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A comparison of the effects of indoramin and yohimbine at pre- and postsynaptic alpha adrenoceptors of the rabbit pulmonary artery

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Indoramin is an antihypertensive agent with selective alpha$_1$-adrenoceptor blocking activity [1–3]. In this study the presynaptic alpha$_2$- and postsynaptic alpha$_1$ blocking actions of indoramin have been compared with those of yohimbine, a compound known to block alpha$_2$-adrenoceptors selectively [4], using a tritiated noradrenaline ([^{3}H]NA) overflow technique [5]. This method allows a measure of alpha$_2$ and alpha$_1$ adrenoceptor blockade to be obtained simultaneously. Alpha$_2$-adrenoceptor blockers are believed to enhance stimulation-evoked release of transmitter by interrupting the presynaptic alpha$_2$-mediated negative feedback control at the nerve terminal [6, 7]. Alpha$_1$ blockade is assessed from the reduction in stimulation-evoked tissue contraction which these agents produce.

Materials and methods. Male rabbits (1.5–3.5 kg) were stunned by a blow on the head and exsanguinated. The pulmonary artery was cut into two parallel, helical strips and each strip immersed in 25 ml Krebs' solution containing 3×10^{-5} M EDTA and 10^{-4} M ascorbic acid (inhibitors of catecholamine oxidation), maintained at 37° and gassed with a mixture of 5% CO_2 in oxygen. The tissues were incubated for 30 min at 37° after addition of $1.5–2 \times 10^{-6}$ M L-[7, 8-^{3}H]noradrenaline hydrochloride to the medium. The strips were washed by immersion for 1 min in each of 6×25 ml samples of the physiological solution. The strips were then mounted vertically in 1 ml organ baths under 2 g tension between two platinum ring electrodes.

Krebs' solution maintained at 37° and gassed with a mixture of 5% CO_2 in oxygen containing EDTA and ascorbic acid together with corticosterone (4×10^{-5} M), desipramine (5×10^{-7} M) and propranolol (4×10^{-6} M) was perfused upwards through the organ bath at a rate of 2 ml/min. The perfusate was collected in 3 min samples using a fraction collector.

After an initial 2 hr perfusion period, each tissue was stimulated electrically with a train of 300 pulses of 1 msec duration at 2 Hz and supramaximal voltage using a square wave stimulator (Square One Instruments). Contractions of the tissue were recorded isometrically using a Grass Strain Gauge (Model FTO3C) connected to a Grass 7D polygraph. A total of five stimulation periods at 24 min intervals was used for each tissue.

A 2 ml aliquot of each 3 min fraction was added to 10 ml NE 260 scintillation fluid (Nuclear Enterprise Ltd) and tritium measured using a Packard Tricarb 2660 scintillation counter. Counts were corrected for quenching. At the end of the experiment, the tissues were solubilized in 0.5 ml Soluene-350 (Packard Instrument Co). The solubilized tissue strips were counted in the same way as the fraction aliquots described above.

Stimulation-evoked overflow of tritium was calculated from the sum of the tritium contents of the three fractions collected following the onset of each electrical stimulation. From this total was subtracted the estimated spontaneous overflow during each of these three fractions. Spontaneous overflow was estimated by interpolation, assuming a linear reduction in spontaneous release with time, between the fraction immediately preceding the onset of that stimulation and the fraction beginning 21 min after the onset of that stimulation period. The difference (stimulation-evoked overflow) in disintegrations per min (dpm) between the total tritium present in these three fractions and the estimated spontaneous overflow was expressed as a percentage of the total tritium present (in dpm) in the tissue at the onset of that stimulation period and is referred to as fractional overflow. Total tritium present in the tissue was calculated as the sum of the tritium present in the tissue at the end of the experiment and the tritium present in all fractions following the onset of that stimulation period.

Indoramin and yohimbine were added to the perfusion stream 12 min before the fourth stimulation period (S_4). Drug effects were assessed by expressing the fractional overflow for the stimulation period in the presence of the drug (S_5) as a ratio to the fractional overflow during the second control stimulation period (S_5/S_2). The effect of the drug on the contraction of the tissue was expressed in a similar way, the ratio S_5/S_2 being calculated using the values of the maximum tensions recorded during these stimulation periods.

