*Br. J. Pharmacol.* (1990), **99**, 97–102 

# No evidence for differences between pre- and postjunctional $\alpha_2$-adrenoceptors in the periphery

Sonia Connaughton & [1]J.R. Docherty

Department of Clinical Pharmacology, Royal College of Surgeons in Ireland, 123, St. Stephen's Green, Dublin 2, Ireland

**1** We have compared prejunctional $\alpha_2$-adrenoceptors in rat and guinea-pig vas deferens and rat and guinea-pig atria with postjunctional $\alpha_2$-adrenoceptors in human saphenous vein and human platelets employing the antagonists yohimbine and SK&F 104078 and other $\alpha_2$-adrenoceptor antagonists.

**2** Yohimbine was approximately 10 times more potent prejuunctionally than SK&F 104078 at antagonizing the inhibition by the $\alpha_2$-adrenoceptor agonist xylazine of stimulation-evoked contractions in rat and guinea-pig vas deferens, and at increasing stimulation-evoked release of tritium in rat and guinea-pig atria pre-incubated with [$^3$H]-noradrenaline.

**3** Yohimbine was approximately 10 times more potent postjunctionally than SK&F 104078 at antagonizing contractions to noradrenaline in human saphenous vein and at displacing [$^3$H]-yohimbine binding in human platelet membranes.

**4** For the antagonists yohimbine, SK&F 104078, prazosin, phentolamine, CH 38083 and urapidil, there was a significant correlation between prejunctional potency in rat vas deferens atrium and postjunctional potency in human platelet, although the correlation was improved by the omission of prazosin.

**5** We have no evidence for differences between functional pre- and postjunctional $\alpha_2$-adrenoceptors in the periphery, although these functional receptors may differ from the ligand binding site in the human platelet.

## Introduction

We have recently examined the prejunctional $\alpha_2$-adrenoceptors of the pithed rat heart and rat isolated atrium and the postjunctional $\alpha_2$-adrenoceptors of the pithed rat vasculature and the human isolated saphenous vein but failed to find differences between these pre- and postjunctional receptors in terms of the potencies of the antagonists yohimbine and SK&F 104078 (Connaughton & Docherty, 1988). Since SK&F 104078 is reported to show selectivity for post- over prejunctional $\alpha_2$-adrenoceptors in a variety of tissues (Ruffolo *et al.*, 1987, Daly *et al.*, 1988; Hieble *et al.*, 1988), we have investigated the actions of this antagonist further in additional human, rat and guinea-pig isolated tissues, including tissues in which SK&F 104078 is reported to have low prejunctional potency (Hieble *et al.*, 1988).

Some of these results have been presented in abstract form (Connaughton *et al.*, 1989).

## Methods

Male Wistar rats (250–350 g) and male Duncan Hartley guinea-pigs (450–500 g) were obtained from BioLabs, Ballina, Ireland and a variety of isolated tissues were used.

### *Rat and guinea-pig vas deferens*

Prostatic portions of rat vas deferens or whole vas deferens of the guinea-pig were placed between platinum electrodes in organ baths (50 ml) and bathed at 37°C in Krebs-Henseleit solution of the following composition (mmol $l^{-1}$): NaCl 119, $NaHCO_3$ 25, (+)-glucose 11.1, KCl 4.7, $CaCl_2$ 2.5, $KH_2PO_4$ 1.2, $MgSO_4$ 1.0. The tissues were attached to myograph transducers for recording of isometric contractions. Responses to single pulse field stimulation (rat vas deferens) or to 3 pulses at 1Hz (guinea-pig vas deferens) (supramaximal voltage, 0.5 ms pulses) were obtained at intervals of 5 min. When consistent control responses had been obtained, antagonist or vehicle was administered and 15 min later the effects of the $\alpha_2$-adrenoceptor agonist xylazine were assessed on nerve-stimulation evoked contractions. Xylazine was added cumulatively in 0.5 log unit increments at intervals of 5 min. Xylazine $IC_{50}$ values (concentration producing 50% of maximum inhibition of the contraction to a single stimulus) were obtained from individual paired experiments in which one tissue received antagonist and the other received vehicle. Antagonist potency was expressed as the dissociation constant $K_B$ from the equation $K_B = [B]/(DR - 1)$, where [B] is the concentration of antagonist and DR is the agonist dose-ratio produced by the antagonist, or as a $pA_2$ value. Antagonist $pA_2$ values were obtained from the x-intercept of the plot of log (agonist DR − 1) against log antagonist concentration, where the slope was not significantly different from negative unity (Arunlakshana & Schild, 1959).

### *Rat and guinea-pig atria*

Isolated atria were preincubated for 1 h in 1 ml medium containing [$^3$H]-noradrenaline (0.5 $\mu$mol $l^{-1}$, specific activity 39 Ci mmol$^{-1}$) before being superfused with [$^3$H]-noradrenaline-free Krebs-Henseleit solution of the same composition as listed above except that ascorbic acid (0.28 mmol $l^{-1}$) tetrasodium EDTA (30 $\mu$mol $l^{-1}$), corticosterone (30 $\mu$mol $l^{-1}$) and propranolol (1 $\mu$mol $l^{-1}$) were present. In addition, cocaine (3 $\mu$mol $l^{-1}$) was present after pre-incubation with [$^3$H]-noradrenaline. Tissues were placed between platinum electrodes in organ baths, and superfused at a rate of 2 ml min$^{-1}$ at 37°C.

