984, Michel *et al.*, 1989b) and neonatal rat lung (Latifpour *et al.*, 1982). cDNA human kidney clone (α_2-C4; Regan *et al.*, 1988) is considered representative of the α_{2B}-adrenoceptor. Affinity values for Praz in the low nM range obtained with labelled Yoh or rauwolscine as ligands, also characterize an α_{2B}-site, although no functional correlate has so far been identified. To date, no postsynaptic α_2-adrenoceptor has been demonstrated to be coupled through phospholipase C and it therefore appears unlikely that the inhibitory effects of Yoh on Phen-stimulated PI are mediated by α_2-adrenoceptors. Finally, the relative agonist selectivities of both BHT-920 and Phen at α_{2A} or α_{2B}-adrenoceptors respectively, would need to be very high to account for the antagonist selectivity found with WB (80 fold selectivity for Phen over BHT contractions) and with Idaz, which retained a 15 fold selectivity for BHT over Phen. The relative selectivity/affinity of these agonists for α_{2A} or α_{2B}-sites, assessed on human α C_{10}/C_4 cloned receptors (Regan *et al.*, 1988), showed a 2 fold selectivity for Phen at the α_{2B}-site while BHT was not selective for either site.

How then can the receptors which mediate contraction and InsP formation to Phen in DSV be characterized? Firstly, the antagonist effects of Yoh against Phen occurred at slightly higher concentrations than those required to antagonize responses to BHT (5–8 fold difference in mean pK_B). The antagonist effects of Yoh against both of these agonists did not, however, show simple competitive antagonism, since the slopes of the Schild plots were less than unity. These observations have important implications, since it clearly shows the necessity of evaluating antagonists using small concentration increments over a wide range, particularly when heterogeneous receptor populations are present. Schild plots with slopes significantly less than unity, or which are biphasic can arise for a number of reasons (Kenakin, 1982; Milnor, 1986), although receptor heterogeneity remains a probability when other factors are controlled. The fact that inclusion of desipramine and corticosterone in the bathing medium did not modify the contractile-response curve to Phen, or change the antagonist affinity or the slope of the Schild plot for Yoh, suggests that Phen is not a good substrate for either neuronal or extraneuronal uptake in DSV. Phen does not appear to have an indirect sympathomimetic action, which by releasing endogenous noradrenaline, might account for the low Schild slopes obtained. The routine absence of uptake blockers in the present study should not therefore confound the results obtained. Nevertheless, a surprising finding in this study was that Praz, although weak (relative to its α_1-antagonist affinity in other tissues) as a blocker of Phen-induced responses in DSV (Alabaster *et al.*, 1985; Akers *et al.*, 1987; Eskinder *et al.*, 1988; Shi *et al.*, 1989), did not increase the antagonist affinity of Yoh against Phen when the two drugs were combined. This might have been expected if only two subtypes of α-

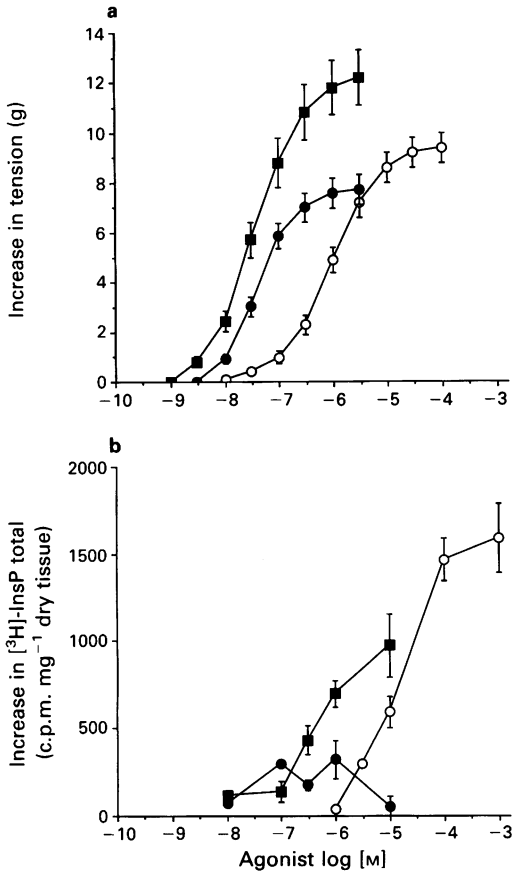

Figure 6 (a) Cumulative concentration-response curves (change tension, g) for cirazoline (■), phenylephrine (○) or BHT-920 (●) in dog saphenous veins in calcium 2.5 mM medium. (b) Concentration-response curves (increase in total [^{3}H]-InsP$_{1-3}$; c.p.m. mg^{-1} dry tissue) for cirazoline (■), phenylephrine (○) or BHT-920 (●) in dog saphenous vein in calcium 2.5 mM medium. Each point represents the mean value obtained from separate dog saphenous vein strips (n = 4–5/group); vertical bars show s.e.mean.

