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The effect of isoprenaline on adrenoceptors in human saphenous vein

I. M. COUPAR

Division of Clinical Pharmacology, the Medical Professorial Unit, St. Bartholomew's Hospital, London E.C.1

Summary

1. The order of potency of sympathomimetic amines in causing contraction of strips of human saphenous vein was (−)-adrenaline>(−)-noradrenaline>(−)-phenylephrine>(±)-isoprenaline.

2. The competitive α-adrenoceptor blocking drugs tolazoline, phentolamine, and thymoxamine and the irreversible blocking drug phenoxybenzamine all blocked noradrenaline and isoprenaline contractions.

3. Contractions produced by 5-hydroxytryptamine were also blocked by phentolamine and thymoxamine. Fenfluramine-induced contractions were not blocked by thymoxamine or phenoxybenzamine.

4. ED50 contracting doses of isoprenaline did not cause consistent relaxation of noradrenaline-contracted strips.

5. It is concluded that human saphenous vein contains a dominant population of α-adrenoceptors which can be stimulated by high doses of isoprenaline, but the occurrence of β-adrenoceptors mediating relaxation is rare.

Introduction

Isoprenaline acts predominantly on β-adrenoceptors (Cobbold, Ginsburg & Paton, 1960; Innes & Nickerson, 1965) but appears to have different actions on venous smooth muscle when studied in different experimental conditions. Kaiser, Ross & Braunwald (1964) measured the volume of venous return in the anaesthetized dog using a complete cardiopulmonary by-pass method and showed evidence of α- and β-adrenoceptors associated with venoconstriction. Infusion of isoprenaline ((4 μg/kg)/min) into the femoral artery caused venoconstriction which was blocked by pronethalol, but the venoconstriction caused by noradrenaline was not completely blocked by phenoxybenzamine. Reflex intervention from the vagus was eliminated by bilateral vagotomy and secondary reflexes that might result from systemic hypotension were prevented by ganglionic blockade. Venoconstriction has also been recorded in man (Eckstein & Hamilton, 1959), but in this case could have resulted from reflex compensation rather than a direct effect on venous smooth muscle. Abboud, Eckstein & Zimmerman (1963) recorded perfusion pressure at constant flow in the dog brachial vein and showed that isoprenaline (0·25–4 μg injected into the brachial artery) caused small venodilatation which was blocked by dichloroisoprenaline (DCI). However, Zsotér (1968), using a more direct measurement of venous function, the distensibility in a single vein, could demonstrate no effect of

isoprenaline in the superficial arm veins of man or the femoral and jugular veins of the anaesthetized dog, but only low doses were used (1–3 μg injected into a segment of vein about 3 cm long isolated by rubber wedges). Plethysmographic studies (Sharpey-Schafer, 1961) have shown that isoprenaline (0·1 μg/min infused intra-arterially) causes a small dilatation of the forearm veins of man (Sharpey-Schafer & Ginsburg, 1962).

In order to eliminate the local and central reflexes that occur *in vivo*, the direct effect of isoprenaline is most easily measured on strips of vein cut spirally or longitudinally. Again conflicting results appear in the literature as vascular smooth muscle is not heterogeneous in its reactivity (Somlyo, Sandberg & Somlyo, 1965; Miller & Lewis, 1969). The contractile effect of isoprenaline on isolated human saphenous vein previously reported as a demonstration to the British Pharmacological Society (Coupar & Turner, 1969) has, therefore, been investigated further.

Methods

Specimens

Fresh specimens of human saphenous vein were collected from the operating theatres in vacuum flasks containing cold Krebs bicarbonate solution (at approximately 5° C) and were taken to the laboratory for dissection. The specimens were taken from patients at operation for ligation and stripping of varicose veins or from below-knee amputations. Specimens were used only if they had been cut out by the surgeon before stripping (usually from the groin) or from pieces taken at operation for ligation and excision. Strips which had been prepared from stripped vein were unresponsive to noradrenaline. Fat and connective tissue were removed and the veins cut spirally using a glass rod and fine scissors. The patients from whom specimens were taken had usually been premedicated with Omnopon (20 mg) and Scopolamine (0·4 mg). Anaesthesia was induced with thiopentone (200–300 mg) and maintained with a nitrous oxide-oxygen or a halothane-oxygen mixture.