In all experiments, tissues were stimulated 4 or 5 times ($S_0$–$S_4$) for 3 min at a frequency of 2Hz at intervals of 27 min, beginning after 99 min of superfusion. Effluent samples were collected in 6 ml aliquots beginning after 120 min of superfusion so that sampling began 6 min before $S_1$. Antagonist drugs or distilled water vehicle were added to the superfusion stream at a rate of 50 $\mu$l min$^{-1}$ in 2 or 3 cumulative concentrations beginning 12 min before $S_2$. At the end of the experiment tissues were made soluble in 1 ml of tissue solubiliser. A

[1] Author for correspondence.

**Table 1** Potencies of a series of α-adrenoceptor antagonists at antagonizing the prejunctional inhibition by xylazine of stimulation-evoked contractions in rat and guinea-pig vas deferens.

| | $K_B$ (−log M) | |
|---|---|---|
| | *Prejunctional rat vas* | *Prejunctional guinea-pig vas* |
| Yohimbine | 7.50 ± 0.35 | 7.66 ± 0.43 |
| SK&F 104078 | 6.39 ± 0.41 | 6.82 ± 0.44 |
| Prazosin | 6.20 ± 0.40 | / |
| Phentolamine | 7.82 ± 0.27 | / |
| CH 38083 | 8.52 ± 0.13 | / |
| Urapidil | 5.33 ± 0.17 | / |

Values are dissociation constant ($K_B$, −log M) and 95% confidence limits from at least 4 experiments.

volume of 1 ml of superfusate or dissolved tissue was added to 9 ml of liquid scintillation solution (Liquiscint) and counted in a liquid scintillation counter. The stimulation-evoked overflow of tritium was calculated by subtraction of the basal outflow, and was expressed as a percentage rate (i.e. the evoked overflow during a given stimulation period was expressed as a percentage of the tritium content of the tissue at the onset of that stimulation period). The effects of antagonist on the stimulation-evoked overflow of tritium were expressed as an $EC_{30}$ (concentration causing 30% enhancement of the stimulation-evoked overflow) in rat atria and as the lowest concentration producing a significant enhancement of stimulation-evoked overflow in both rat and guinea-pig atria.

### *Human saphenous vein*

Human saphenous veins were obtained from patients (aged 34–59 years) undergoing surgical removal of varicose veins, and apparently healthy segments were chosen for experimentation. Rings were attached to myograph transducers under 1g tension for isometric tension recordings in organ baths at 37°C in Krebs-Henseleit solution of the same composition as used in superfusion experiments (see above). Rings were exposed to noradrenaline (10 $\mu$mol $l^{-1}$) for 5 min, and thereafter bathing fluid was changed every 15 min except during agonist administration. In the first group of experiments, after 30 min a cumulative concentration-response curve was carried out to noradrenaline in 0.5 log unit increments beginning with 1 nmol $l^{-1}$. Once a maximum response to noradrenaline had been reached, bathing fluid was changed, and 120 min later, following 60 min exposure to antagonist or distilled water vehicle, a second cumulative concentration-response curve to NA was obtained. Effects of antagonist on the $EC_{50}$ (concentration of noradrenaline producing 50% of maximum contraction) and maximum contractile response of noradranline were corrected for changes occurring in vehicle experiments. Antagonist $pA_2$ values were obtained from the x-intercept of the plot of log (agonist dose ratio − 1) against log antagonist concentration, when the slope was not significantly different from negative unity (Arunlakshana & Schild, 1959).

In the second group of experiments, 60 min after a first concentration-response curve to noradrenaline, a cumulative concentration-response curve was obtained to the test antagonist to assess possible partial agonist properties and the maximum vasoconstrictor response to the antagonist was expressed as a fraction of the maximum to noradrenaline.

### *Human platelet membranes*

Radioligand binding studies were carried out by a method adapted from that described by Buckley *et al.* (1986): 60 ml blood was drawn from an antecubital vein of healthy young adult subjects into lithium heparin tubes. The blood was centrifuged at 160 *g* for 10 min and platelet-rich plasma was removed. Isotonic buffer (Tris-HCl 50 mmol $l^{-1}$, NaCl 100 mmol $l^{-1}$, EDTA 5 mmol $l^{-1}$) was mixed with the remaining blood and the tubes were centrifuged again. The supernatant and platelet-rich plasma were combined and centrifuged at 27,000 *g* for 10 min at 4°C. The pellets were resuspended in lysing buffer (Tris-HCl 1 mmol $l^{-1}$ EDTA 0.5 mmol $l^{-1}$, pH 7.4) at 4°C and homogenized. After standing for 10 min in lysing buffer, the membranes were centrifuged at 27,000 *g* for 10 min. The resultant pellet was used immediately or stored at −20°C for later use. Pellets were reconstituted in incubation buffer (Tris-HCl 50 mmol $l^{-1}$, $MgCl_2$ 8 mmol $l^{-1}$, EGTA 5 mmol $l^{-1}$), homogenized and centrifuged at 27,000 *g* for 10 min. Pellets were again reconstituted in 2.5 ml incubation buffer. Aliquots were incubated with various concentrations of [$^3$H]-yohimbine at 37°C. Specific binding was estimated by subtracting non-specific binding obtained in the presence of phentolamine 100 $\mu$mol $l^{-1}$. In competition studies, [$^3$H]-yohimbine (5 nmol $l^{-1}$) was incubated with displacing ligands in concentrations from 1 nmol $l^{-1}$ to 100 $\mu$mol $l^{-1}$ in 0.5 log unit increments for 30 min. Assays were terminated by filtration through Whatman GF/C filters.