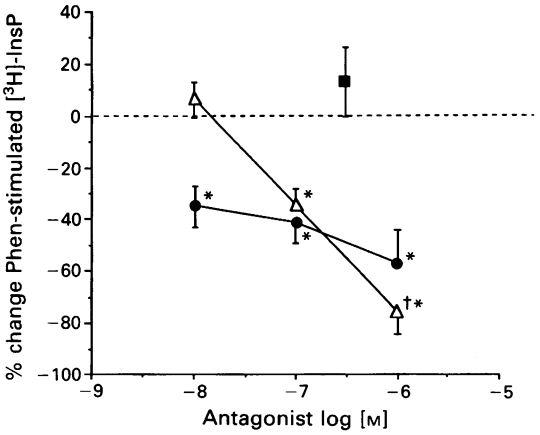

Figure 7 Inhibitory effects of yohimbine (△), prazosin (●) or idazoxan (■) on phenylephrine (Phen 100 μM)-stimulated total [^{3}H]-inositolphosphate production (InsP$_{1-3}$) in separate groups of dog saphenous vein strips. Inhibitory effects were calculated with respect to non-treated Phen-stimulated strips after correction for basal InsP values. 100% control values for Phen were 1887 ± 161 c.p.m. mg^{-1} for the yohimbine- and prazosin-treated groups and 1666 ± 104 c.p.m. ms^{-1} for the idazoxan group. Vertical bars indicate s.e.mean; n = 3–6 strips/point.
* Significant reduction from control tissues (P less than 0.05).
† Significant difference from yohimbine 10^{-7} M data (P less than 0.05).

adrenoceptor are present in this tissue and Phen was acting as a non-selective agonist. Indeed in rabbit saphenous vein where both α_1- and α_2-subtypes can be clearly identified using functional responses (Daly *et al.*, 1988a,b,c), the combination of rauwolscine and Praz did increase the potency of the α_2-adrenoceptor antagonist. It remains plausible that in DSV, the contractions to Phen which occur over the concentration-range 1–300 μM in the presence of both Praz (0.3 μM) and a high concentration of Yoh (3 μM) are not adrenoceptor-mediated.

The question of the 5-HT$_{1A}$ affinity of WB has previously been addressed by Morrow & Creese (1986), in considering the nanomolar affinity of this antagonist at α_{1B}-sites. Certainly WB has relatively high affinity for 5-HT$_{1A}$ receptors (Norman *et al.*, 1985); however, in DSV 5-HT-induced responses are mediated by 5-HT$_1$ like-receptors which are apparently not 5-HT$_{1A}$ (Humphrey *et al.*, 1988). Further, Phen has not been reported to have affinity at 5-HT sites to our knowledge.

The possibility that high concentrations of Phen might release ATP to cause contraction was also considered. Thus incubation of DSV rings with a combination of Praz (0.3 μM) and the P$_2$-receptor desensitizing agent α,β-MeATP (Sneddon & Burnstock, 1984) at a concentration that blocked the contractions of the P$_2$-receptor agonist β,γ-MeAPT, failed to modify either the contractile-response curve to Phen in the presence of Praz, or the antagonist affinity of Yoh. Phen does not therefore indirectly release ATP to cause sustained contraction through P$_2$-receptor mechanisms in DSV.

The range of antagonist dissociation constants reported in the literature for Yoh is considerable (Ruffolo *et al.*, 1981; Drew, 1985; Flavahan & Vanhoutte, 1986b) and it is clear that this antagonist shows higher potency in DSV against selective α_2-adrenoceptor agonists (pA$_2$ or pK$_B$ 8.2–8.7) than at prejunctional α_2-adrenoceptors (pA$_2$ or pK$_B$ 7.3–7.8) in other preparations. On the other hand Yoh was a weak antagonist against contractions induced by Cir (pK$_B$ 6.3; present study), in close agreement with values obtained by Cavero *et al.* (1983). This indicates further differences in the α-adrenoceptor antagonist affinities of Yoh and suggests that the receptor mediating Phen-induced contractions is not a 'classical' α_1-subtype. The contractile effects of Phen in DSV have been reported to be more sensitive to inhibition by phenoxybenzamine (Constantine *et al.*, 1982; Hicks *et al.*, 1988) than those induced by α_2-adrenoceptor agonists (Flavahan *et al.*, 1984; Ruffolo & Zeid, 1985; Guimarães *et al.*, 1987) and a greater receptor reserve exists for both Phen and other α_1-adrenoceptor agonists (Cooke *et al.*, 1985; Ruffolo & Zeid, 1985) compared with α_2-adrenoceptor agonists in this vessel. These findings would be entirely consistent with the greater α_1-adrenoceptor selectivity for phenoxybenzamine (Dubocovitch & Langer, 1974).

