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Evidence for neuro-effector transmission through postjunctional α_2-adrenoceptors in human saphenous vein

James R. Docherty & Lisa Hyland

Department of Clinical Pharmacology, Royal College of Surgeons in Ireland, Dublin 2, Ireland

1 The effects of the α_1-adrenoceptor antagonist prazosin and the α_2-adrenoceptor antagonist yohimbine were examined against stimulation-evoked contractions in human isolated saphenous veins.

2 The concentration of yohimbine producing 30% inhibition of stimulation-evoked contractions (IC_{30}) was 13.2 nM, whereas the IC_{30} of prazosin was greater than 250 nM.

3 The inhibition of stimulation-evoked contractions by yohimbine was not prejunctionally mediated since yohimbine (0.01–0.1 μM) significantly potentiated the stimulation-evoked overflow of tritium in tissues pre-incubated with [^{3}H]-noradrenaline.

4 The high potency of yohimbine and the low potency of prazosin indicate that neuro-effector transmission in human saphenous vein is mediated predominantly by postjunctional α_2-adrenoceptors.

Introduction

Evidence has accumulated in recent years demonstrating that more than one type of postjunctional α-adrenoceptor is present on vascular smooth muscle (Starke, 1981; McGrath, 1982). While the α_1-subtype is the prototype postjunctional receptor, evidence has been obtained in several animal species and in man for α_2-receptor mediated contractions to exogenous agonists both *in vivo* (Drew & Whiting, 1979; Docherty & McGrath, 1980; Elliott & Reid, 1983) and, less easily, *in vitro* (Stevens & Moulds, 1981; Constantine *et al.*, 1982; Schümann & Lues, 1983).

Although there is a wealth of data showing α_2-mediated contractions to exogenously applied agonists there is little evidence for an α_2-receptor involvement in contractions evoked by nerve stimulation. In a previous study, we obtained results in rabbit portal vein which could best be explained in terms of a mixed population of α_1- and α_2-receptors mediating stimulation-evoked contraction (Docherty & Starke, 1982); however, that demonstration required the elimination of α_1-mediated contractions by use of the irreversible antagonist phenoxybenzamine. In this present study, we have examined the α-receptors involved in contractions evoked by nerve stimulation in human saphenous vein employing the selective α_1-antagonist prazosin (Cambridge *et al.*, 1977) and the selective α_2-antagonist yohimbine (Weitzell *et al.*, 1979).

Methods

Human saphenous veins were obtained as leftovers from coronary artery bypass grafts of male patients (aged 38–64 years). Tissues were cut spirally into strips approximately 3 mm wide and 20–30 mm long, were placed between platinum electrodes in organ baths and superfused at 37°C in Krebs-Henseleit solution of the following composition (mM): NaCl 119, $NaHCO_3$ 25, (+)-glucose 11.1, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.0, ascorbic acid 0.28 and tetrasodium EDTA 0.03. Tissues were attached to myograph transducers under 1 g tension for isometric tension recordings.

In some experiments, tissues were pre-incubated for 1 h in 1 ml medium containing [^{3}H]-noradrenaline (1 μM, specific activity 39 Ci mmol^{-1}) before placing between platinum electrodes in organ baths and beginning superfusion. The superfusate was collected in 3 min fractions, and stimulation-evoked overflow of total tritium produced by field stimulation for 3 min at 5 Hz was calculated by subtraction of the basal overflow. At the end of experiments, tissues were solubilized in 2 ml of tissue solubilizer and the radioactivity in superfusate samples and tissues was determined by liquid scintillation counting. The basal outflow of tritium and the stimulation-evoked overflow of tritium were expressed as a percentage of the

tritium content of the tissue at the start of the respective stimulation period (see Borowski *et al.*, 1977).

In all experiments, after 2 h equilibration, responses to field stimulation for 3 min at a frequency of 5 Hz (0.5 ms pulses, supramaximal voltage) were obtained at intervals of 30 min. After two control stimulation periods (S_1 and S_2), test drugs were added to the superfusion stream in three increasing concentrations beginning 18 min before subsequent stimulation periods (S_3-S_5). The rate of infusion of test drug or, in control experiments, of vehicle was 16 $\mu l\,min^{-1}$.

Drugs

The following were used: prazosin hydrochloride (gift: Pfizer); yohimbine hydrochloride (Sigma). Drug stocks and dilutions were dissolved in distilled water, and in control experiments, distilled water was administered.