The inhibition constant ($K_i$) for displacement of radiolabelled ligand was determined from the formula: $K_i = IC_{50}/(1 + [^3H - Y/K_D])$ where $IC_{50}$ is the concentration of competing ligand that inhibits radioligand binding by 50%, $K_D$ is the dissociation constant for the radioligand (5.75 ± 0.78 nmol $l^{-1}$, $n = 6$), and $^3H - Y$ is the concentration of tritiated yohimbine employed (5 nmol $l^{-1}$).

### *Statistical evaluation*

Results are expressed as mean ± s.e. mean or mean and 95% confidence limits. Effects of antagonist on agonist $IC_{50}$, $EC_{50}$ or maximum tension, or on the stimulation-evoked overflow of tritium were compared with the effects of vehicle by Student's *t* test for unpaired data, or Student's *t* test for paired data where appropriate, and Analysis of Variance. Slopes and elevation of Schild regressions were compared by covariance analysis (see Snedecor & Cochrane, 1980).

### *Drugs*

The following drugs were used: CH-38083 (7,8-(methylenedioxi)-14-x-hydroxyalloberbane hydrochloride; (gift: Chinoin, Budapest, Hungary); cocaine hydrochloride (Sigma, Poole, U.K.); corticosterone (Sigma); noradrenaline bitartrate (Sigma); phentolamine mesylate (gift: CIBA, Horsham, U.K.); prazosin hydrochloride (gift: Pfizer, Sandwich, U.K.); (±)-propranolol hydrochloride (Sigma); SKF 104078 (6-chloro-9-(3-methyl-2-butenyl) oxyl-3-methyl-1H-2,3,4,5-tetrahydro-3-benzazepine maleate; gift: Smith Kline & French, King of Prussia, PA, U.S.A.), xylazine hydrochloride (gift: Bayer, Ireland); urapidil (gift: Byk Gulden, Konstanz, F.R.G.) yohimbine hydrochloride (Sigma).

Drug stocks were dissolved in distilled water and dilutions made up in distilled water, with the exception of corticosterone which was dissolved in 100% ethanol.

## Results

### *Vas deferens*

In prostatic portions of the rat vas deferens, single pulse electrical stimulation produced an isometric contraction of 1.13 ± 0.07 g ($n = 37$). Antagonists in the concentrations employed did not significantly affect the response to a single stimulus. In vehicle experiments, xylazine (1–1000 nmol $l^{-1}$) produced concentration-dependent inhibition of the

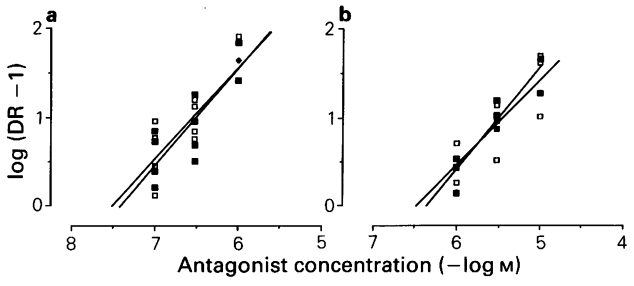


**Figure 1** Schild plots of the relationship between log (agonist dose-ratio −1) and antagonist concentration (−log M) for the antagonists yohimbine (a) and SK&F 104078 (b) against the prejunctional inhibitory actions of xylazine in rat vas deferens (filled symbols) or against postjunctional isometric contractions to noradrenaline in the human saphenous vein (open symbols). Each point is the result of an individual experiment. $pA_2$ values and slopes of Schild plots are shown in Table 2.

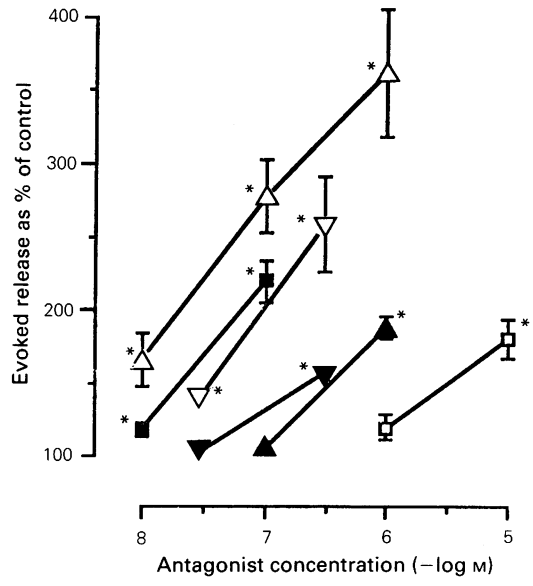


**Figure 2** Effects of yohimbine (■), SK&F 104078 (▲), phentolamine (▽), prazosin (▼), CH 38083 (△) and urapidil (□) on the stimulation-evoked overflow of tritium from rat atria pre-incubated with [$^3$H]-noradrenaline and superfused in [$^3$H]-noradrenaline-free medium. Effects of drug are expressed as percentage increase in stimulation-evoked release of tritium, and were corrected for changes occurring in vehicle experiments. Vertical bars denote s.e.mean from at least 4 experiments. Asterisks denote effects of antagonist significantly different from vehicle (Student's $t$ test; $^*P < 0.05$).

stimulation-evoked isometric contractions, with a maximum inhibition of 95.5 ± 1.2% ($n = 37$) and an $IC_{50}$ of 66.1 nmol $l^{-1}$ (95% confidence limits of 51.3–85.1 nmol $l^{-1}$). Antagonist potencies at antagonizing the inhibitory effects of xylazine are shown as $-\log K_B$ values in Table 1. Additionally, Schild plots were constructed for the antagonists yohimbine and SK&F 104078 (Figure 1). Slopes of Schild plots were not significantly different from negative unity, and the $pA_2$ obtained for yohimbine was approximately 1 log unit higher than the $pA_2$ value for SK&F 104078 (Table 2).