Phen-induced contractions in DSV are at least partly dependent on calcium entry, although not entirely through dihydropyridine-sensitive (L-type) calcium channels (Jim *et al.*, 1985). However, unlike the α_2-agonist BHT-920, Phen can also stimulate intracellular calcium release as evidenced by increased $^{45}Ca^{2+}$ efflux (Janssens & Verhaeghe, 1984; Jim & Matthews, 1985) and responses of DSV to these agonists in calcium-free medium (Janssens & Verhaeghe, 1984; present study). The present results which show that nitrendipine (1 μM) had minimal inhibitory effects on Phen-induced contractions entirely support these data. The fact that Phen-induced contractions can be evoked in calcium-free medium, confirms the significant role of receptor-mediated intracellular Ca^{2+} release in these responses. The relatively high antagonist potency of Yoh (0.1 μM) shown on Phen-induced contractile responses in zero calcium was not anticipated, but was difficult to quantify in terms of affinity, since the Phen-induced response curves in the presence of Yoh were displaced in a non parallel manner in these experiments. Nevertheless Yoh (0.1 μM) clearly antagonized the effects of Phen under zero extracellular calcium conditions.

The demonstration that Phen caused a concentration-

dependent increase in total InsP formation, suggests that this agonist stimulates a receptor which could be coupled through phospholipase C in DSV. Our assumptions are based on the increase in $InsP_1$ which makes up the largest percentage increase in total InsPs measured (71%) after 10 min of Phen stimulation in the presence of lithium. We have not attempted in this study to measure $Ins(1,4,5)P_3$, since the methodology of Berridge (1983), does not allow the separation of $InsP_3$ isomers. Preliminary results obtained with high performance liquid chromatography (h.p.l.c.), indicate that 74% of total $InsP_3$ was recovered as $Ins(1,4,5)P_3$ and 26% as $Ins(1,3,4)P_3$ following 10 min stimulation with Phen(100 μM) in DSV.

α_1-Adrenoceptors have recently been subclassified from functional studies (Han *et al.*, 1987) into α_{1A} (rat vas deferens) or α_{1B} (rat spleen), based on different molecular mechanisms used to evoke these responses. The high pM antagonist effects of WB on contractions to Phen and Cir are consistent with the presence of α_{1A}-sites in DSV and are close to the reported affinity of WB at this site as defined by binding studies (Minneman *et al.*, 1988; Michel *et al.*, 1989a). Although previous reports on Praz in DSV indicate a rather weak antagonist effect on Phen contractions, our present data indicate that Praz also identifies two sites in this vessel, with a high pK_B (8.4 at 10 nM) which is close to the pK_B of 8.2 calculated using Cir as the agonist. The high nM antagonist effects of Praz on Phen contractions (pK_B 7.7) appear to be competitive in nature and are close to previously reported values (Alabaster *et al.*, 1985; Akers *et al.*, 1987). It is tempting to speculate that the antagonist effects of both WB and Praz on Cir reflect an α_{1A}-selectivity of this agonist in this tissue.

In DSV the α-adrenoceptor stimulated by Phen to increase InsP shows similarities to the α_{1B} subtype in rat spleen (Han *et al.*, 1987), except that in DSV the increase in InsP formation was antagonized by yohimbine. Where tested, α-adrenoceptor-mediated InsP production (Minneman & Johnson, 1984; Fox *et al.*, 1985; Chiu *et al.*, 1987) or [^{3}H]-P incorporation into PtdIns (Zeleznikar *et al.*, 1983), were antagonized by Praz, but data reported thus far show that Yoh has low potency as an antagonist of α-adrenoceptor-mediated PI (Legan *et al.*, 1985; Han *et al.*, 1987) consistent with its affinity (pK_D 5.6) at cloned α_1 sites from the hamster (Cotecchia *et al.*, 1988). The absolute affinity of Praz for PI responses is, however, not easy to ascertain from the literature, since relatively high concentrations of Praz have often been used. Recently Michel *et al.* (1990), have reported complete inhibition by Praz (0.1 μM) of NA-stimulated PI in rat cerebral cortex. In DSV the inhibitory effects of Praz on Phen-stimulated PI were weaker than would be expected. Thus a 30% inhibition of this effect was obtained with 10 nM Praz, but even at 1 μM, Praz only inhibited this response by 60%. Furthermore, at concentrations of 0.1 and 1 μM, Praz and Yoh were equipotent (see Figure 7).