Recording

The strips (approximately 3–5 cm long) were mounted on a 10 ml tissue bath containing Krebs bicarbonate solution of the following composition: NaCl 6·87 g, KCl 0·4 g, $MgSO_4.7H_2O$ 1·4 g, $CaCl_2$ 0·606 g, KH_2PO_4 0·16 g, $NaHCO_3$ 0·52 g and glucose 2 g, distilled water to 1 litre. A 95% oxygen, 5% carbon dioxide gas mixture was bubbled through the tissue bath and the temperature was maintained at 37° $\pm$0·25° C. Isotonic responses of the smooth muscle strips (the circular muscle) were recorded on a smoked drum with a frontally writing lever arranged to apply a tension of 0·5–1·0 g at a lever magnification of approximately $\times$ 10. Preparations were left for at least 1 h before any drugs were added to the bath. Solutions of sympathomimetic amines were stabilized against autoxidation by the addition of approximately 200 μg/ml of ascorbic acid.

Drugs

Adrenaline acid tartrate B.P., cocaine hydrochloride B.P., fenfluramine ("Ponderax", Selpharm Laboratories), 5-hydroxytryptamine creatinine sulphate (Koch-Light Laboratories), isoprenaline sulphate B.P., noradrenaline ("Levophed",

Bayer Products Co.), phenoxybenzamine hydrochloride (" Dibenyline ", Smith, Kline & French Laboratories Ltd.), phentolamine (" Rogitine ", Ciba Laboratories Ltd.), phenylephrine hydrochloride B.P. (Boots Pure Drug Co. Ltd.), propranolol (" Inderal ", I.C.I. Ltd.), thymoxamine (" Opilon ", William Warner Ltd.), tolazoline (" Priscol ", Ciba Laboratories Ltd.) and tyramine hydrochloride (B.D.H. Ltd.).

Relative potencies

The potencies of adrenaline, isoprenaline and phenylephrine were measured relative to noradrenaline by the method of Furchgott (1967). Cumulative dose response curves were produced and the ED50 concentrations of the free base compared. Cocaine hydrochloride (5 μg/ml) was included in the Krebs solution to block the amine uptake system of sympathetic nerve terminals.

Experiments with α-adrenoceptor blocking drugs

The competitive α-adrenoceptor blocking drugs tolazoline, phentolamine and thymoxamine and the irreversible blocking drug phenoxybenzamine were used to determine the mode of action of isoprenaline in causing contraction of human saphenous vein. Approximately 50% contractions were produced with noradrenaline and isoprenaline by adding a contracting dose (ED50) determined previously from a dose response curve. Agonist responses were recorded and when constant were measured in the presence of the blocking drugs. The competitive blocking drugs were added to the bath fluid 5 min before the agonist was due to be added and were washed out with the agonist at the end of the response. Phenoxybenzamine was incubated with strips for 10 min. Noradrenaline responses were recorded to test the efficiency of the blocking drugs, but an apparent partial blockade could result from loss of sensitivity of the tissue. To show that the effects of the antagonists were real and not a result of such a loss of sensitivity two procedures were used:

(*a*) The demonstration that the responses to doses of noradrenaline and isoprenaline (ED50) were restored to their original magnitude after washing out the antagonists.

(*b*) The use of an agonist that produced a contraction by a mechanism not involving α-adrenoceptors (referred to here as the " marker " agonist). Initially 5-hydroxytryptamine (5-HT) was used as the marker agonist (Table 1), but as its action was blocked by the α-adrenoceptor antagonists fenfluramine was used in later experiments (Table 2).

The drug contact time and dose cycles for each tissue strip were kept constant. The selection of times for each strip depended upon response time and relaxing time.

Results

Relative potencies of sympathomimetic amines

Cumulative dose response curves of sympathomimetic amines showed that the order of potency in causing contraction was (−)-adrenaline>(−)-noradrenaline (−)-phenylephrine>(±)-isoprenaline. The maximum contraction heights produced by phenylephrine and isoprenaline were lower than those caused by adrenaline and noradrenaline (Figs. 1 and 2, Table 1).