In guinea-pig vas deferens, stimulation at 1 Hz with 3 pulses produced discrete peaks for the second and third pulses. The peak response to the third pulse was 1.03 ± 0.11 g ($n = 20$). SK&F 104078 (3 μmol $l^{-1}$) but not yohimbine (0.3 μmol $l^{-1}$) significantly increased the stimulation-evoked contraction to 132.0 ± 17.8% ($n = 5$) of control as compared to the effects of vehicle ($P < 0.05$). In vehicle experiments, xylazine (10–1000 nmol $l^{-1}$) produced concentration-dependent inhibition of the stimulation-evoked contraction with a maximum inhibition of 45.8 ± 4.0% ($n = 10$) and an $IC_{50}$ of 40.7 nmol $l^{-1}$ (95% confidence limits of 24.0–69.2 nmol $l^{-1}$). Yohimbine was approximately 10 times more potent than SK&F 104078 at antagonizing the inhibitory effects of xylazine (Table 1).

### *Isolated atria*

Field stimulation of rat isolated atria at a frequency of 2 Hz for 3 min produced an evoked overflow of tritium at $S_1$ of 2.02 ± 0.13% of tissue tritium ($n = 32$). Basal outflow of tritium was 0.18 ± 0.03% of tissue tritium per min ($n = 32$) before $S_1$. Yohimbine (10–100 nmol $l^{-1}$), SK&F 104078 (1 μmol $l^{-1}$), phentolamine (0.03–0.3 μmol $l^{-1}$), CH 38083 (0.01–1 μmol $l^{-1}$), prazosin (0.3 μmol $l^{-1}$) and urapidil (10 μmol $l^{-1}$) significantly increased the stimulation-evoked overflow of tritium without increasing basal outflow (Figure 2). SK&F 104078 (10 μmol $l^{-1}$) and prazosin (3 μmol $l^{-1}$) significantly increased basal outflow, so that the effects of these concentrations of the respective antagonists are not shown in Figure 2. Potency of antagonist was expressed as an $EC_{30}$ value (concentration producing a 30% increase in stimulation-evoked overflow of tritium) and values are shown in Table 3. Yohimbine was approximately 10 times more potent than SK&F 104078 (Table 3).

Field stimulation of guinea-pig isolated atria at a frequency of 2 Hz for 3 min produced an evoked overflow of tritium at $S_1$ of 1.65 ± 0.15% of tissue tritium ($n = 16$). Basal outflow of tritium was 0.13 ± 0.01% of tissue tritium per min ($n = 16$) before $S_1$. Yohimbine (100–1000 nmol $l^{-1}$) and SK&F 104078 (1 μmol $l^{-1}$) significantly increased the stimulation-evoked

**Table 2** Prejunctional $pA_2$ values obtained against xylazine in the rat vas deferens and postjunctional $pA_2$ values obtained against noradrenaline in the human saphenous vein for yohimbine, SK&F 104078 and prazosin

| | *Yohimbine* | *SK&F 104078* | *Prazosin* |
|---|---|---|---|
| Prejunctional $pA_2$ | 7.50 (6.82 – 8.87) | 6.45 (5.66 – 8.77) | 6.20[+] |
| Slope | −1.06 ± 0.25 | −0.98 ± 0.26 | / |
| Postjunctional $pA_2$ | 7.40 (6.74 – 8.71) | 6.33 (5.91 – 6.92) | 6.45 (5.83 – 7.59) |
| Slope | −1.14 ± 0.26 | −1.18 ± 0.15 | −0.94 ± 0.19 |

Values are mean and 95% confidence limits (−log M) for $pA_2$ and mean ± s.e.mean for slope of Schild plots.
[+] $K_B$ (−log M) from Table 1.

**Table 3** Prejunctional potencies of a series of α-adrenoceptor antagonists in rat atria

| | *Rat atrium* $EC_{30}$ (−log M) |
|---|---|
| Yohimbine | 7.89 (7.75 – 8.03) |
| SK&F 104078 | 6.68 (6.52 – 6.84) |
| Prazosin | 6.97 (6.71 – 7.23) |
| Phentolamine | 7.60 (7.37 – 7.83) |
| CH 38083 | 8.27 (7.83 – 8.71) |
| Urapidil | 5.80 (5.28 – 6.32) |

Potency is expressed as an $EC_{30}$ value (concentration producing 30% increase in stimulation-evoked overflow of tritium). $EC_{30}$ values are mean and 95% confidence limits (−log M) from at least 4 experiments.

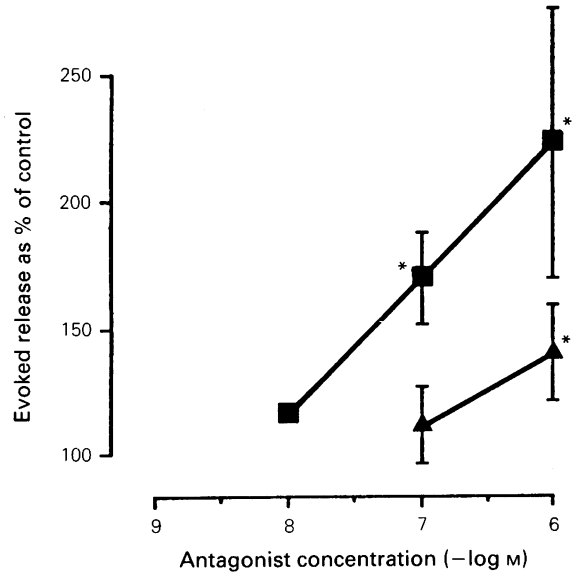


**Figure 3** Effects of yohimbine (■) and SK&F 104078 (▲) on the stimulation-evoked overflow of tritium from guinea-pig atria pre-incubated with [$^3$H]-noradrenaline and superfused in [$^3$H]-noradrenaline-free medium. Effects of drug are expressed as percentage increase in stimulation-evoked release of tritium, and were corrected for changes occurring in vehicle experiments. Vertical bars denote s.e.mean from at least 4 experiments. Asterisks denote effects of antagonist significantly different from vehicle (Student's $t$ test: $^*P < 0.05$).