Statistics

Effects of a given concentration of test drug on stimulation-evoked contractions or stimulation-evoked overflow of tritium were compared with the equivalent effects of vehicle (i.e. effects of 0.01 μM were compared with vehicle given before S_3, 0.1 μM with vehicle given before S_4, 1 μM with vehicle given before S_5) by Student's *t* test for unpaired data.

Results

Field stimulation of human isolated saphenous vein for 3 min at a frequency of 5 Hz produced an isometric contraction of 1.00 ± 0.19 g ($n = 14$). Infusion of distilled water vehicle did not significantly affect the stimulation-evoked contraction over 3 stimulation periods ($n = 4$). The α_2-adrenoceptor antagonist yohimbine produced a significant inhibition of this contraction over the concentration range 0.01 – 1 μM, whereas the α_1-adrenoceptor antagonist prazosin produced a significant inhibition only at concentrations of 0.1 μM and above (Figure 1). The concentration of yohimbine producing 30% inhibition of the stimulation-evoked contraction (IC_{30}) was calculated by linear regression analysis as 13.2 nM (95% confidence limits 6.6–26.3 nM; $n = 4$). For prazosin, an IC_{30} could not be obtained in 2 of 6 experiments in which the inhibition by prazosin (up to 1 μM) was small and not concentration-dependent: in these two experiments prazosin was assigned an IC_{30} of 1 μM, giving a mean IC_{30} of prazosin of not less than 251 nM ($n = 6$).

The inhibition of the stimulation-evoked contraction by yohimbine was postjunctionally mediated since prejunctionally yohimbine (0.01 – 1 μM) did not cause any significant reduction in stimulation-evoked tritium overflow in tissues pre-incubated with [^{3}H]-noradrenaline. Yohimbine (0.01 – 0.1 μM) significantly potentiated the stimulation-evoked overflow (Figure 2), presumably by blockade of endogenous α_2-mediated inhibition by neurotransmitter noradrenaline. Prazosin (0.01 – 0.1 μM) did not significantly alter stimulation-evoked tritium overflow (Figure 2). Prazosin (1 μM, administered before S_5) significantly increased the basal outflow of tritium by approximately 20% (basal outflow in the 3 min before S_5 as a percentage of the basal outflow in the 3 min before S_2: vehicle $84.0 \pm 2.5\%$, $n = 5$; prazosin 1 μM $99.9 \pm 4.7\%$, $n = 4$; Student's *t* test, $P < 0.05$). Effects of prazosin (1 μM) on stimulation-evoked overflow of tritium were not calculated since the increase in basal outflow produced by this concentration makes interpretation of its effects on stimulation-evoked overflow difficult.

Discussion

Investigations into the interaction of α-adrenoceptor agonists and antagonists on some human isolated

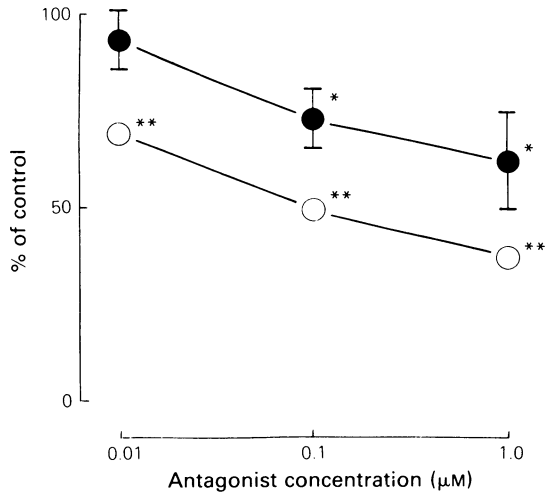

Figure 1 Concentration-response curves for the effects of yohimbine and prazosin on the isometric contraction of human saphenous vein to field stimulation for 3 min at a frequency of 5 Hz. Responses are expressed as a percentage of pre-drug response. Vertical bars representing s.e.mean are shown except when contained within the symbol. Symbols: yohimbine, $n = 4$ (○); prazosin, $n = 6$ (●). Asterisks denote effect of drug significantly different from effect of distilled water vehicle (Student's *t* test: $^*P < 0.05$; $^{**}P < 0.01$).