The ratios S_5/S_2 for drug treated groups ($n = 4$ at each concentration) were compared with the same ratios from an untreated control group ($n = 10$) using Student's unpaired t-test.

The following drugs were used: L-ascorbic acid (BDH); corticosterone (Sigma); desipramine HCl (CIBA); EDTA (BDH); propranolol HCl (ICI); L-[7, 8-^{3}H]noradrenaline HCl, 8–15 Ci per mmole (Radiochemical Centre, Amersham, Bucks, U.K.), L-noradrenaline bitartrate (Koch-Light), yohimbine HCl (Sigma).

Results. In the two groups of ten control tissues, spontaneous overflow of tritium in the three fractions (9 min) preceding S_2 was $1.17 \pm 0.085\%$ (mean ± S.E.) expressed as fractional overflow. In the three fractions following the beginning of the S_2 period the stimulation-evoked fractional overflow (after subtraction of calculated spontaneous efflux) was $1.50 \pm 0.140\%$ (mean ± S.E.) The maximum tension recorded in this group of tissues during S_2 was 0.37 ± 0.051 g (mean ± S.E.).

Indoramin had little effect on stimulation-evoked overflow in the concentration range tested (3×10^{-8}–10^{-5} M; Fig. 1). A small reduction in overflow was observed at 10^{-5} M. Contractions of the tissue were reduced in a concentration-related manner by indoramin in the range 10^{-7}–10^{-5} M. (Fig. 2).

In contrast, tritium efflux was significantly increased in the presence of yohimbine in the concentration range 10^{-7}–3×10^{-6} M (Fig. 1). The peak effect was at 3×10^{-7} when efflux was 2.14 times greater than before exposure to yohimbine. A 50% increase in efflux was estimated graphically at 1.1×10^{-7} M. At higher concentrations of yohimbine the increase in efflux was reduced. At 10^{-5} M efflux had returned to the value observed before exposure to yohimbine and at 3×10^{-5} M it was significantly less than this value. Contractions of the tissue were not significantly enhanced by yohimbine at the concentrations at which efflux was enhanced, but at higher concentrations (10^{-5} and 3×10^{-5} M) contractions of the tissue were greatly reduced (Fig. 2).

Both indoramin (10^{-6}–10^{-5} M) and yohimbine (3×10^{-5} M only) produced small increases in spontaneous (unstimulated) tritium release.

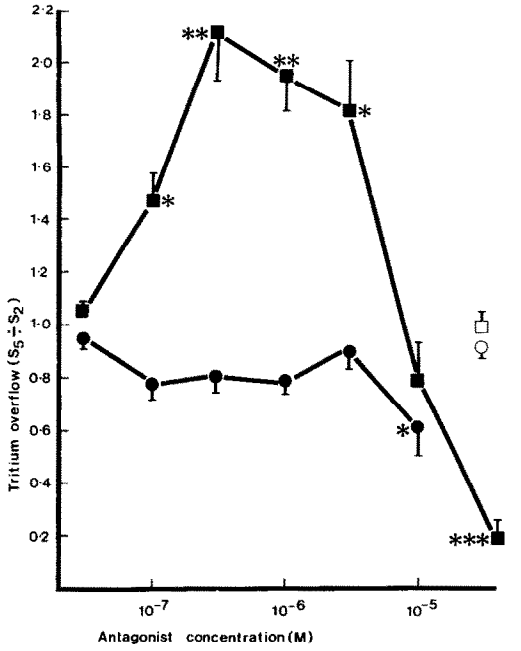

Fig. 1. The effects of indoramin (closed circles) and yohimbine (closed squares) on stimulation-induced efflux of tritium from rabbit isolated pulmonary artery preincubated with [^{3}H]NA. Results are expressed as the ratio of response in the presence of drug (S_5) to that in the absence of drug (S_2). Each point represents the mean of four determinations. Statistical comparisons were made with the same ratios from an untreated control group ($n = 10$) using Student's unpaired t-test. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Open symbols show control group values obtained with the indoramin and yohimbine test groups. Vertical bars represent S.E.M.