release of tritium (Figure 3). Higher concentrations of SK&F 104078 significantly increased the basal outflow of tritium, so that effects on stimulation-evoked release were not calculated. Since the magnitude of the increase in evoked overflow produced by SK&F 104078, and to a lesser extent yohimbine, was relatively small, potency was not expressed as an $EC_{30}$. Yohimbine was again approximately 10 times more potent than SK&F 104078, in terms of threshold concentration for producing a significant increase in stimulation-evoked release of tritium (Figure 3).

### *Human saphenous vein*

In human saphenous vein, noradrenaline produced isometric contractions with an $EC_{50}$ of 0.63 $\mu$mol l$^{-1}$ (95% confidence limits of 0.28–1.44 $\mu$mol l$^{-1}$, $n = 40$ tissues from 13 patients), and a maximum contraction of 2.17 ± 0.20g. Yohimbine (0.1, 0.3 and 1 $\mu$mol l$^{-1}$), SK&F 104078 (1 and 3 $\mu$mol l$^{-1}$) and prazosin (1–10 $\mu$mol l$^{-1}$) produced approximately parallel shifts in the concentration-response curve for noradrenaline without significantly affecting the maximum contraction to noradrenaline. However, SK&F 104078 (10 $\mu$mol l$^{-1}$) contracted the human saphenous vein producing contractions of 35.4 ± 2.2% ($n = 3$) of the maximum contraction to noradrenaline (response measured during 1 h exposure to SK&F 104078). The slopes of Schild plots for the effects of yohimbine and SK&F 104078 were not significantly different from negative unity, and the $pA_2$ obtained for yohimbine was approximately 1 log unit higher than for SK&F 104078 (Figure 1 and Table 2). Prazosin was more than 10 times less potent than yohimbine, confirming that responses to noradrenaline in the human saphenous vein are mediated predominantly by $\alpha_2$-adrenoceptors (Table 2). Note that the Schild plots obtained for yohimbine and SK&F 104078 at postjunctional $\alpha_2$-adrenoceptors in human saphenous vein are superimposable on Schild plots obtained for the same antagonists at prejunctional $\alpha_2$-adrenoceptors in rat vas deferens (Figure 1).

In a second series of experiments, cumulative concentration-response curves were carried out for antagonist drugs to assess their ability to contract the human saphenous vein, and response was expressed relative to the maximum response to noradrenaline. SK&F 104078 (30 $\mu$mol l$^{-1}$) contracted the human saphenous vein with an intrinsic activity 52.2 ± 8.5% ($n = 4$) of that to noradrenaline. Other antagonists in concentrations of up to 30 $\mu$mol l$^{-1}$ produced no significant contractions.

**Table 4** $K_i$ values obtained to α-adrenoceptor antagonists for the displacement of [$^3$H]-yohimbine binding in human platelet membranes

| | $K_i$ (−log M) |
|---|---|
| Yohimbine | 8.04 ± 0.25 |
| SK&F 104078 | 6.52 ± 0.52 |
| Prazosin | 5.62 ± 0.40 |
| Phentolamine | 7.61 ± 0.28 |
| CH 38083 | 8.65 ± 0.41 |
| Urapidil | 5.52 ± 0.76 |

Values are mean and 95% confidence limits from at least 4 experiments.

### *Human platelet membranes*

$K_i$ values for the displacement by α-adrenoceptor antagonists of [$^3$H]-yohimbine binding to human platelet membranes are shown in Table 4. Yohimbine was more than 10 times more potent than SK&F 104078.

### *Correlation between tissues*

Figure 4 shows the correlation between postjunctional $K_i$ in human platelet membranes and prejunctional $K_B$ or $EC_{30}$ obtained in rat vas deferens and rat atrium for 6 antagonists. There was a significant correlation between platelet $K_i$ and potency in rat vas deferens ($r = 0.96$, $P < 0.01$, slope = 0.87 ± 0.13) and atrium ($r = 0.91$, $P < 0.05$, slope = 0.63 ± 014). However, the correlation coefficients were 0.98 and 1.00, and the slopes were 0.98 ± 0.12 and 0.80 ± 0.03 for platelet versus vas deferens and atrium, respectively, when prazosin was omitted from the calculations. The correlation between potency in rat was deferens and atrium was $r = 0.96$, slope = 1.26 ± 0.18, $P < 0.01$ (data not shown).