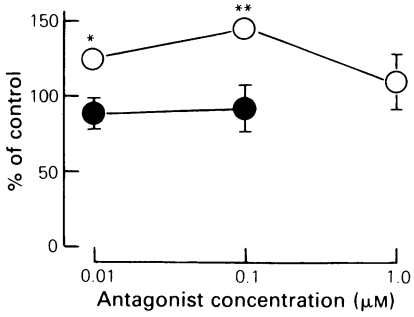

Figure 2 Concentration-response curves for the effects of yohimbine and prazosin on the overflow of tritium evoked by field stimulation for 3 min at a frequency of 5 Hz in human saphenous veins preincubated with [^{3}H]-noradrenaline. Responses are expressed as a percentage of pre-drug response. Vertical bars representing s.e.mean are shown except when contained within the symbol; $n = 4$, each. Symbols: yohimbine (○); prazosin (●). Asterisks denote effect of drug significantly different from effect of distilled water vehicle (Student's t test: $^*P < 0.05$; $^{**}P < 0.01$).

blood vessels, particularly veins, have produced results which can best be explained in terms of a mixed population of α_1- and α_2-adrenoceptors on the vascular smooth muscle (Stevens & Moulds, 1981; Glusa & Markwardt, 1983; Steen *et al.*, 1984). Evidence that endogenous noradrenaline neurotransmitter acts on postjunctional α_2-receptors has proved difficult to obtain even in animal experiments (see Docherty & Starke, 1982) or has proved negative (see Langer *et al.*, 1980; Wilffert *et al.*, 1982).

We are now able to demonstrate in human saphenous vein that the α_2-selective antagonist yohimbine is at least 20 times more potent than the α_1-selective antagonist prazosin at inhibiting nerve stimulation-evoked isometric contractions. The postjunctional potency of yohimbine obtained in this study was all the more remarkable since yohimbine (0.01–0.1 μM) increased stimulation-evoked transmitter overflow by a prejunctional action. In tissues such as rabbit pulmonary artery and aorta, an increase in the stimulation-evoked transmitter overflow by α_2-antagonists results in a potentiation of the stimulation-evoked isometric contraction (Weitzell *et al.*, 1979; Docherty & Starke, 1982). If the human saphenous vein contained only α_1-receptors postjunctionally, a potentiation of stimulation-evoked overflow by yohimbine should have resulted in a potentiation of the stimulation-evoked contraction.

The evidence in favour of an involvement of postjunctional α_2-receptors in stimulation-evoked contractions in human saphenous vein can be discussed in terms of the absolute potencies of antagonists, the potencies of antagonists relative to each other and the pre- and postjunctional potencies of antagonists. The absolute postjunctional potency of yohimbine obtained in this study agrees well with its reported potency at prejunctional α_2-receptors in the rabbit pulmonary artery (8 nM: Starke, 1981) and contrasts with its postjunctional potency at α_1-receptors in the same tissue (400 nM: Starke, 1981). The postjunctional potency of prazosin obtained in this study is much lower than its reported potency against exogenous α_1-agonists in rabbit pulmonary artery (2 nM: Starke, 1981) and its IC_{30} against stimulation-evoked contractions in rabbit aorta (3.7 nM: adapted from Docherty & Starke, 1982). Even in rabbit portal vein, where stimulation-evoked contractions may involve both α_1- and α_2-receptors, tne IC_{30} of prazosin was 12.9 nM (adapted from Docherty & Starke, 1982). In contrast, the prejunctional potency of prazosin at α_2-receptors is low but difficult to establish since prazosin at up to 100 nM had no effect on stimulation-evoked overflow in rabbit pulmonary artery (Starke, 1981) and human saphenous vein (present results) but higher concentrations increased the basal outflow of tritium. Hence prazosin is not an α_2-antagonist in concentrations of up to 100 nM.

In rabbit pulmonary artery, prazosin is 200 times more potent than yohimbine at antagonising contractions to α_1-agonists, but is at least 10 times less potent than yohimbine at prejunctional α_2-receptors (see Starke, 1981). The potency of yohimbine relative to prazosin in human saphenous vein would suggest that the postjunctional receptors are predominantly of the α_2-subtype.

In the human saphenous vein, yohimbine inhibited contractions over the same concentration range at which it increased the stimulation-evoked overflow: since prejunctional α-receptors were the prototype α_2-receptors this would suggest that these postjunctional receptors are also predominantly α_2. In contrast, prazosin inhibited contractions at a concentration (100 nM) only slightly lower than the concentration (1 μM) which produced non-specific prejunctional effects. Indeed, in two preparations prazosin at up to 1 μM had little effect on stimulation-evoked isometric contractions.

In conclusion, the data suggest that neuro-effector transmission in human saphenous vein is mediated predominantly through postjunctional α_2-adrenoceptors.

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