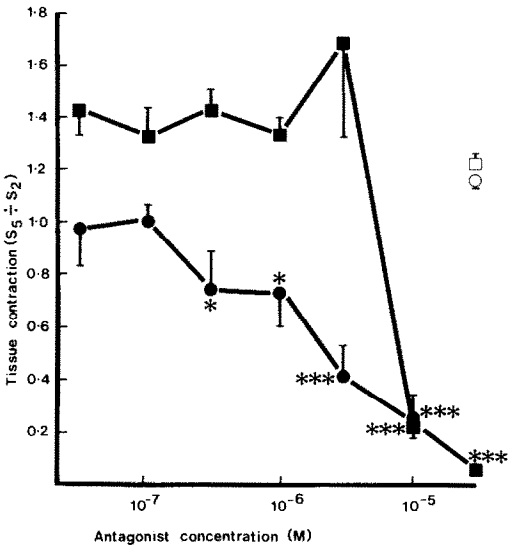

Fig. 2. The effects of indoramin (closed circles) and yohimbine (closed squares) on stimulation-induced contractile responses of the rabbit isolated pulmonary artery. Each point represents the mean of four determinations. S_5/S_2 ratios were derived and statistical analysis made as described in Fig. 1. Open symbols show control group values ($n = 10$) obtained with the indoramin and yohimbine test groups. Vertical bars represent S.E.M.

Discussion. The observation that indoramin did not enhance stimulation-evoked overflow of tritium from rabbit pulmonary artery, previously incubated with [^{3}H]NA, at concentrations which markedly reduced the contractile response of the tissue to stimulation supports the view that indoramin is a selective antagonist at the postsynaptic ($alpha_1$) adrenoceptor. It has previously been reported from this laboratory [1] that indoramin has only weak activity at the presynaptic ($alpha_2$) site of the rat vas deferens using clonidine as agonist; the failure of indoramin to enhance stimulation-evoked tritium release at concentrations of up to 10^{-5} M is in agreement with this.

The results obtained with yohimbine contrast markedly with those for indoramin and are in general agreement with those of other authors [8]. Thus, enhanced release of tritium with little effect, or small enhancements, of contractile response were observed at moderate concentrations of yohimbine as expected for a selective $alpha_2$ adrenoceptor blocker. In the present study, yohimbine at 10^{-5} M had much less effect on electrically-evoked tritium overflow than did lower concentrations, and at 3×10^{-5} M overflow was significantly less than that of controls. Inhibition of transmitter release with large concentrations of yohimbine has been noted by other workers [9] though the mechanism is unclear.

The increase in basal outflow of tritium with alpha adrenoceptor blockers has been noted previously and is believed to consist mainly of noradrenaline metabolites [8]. An increase in basal overflow was observed with both yohimbine and indoramin in this study but no attempt was made to separate [^{3}H]NA from its tritiated metabolites.

Indoramin thus reduced contractile responses to stimulation of the isolated rabbit pulmonary artery with little effect on evoked overflow of tritium. This would support the view that indoramin is a selective $alpha_1$ adrenoceptor blocker. Yohimbine produced a marked increase in stimulation-evoked overflow of tritium at concentrations which did not inhibit the contractile response, in keeping with its $alpha_2$ adrenoceptor selectivity.

It has been suggested that the disappointing clinical results in hypertension with non-selective alpha antagonists are a consequence of impaired feedback [10]. The results of the present study suggest that indoramin would not have this disadvantage.

In summary, the effects of indoramin and yohimbine on $alpha_1$ and $alpha_2$ adrenoceptors have been compared using the tritium overflow technique on the rabbit isolated pulmonary artery preincubated with [^{3}H]NA. Indoramin did not increase electrically-evoked tritium overflow over the concentration range 3×10^{-8}–10^{-5} M, but inhibited tissue contractions in the range 10^{-7}–10^{-5} M. In contrast, yohimbine increased tritium efflux over the range 10^{-7}–3×10^{-6} M and tissue contractions were reduced at concentrations of 10^{-5} and 3×10^{-5} M. The results confirm the selectivity of indoramin for $alpha_1$-adrenoceptors.