## Discussion

We have previously shown that SK&F 104078 does not distinguish between prejunctional $\alpha_2$-adrenoceptors in rat iso-

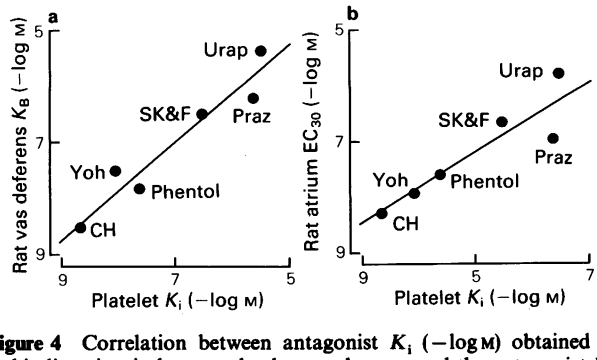


**Figure 4** Correlation between antagonist $K_i$ (−log M) obtained at $\alpha_2$-binding sites in human platelet membranes, and the antagonist $K_B$ or $EC_{30}$ obtained at prejunctional $\alpha_2$-adrenoceptors in rat vas deferens (a) or rat atria (b): Urap, urapidil; Yoh, yohimbine; Praz, prazosin; Phentol, phentolamine, SK&F, SK&F 104078; CH, CH 38083.

lated atrium and postjunctional $\alpha_2$-adrenoceptors in the human saphenous vein and between pre- and postjunctional $\alpha_2$-adrenoceptors in the pithed rat (Connaughton & Docherty, 1988). However, these results do not preclude the possibility that prejunctional $\alpha_2$-adrenoceptors in the rat heart are atypical, since there are reports that SK&F 104078 has low potency at prejunctional $\alpha_2$-adrenoceptors in guinea-pig ileum, guinea-pig atrium and rat vas deferens (Ruffolo *et al.*, 1987; Hieble *et al.*, 1988). Hence, we have carried out additional investigations of the prejunctional actions of SK&F 104078 and other $\alpha_2$-adrenoceptor antagonists at prejunctional $\alpha_2$-adrenoceptors in rat and guinea-pig vas deferens and at postjunctional $\alpha_2$-adrenoceptors in the human platelet.

In the rat vas deferens, SK&F 104078 and yohimbine behaved as competitive antagonists of the prejunctional $\alpha_2$-adrenoceptor-mediated inhibitory actions of xylazine, with slopes of Schild plots not significantly different from negative unity. Schild plots for the prejunctional actions of these antagonists in rat vas deferens were superimposable on their Schild plots for postjunctional actions at $\alpha_2$-adrenoceptors in human saphenous vein. Likewise, the potency ($EC_{30}$) of a range of $\alpha_2$-adrenoceptor antagonists at prejunctional $\alpha_2$-adrenoceptors in rat atrium gave a very significant correlation with their affinity ($K_i$) at $\alpha_2$-adrenoceptor ligand binding sites in the human platelet. The potencies of yohimbine, phentolamine, idazoxan and CH-38083 obtained here are in agreement with published values at prejunctional $\alpha_2$-adrenoceptors in the rat vas deferens (Vizi *et al.*, 1986). Hence, we have no evidence for differences between pre- and postjunctional receptors. In contrast, Heible *et al.* (1988) found that SK&F 104078 had low potency and acted non-competitively at prejunctional $\alpha_2$-adrenoceptors in the rat vas deferens. However, there were methodological differences between the present results and those of Hieble *et al.* (1988): single pulse versus 0.1 Hz stimulation; prostatic portion versus whole vas; xylazine versus UK 14,304 as agonist. The isometric contraction of the prostatic portion of the rat vas deferens is non-adrenergic and unaffected by postjunctional $\alpha$-adrenoceptor antagonism but can be augmented by postjunctional $\alpha_1$-adrenoceptor agonism (see Docherty & Warnock, 1985). A similar prejunctional potency to the present result for SK&F 104078 in the rat vas deferens was obtained by Bertie *et al.*, (1988). In the guinea-pig vas deferens SK&F 104078 had similar prejunctional potency to its prejunctional potency in rat vas deferens, and had a similar potency relative to yohimbine as in the rat vas deferens. We have also examined stimulation-evoked contractions in longitudinal muscle from guinea-pig ileum but since SK&F 104078 (1–3 $\mu$mol $l^{-1}$) inhibited and SK&F 104078 (10 $\mu$mol $l^{-1}$) abolished stimulation-evoked contractions it was not possible to examine the antagonism of the inhibitory effects of xylazine (Lonart and Docherty, unpublished).

We have previously demonstrated that the relative potencies of yohimbine and SK&F 104078 at increasing the stimulation-evoked overflow of tritium in rat atria pre-incubated with [$^3$H]-noradrenaline are similar to their relative potencies postjunctionally in the human saphenous vein, suggesting that SK&F 104078 does not distinguish between these receptors (Connaughton & Docherty, 1988). However, Hieble *et al.* (1988) found that, in guinea-pig vas deferens and atrium, SK&F 104078 at concentrations of 1–3 $\mu$mol $l^{-1}$ did not significantly potentiate the stimulation-evoked release of tritium in tissues pre-incubated with [$^3$H]-noradrenaline. We have re-investigated the actions of SK&F 104078 on stimulation-evoked overflow of tritium in guinea-pig atrium, and found that SK&F 104078 (1 $\mu$mol $l^{-1}$) produced a small but significant potentiation. As in rat atrium, higher concentrations of SK&F 104078 increased the basal outflow of tritium. Hence, the difference between rat and guinea-pig atria is not in the potency of SK&F 104078 at potentiating stimulation-evoked overflow, but in the magnitude of the potentiation. We feel that this difference is due to differences between rat and guinea-pig atria in the operation of negative feedback by noradrenaline and to the partial agonist nature of the compound (see below), rather than to pharmacological differences in prejunctional $\alpha_2$-adrenoceptors.