The antagonist potency of WB reported at α_{1B}-adrenoceptors in rat spleen against NA-induced contraction or InsP formation (pK_B 8.2: Han *et al.*, 1987) are close to the intermediate affinity values shown in DSV against Phen-induced contractions (Akers *et al.*, 1987; present study pA_2 8.4) and are also close to the low nM affinity WB sites identified with [^{3}H]-BE-2254 in other peripheral tissues i.e. vas deferens, liver. All of these affinity estimates for WB are intermediate between the high pM affinity WB sites (Minneman *et al.*, 1988; Michel *et al.*, 1989a) and the currently reported low potency α_{2A}-antagonist effects in DSV (pK_B 7.1) and suggest that α_{1B}-receptors are indeed present in DSV. In functional tests the α_2-adrenoceptor antagonist effects of WB (pA_2 6.6 against inhibition of the twitch response of rat proximal vas deferens by clonidine; Pigini *et al.*, 1988, or facilitation of stimulation-evoked release of [^{3}H]-NA in cat isolated spleen; IC_{50}: 0.2 μM: Massingham *et al.*, 1981) closely approximate the pK_B calculated on BHT-induced contractions in the present study. The effects of WB on Phen-stimulated PI are currently being examined in DSV.

The present results therefore agree with previously published data in DSV, which show that Phen-induced contractions are antagonized by Yoh at concentrations similar, but not identical to those required to block responses induced by α_2-adrenoceptor agonists. Idaz was significantly less potent against Phen that BHT-mediated contractions. WB was the most potent and selective antagonist tested against the contractile effects of Phen and identified two high affinity sites. The high pM affinity value was close to that identified when Cir was used as the agonist and probably represents effects at the α_{1A}-adrenoceptor. The low nM affinity site identified for WB on Phen could therefore be the α_{1B}-adrenoceptor. Prazosin has previously been reported to show low potency against Phen-stimulated contractions in DSV, but like WB also appears to identify two sites in this tissue. Both antagonists demonstrate competitive antagonism against the low affinity site stimulated by Phen. Finally, BHT-920 appears to be a relatively selective α_{2A}-adrenoceptor agonist in DSV. These data provide little support for the contention that Phen mediates contraction in DSV by stimulating 'classical' α_2-adrenoceptors (Guimarães *et al.*, 1987), although a receptor which shows some homology with the α_{2B}-subtype cannot yet be excluded, since the nM antagonist potency of Praz was similar to the potency of Yoh on Phen-stimulated contraction. Nevertheless, only high concentrations of Idaz antagonized the contractile effects of Phen and neither Yoh nor Idaz antagonized the effects of Cir at concentrations less than 1 μM.

The increase in InsPs in response to Phen and Cir indicates that InsPs are involved in adrenoceptor-response coupling in this tissue. By use of the PI response, further differentiation can be made between Phen and the α_{2A}-adrenoceptor agonist BHT. The fact that contractions evoked by BHT, but not Phen, were abolished in the absence of extracellular calcium, demonstrates an important role for receptor-mediated intracellular Ca^{2+}-release in the contractile effects of Phen. Current theory would predict that α-adrenoceptor-mediated increases in InsP are mediated by α_{1B}-adrenoceptors.

The results imply that multiple α-adrenoceptor subtypes exist in DSV. The receptor which is stimulated by Cir to cause contraction is competitively antagonized by WB with a pA_2 of 9.6 and by Praz with a pA_2 of 8.2 and is probably the α_{1A}-adrenoceptor. Yoh and Idaz both show antagonist effects in the μM range at this site. Contractile effects of BHT-920 are antagonized by Yoh and Idaz at concentrations consistent with known α_2-affinities of these compounds, while Praz was inactive at 1 μM. These data indicate the presence of α_{2A}-adrenoceptors on DSV. The receptors that are stimulated by Phen to evoke contraction in this tissue show certain characteristics of the α_{1A}-adrenoceptor, since WB demonstrated high pM antagonist affinity against these responses and the contractions were only partially inhibited by the L-channel calcium antagonist nitrendipine. Although a low nM antagonist affinity was shown with Praz on Phen and Cir-induced contractions, the predominant antagonist effect of Praz on Phen-induced responses, although competitive in nature, occurred at higher concentrations of the antagonist and was equivalent to the antagonist affinity of Yoh. Both the contractile effects of Phen in the absence of extracellular calcium and the InsP stimulating effects of Phen were antagonized by Yoh at 0.1–1 μM, but not by the imidazoline α_2-adrenoceptor antagonist Idaz. The inhibitory effects seen with Praz (10 nM) on Phen-stimulated InsP formation in DSV could represent α_{1B}-adrenoceptor antagonist effects of Praz; however, the fact that higher concentrations of Praz (0.1–1 μM) only inhibited this response up to 60%, suggests that these latter effects of Praz and those of Yoh on PI cannot readily be equated to the α_{1B}-receptor as currently defined. We consider that this site could represent an 'atypical' α-adrenoceptor. Further characterization of this site requires the identification of more selective antagonists.

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