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Volatile hydrocarbons from hydrogen peroxide-induced lipid peroxidation of erythrocytes and their cell components

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The release of volatile alkanes, such as pentane and ethane, has recently been proposed as a reliable and sensitive index for lipid peroxidation [1–5]. It is generally accepted that these alkanes originate from the decomposition of lipid hydroperoxides [6]. In erythrocytes, lipid peroxidation is currently studied by measuring the formation of thiobarbituric acid-reactive material and fluorescent chromolipids. In the present study we describe the release of short-chain volatile hydrocarbons formed during hydrogen peroxide-induced peroxidation of the various cell components of erythrocytes.

Materials and methods

Red cells were isolated from the blood of healthy donors, as described by Beutler *et al.* [7], with microcristalline cellulose and α-cellulose (1:1, w/w). As anticoagulant, sodium citrate (3.13%) was used (1:5, v/v). The analysis of hydrocarbons in the head-space vials, used for the incubation of red cells with peroxidizing agents, was performed as previously described [4, 8]. Ghost membranes were isolated as described by Burton *et al.* [9]. Haemolysates were prepared by the lysis of erythrocytes with phosphate buffer (5 mM, pH 8.0) followed by centrifugation at 22,000 *g* for 10 min at 4° to remove the membranes. Haemoglobin was purchased from Sigma (human type IV). Experiments with erythrocytes, ghost membranes, ghost-free haemolysates and haemoglobin were performed with 10 mM hydrogen peroxide as peroxidizing agent. Erythrocytes and haemolysates were incubated with 0.25 mM sodium azide for the inhibition of catalase. Lipid peroxidation of ghost membranes was initiated in the presence of 2.0 mM $FeSO_4$ and 4.0 mM EDTA. Erythrocyte suspension (haematocrit 2.5%) was treated with hydrogen peroxide for 2 hr in a shaking water bath at 37°. Haemolysates and haemoglobin were incubated similarly, using equal concentrations of haemoglobin (0.8 g/dl) as used for the red cell incubation. Ghost membranes were treated with iron for 2 hr in the head-space vials shaken in a water bath at 37°. Protein content of ghost membranes was estimated by the method of Lowry *et al.* [10].

Results and discussion

The mean amounts (± S.E.M.) of hydrocarbon gases produced from the erythrocytes, ghost-free haemolysates and haemoglobin are shown in Table 1. Pentane and ethane were not obtained in the absence of erythrocyte membranes, indicating that they originate from the peroxidation of polyunsaturated fatty acids of membrane lipids. Ethylene, *n*-butane and *iso*-butane, formed during hydrogen peroxide-catalysed peroxidation of haemolysates and haemoglobin, were produced in concentrations comparable to those measured in the erythrocytes. The amount of propane produced by haemolysates and haemoglobin was less than that produced by erythrocytes.

Incubation of ghost membranes with Fe^{2+}-EDTA yielded mainly pentane, ethylene and ethane, but also small amounts of propane and *n*-butane (Table 2). The release of the latter gases could be particularly explained by the degradation of haemoglobin residues in the ghost membrane fraction. The high amount of ethylene could derive principally from the β-scission of lipid peroxides from ω3- and ω6- fatty acids. On the other hand, ethylene is known to be a degradation product from methionine [11] and could derive from haemoglobin residues and membrane proteins. *Iso*-butane was not observed with Fe^{2+}-EDTA, indicating that this gas may originate from amino acid

Table 1. Production of volatile hydrocarbons from erythrocytes, haemolysates and haemoglobin

Source	Ethane	Ethylene	Propane	*n*-Butane	*i*-Butane	Pentane
Erythrocytes $n=38$	0.19 ± 0.01	0.45 ± 0.01	0.41 ± 0.01	0.47 ± 0.03	0.18 ± 0.01	0.79 ± 0.02
Haemolysates $n=5$	0	0.42 ± 0.06	0.20 ± 0.04	0.40 ± 0.04	0.19 ± 0.01	0
Haemoglobin $n=3$	0	0.31 ± 0.01	0.34 ± 0.02	0.45 ± 0.01	0.18 ± 0.001	0

Incubation with 10 mM hydrogen peroxide for 2 hr at 37°. Erythrocytes and haemolysates were incubated with 0.25 mM sodium azide for inhibition of the catalase. Mean values ± S.E.M. Hydrocarbons are expressed as nmole/gHb per 2 hr. Experiments with erythrocytes and haemolysates were performed with blood from different donors.