Contractions to noradrenaline and nerve stimulation-evoked contractions in the human saphenous vein are mediated predominantly by $\alpha_2$-adrenoceptors based both on the absolute and relative potencies of yohimbine and prazosin (Muller-Schweinitzer, 1984; Steen *et al.*, 1984; Docherty & Hyland, 1985; Docherty, 1987). However, Eskinder *et al.* (1988) have reported that contractions to phenylephrine in the human saphenous vein are mediated predominantly by $\alpha_1$-adrenoceptors, suggesting that $\alpha_1$-adrenoceptors may additionally be present in the human saphenous vein. However, $\alpha_2$-adrenoceptor antagonist potency in the human saphenous vein for antagonists such as yohimbine and prazosin correlates well with potencies at prejunctional $\alpha_2$-adrenoceptors in the rat vas deferens and rat atrium, so that contractions to noradrenaline in the human saphenous vein do reflect $\alpha_2$-adrenoceptor function. Incidentally, since the $\alpha_2$-adrenoceptors of tissues such as the human platelet with low affinity for prazosin (250 nM) has been classified as the $\alpha_{2A}$ adrenoceptor (Bylund, 1985; 1988), this might suggest that all the $\alpha_2$-adrenoceptors examined in this study resemble the $\alpha_{2A}$, based on absolute potency of prazosin. However, when potency of prazosin is expressed relative to yohimbine, prazosin is 20 (rat vas deferens), 9 (human saphenous vein) or 8 (rat atrium) times less potent than yohimbine in our functional studies, similar to the potency ratio found by Bylund for the $\alpha_{2B}$ subtype as in neonatal rat lung or for the $\alpha_{2C}$ subtype as in the OK cell line (Murphy & Bylund, 1988) with high affinity for prazosin (5–7.6 nM), but prazosin was 260 times less potent than yohimbine at the $\alpha_{2A}$ of human platelet (present data and Bylund, 1988). We cannot identify our receptors with any of the $\alpha_{2A}$, $\alpha_{2B}$ or $\alpha_{2C}$ subtypes due to the limited number of antagonists used in the present study, although the best correlation between our prejunctional potencies in rat vas deferens and atrium and published $K_i$ values is with the $\alpha_{2B}$ (Bylund, 1988). Gene cloning techniques have shown that the gene coding for the human platelet $\alpha_2$-adrenoceptor derives from chromosome 10 ($\alpha_2$–$C_{10}$) (Kobilka *et al.*, 1987), and can be identified with the $\alpha_{2A}$ (Bylund, 1988). Whereas Bylund (1988) assumes that all $\alpha_2$-adrenoceptors are coupled to the guanine nucleotide-binding regulatory protein $G_i$, usually involving inhibition of adenylate cyclase, there is some evidence that the prejunctional $\alpha_2$-adrenoceptor of the rat vas deferens may not be coupled to $G_i$ since pertussis toxin does not prevent the inhibitory actions of $\alpha_2$-adrenoceptor agonists (Docherty 1988; see also Musgrave *et al.*, 1987) even at pertussis toxin doses higher than those which abolish the negative inotropic effects of acetylcholine in rat left atrium (Docherty, unpublished). Hence, essentially identical receptors pharmacologically may differ in their coupling to G proteins. Alternatively, there may be problems of access for pertussis toxin into peripheral nerve terminals, so that neuronal $G_i$ shows apparent resistance to the toxin.

Unlike yohimbine, SK&F 104078 showed agonist characteristics in that it contracts the human saphenous vein (present results) and raises diastolic blood pressure in the pithed rat (Connaughton & Docherty, 1988). Partial agonist properties of this drug may explain why SK&F 104078 produced a marked potentiation of stimulation-evoked overflow of tritium in rat but not in guinea-pig atria. Differences between species in the level of activation by noradrenaline of prejunctional $\alpha_2$-adrenoceptors in atria would result in a partial agonist behaving more like an agonst in situations in which the level of noradrenaline at the prejunctional receptors is low. Admittedly, SK&F 104078 does not inhibit the stimulation-evoked isometric contraction in rat or guinea-pig vas deferens, but rather augmented the contraction significantly in guinea-pig vas deferens where yohimbine was without effect. However, postjunctional actions of SK&F 104078, perhaps at $\alpha_1$-adrenoceptors, may explain the augmentation of the response in the guinea-pig vas deferens.

To summarise our findings, there are three important conclusions. Firstly, the dissociation constant of SK&F 104078 and its potency relative to yohimbine were similar at all the pre- and postjunctional $\alpha_2$-adrenoceptors examined in this study. Differences between tissues in the effects of SK&F 104078 may reflect partial agonist properties of the drug. Secondly, a whole series of $\alpha_2$-adrenoceptor antagonists had similar dissociation constants at pre- and postjunctional $\alpha_2$-adrenoceptors, so that none of the drugs examined distinguished between pre- and postjunctional receptors. Thirdly, based on absolute prazosin potency and its potency relative to yohimbine, all functional $\alpha_2$-adrenoceptors examined resemble each other, but differ from the human platelet binding sites. The functional receptors examined in the present study could not be classed as $\alpha_{2A}$, $\alpha_{2B}$ or $\alpha_{2C}$ (Bylund, 1988), due partly to the limited number of antagonists examined, but seem to resemble the $\alpha_{2B}$.

Supported by the Irish Heart Foundation and by the Royal College of Surgeons in Ireland. Sonia Connaughton is an IHF scholar. We thank Theresa Walsh for kindly carrying out ligand binding assays, and Dr Gorgy Lonart for his expertise with the guinea-pig ileum. We gratefully acknowledge the kind assistance of Drs D. Moore, M. Sugrue, E. Prendiville and P. Burke of St. James's Hospital, Dublin, in supplying us with human saphenous vein samples.

## References

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmacol. Chemother.* **14**, 48–58.

BERTIE, K., DAVIS, J.A., ROACH, A.G., ROWLAND, P. & SMITH, C.F.C. (1988). SK&F 104078 does not selectively antagonise $\alpha_2$-adrenoceptors in the rat. *Br. J. Pharmacol.*, **94**, 433P.

BUCKLEY, C., CURTIN, D., WALSH, T. & O'MALLEY, K. (1986). Ageing and platelet $\alpha_2$-adrenoceptors. *Br. J. Clin. Pharmacol.*, **21**, 721–722.

BYLUND, D.B. (1985). Heterogeneity of alpha-2 adrenergic receptors. *Pharmacol. Biochem. Behav.* **22**, 835–843.

BYLUND, D.B. (1988). Subtypes of $\alpha_2$-adrenoceptors: pharmacological and molecular biological evidence converge. *Trends Pharmacol. Sci.*, **9**, 356–361.

CONNAUGHTON, S., & DOCHERTY, J.R. (1988). Evidence that SK&F 104078 does not differentiate between pre- and postjunctional $\alpha_2$-adrenoceptors. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **338**, 379–382.

CONNAUGHTON, S., WALSH, T., & DOCHERTY, J.R. (1989). No evidence for differences between pre- and postjunctional $\alpha_2$-adrenoceptors in the periphery. *Br. J. Pharmacol.*, **96**, 127P.

DALY, R.N., SULPIZIO, A.C., LEVITT, B., DEMARINIS, R.M., REGAN, J.W., RUFFOLO, R.R. & HIEBLE, J.P. (1988). Evidence for heterogeneity between pre- and postjunctional alpha-2 adrenoceptors using 9-substituted 3-benzazapines. *J. Pharmacol. Exp. Ther.* **247**, 122–128.

DOCHERTY, J.R. (1987). The use of the human saphenous vein in pharmacology. *Trends Pharmacol. Sci.*, **8**, 358–361.

DOCHERTY, J.R. (1988). Pertussis toxin and pre-junctional $\alpha_2$-adrenoreceptors in rat heart and vas deferens. *J. Auton. Pharmacol.*, **8**, 197–201.

DOCHERTY, J.R. & HYLAND, L. (1985). Evidence for neuro-effector transmission through postjunctional $\alpha_2$-adrenoceptors in human saphenous vein. *Br. J. Pharmacol.*, **84**, 573–576.

DOCHERTY, J.R. & WARNOCK, P. (1985). An investigation of $\alpha$-adrenoceptor responsiveness in the vas deferens of spontaneously hypertensive rats. *Br. J. Pharmacol.*, **86**, 327–333.

ESKINDER, H., HILLARD, C.J., OLINGER, G.N., CHRISTENSEN, C.W., BAKER, J.E., WARLTIER, D.C. & GROSS, G.J. (1988). Alpha adrenoceptor subtypes and receptor reserve in human versus canine saphenous vein: sensitivity to blockade by nitroglycerin. *J. Pharmacol. Exp. Ther.*, **247**, 941–948.

HIEBLE, J.P., SULPIZIO, A.C., NICHOLS, A.J., WILLETTE, R.N. & RUFFOLO, R.R. (1988). Pharmacologic characterization of SK&F 104078, a novel alpha-2 adrenoceptor antagonist which discriminates between pre- and postjunctional alpha-2 adrenoceptors. *J. Pharmacol. Exp. Ther.*, **247**, 645–652.

KOBILKA, B.K., MATSUI, H., KOBILKA, T.S., YANG-FENG, T.L., FRANCKE, U., CARON, M.G., LEFKOWITZ, R.J., & REGAN, J.W. (1987). Cloning, sequencing and expression of the gene coding for the human platelet $\alpha_2$-adrenergic receptor. *Science*, **238**, 650–656.

MULLER-SCHWEINITZER, E. (1984). Alpha-adrenoceptors, 5-hydroxytryptamine receptors, and the actions of dihydroergotamine in human venous preparations obtained during saphenectomy procedures for varicose veins. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **327**, 299–303.

MURPHY, T.J. & BYLUND, D.B. (1988). Characterization of alpha-2 adrenergic receptors in the OK Cell, an opossum kidney cell line. *J. Pharmacol. Exp. Ther.*, **244**, 571–578.

MUSGRAVE, I., MARLEY, P., MAJEWSKI, H. (1987). Pertussis toxin does not attenuate $\alpha_2$-adrenoceptor mediated inhibition of noradrenaline release in mouse atria. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **336**, 280–286.

RUFFOLO, R.R., SULPIZIO, A.C., NICHOLS, A.J., DEMARINIS, R. & HIEBLE, J.P. (1987). Pharmacologic differentiation between pre- and postjunctional $\alpha_2$-adrenoceptors by SK&F 104078. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **336**, 415–418.

SNEDECOR, G.W. & COCHRAN, W.G. (1980). *Statistical Methods*, 7th ed. Ames, Iowa: Iowa State University Press.

STEEN, S., SJOBERG, T., SKARBY, T., NORGREN, L. & ANDERSSON, K.-E. (1984). The postjunctional $\alpha$-adrenoceptors of the human saphenous vein. *Acta Pharmacol. Toxicol.*, **55**, 351–357.

VIZI, E.S., HARSING, L.G., GAAL, J., KAPOSCI, J., BERNATH, S. & SOMOGYI, G.T. (1986). CH-38083, a selective, potent antagonist of alpha-2 adrenoceptors. *J. Pharmacol. Exp. Ther.*, **238**, 701–706.

*(Received June 15, 1989*
*Revised September 12, 1989*
*Accepted September 18, 1989)*