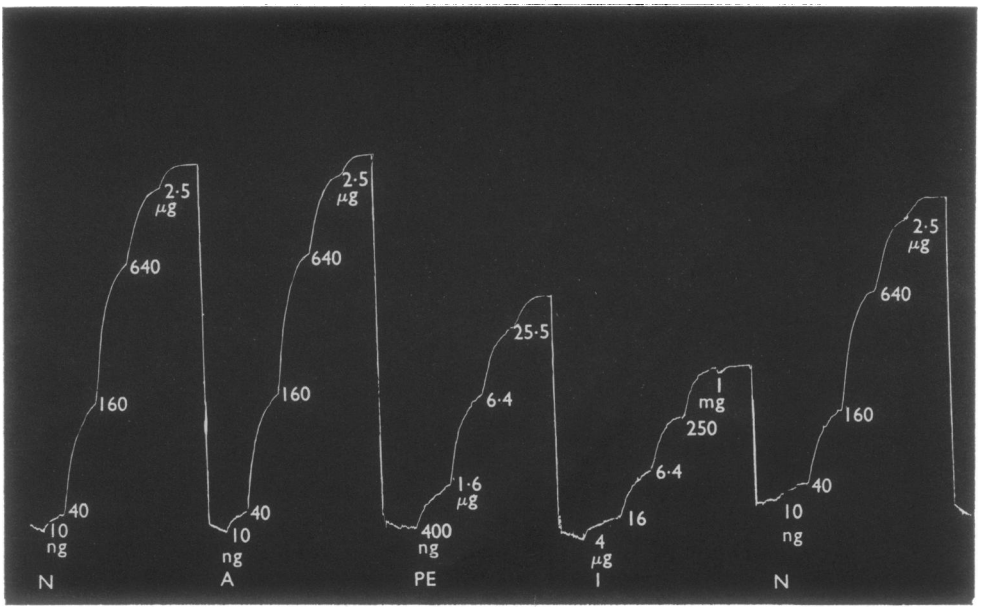

FIG. 1. Contractions caused by cumulative addition of (−)-adrenaline (A), (−)-noradrenaline (N), (−)-phenylephrine (PE) and (±)-isoprenaline (I) on human saphenous vein (strip number 2). Each dose level of sympathomimetic amine was left in contact with the tissue for 4 min before adding the next higher dose. Each determination was made 20 min after washing out when the tissue had relaxed to the base-line. Contractions produced by noradrenaline were repeated at the end of the experiment to check that the sensitivity of the tissue had not changed.

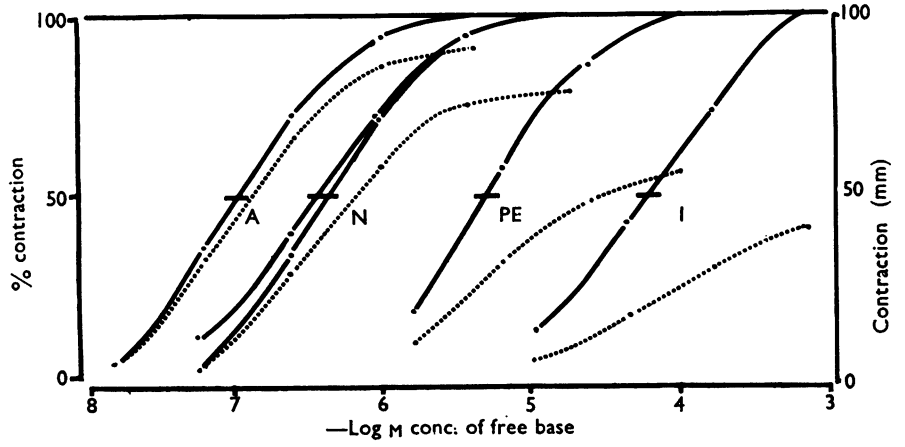

FIG. 2. Dose response curves plotted from Fig. 1 showing that the order of potency of sympathomimetic amines in causing contraction of human saphenous vein was (−)-adrenaline (A) > (−)-noradrenaline (N) > (−)-phenylephrine (PE) > (±)-isoprenaline (I). Doses are expressed as molar concentrations of the free bases and response as the percentage of the maximum response (——) for the determination of the molar ED50s. - - - - represents the heights recorded on the smoked paper in mm.

α-Adrenoceptor antagonists

Preliminary experiments with tolazoline and phentolamine showed that both noradrenaline and isoprenaline responses were blocked by these drugs (Table 2). The responses of the agonists were restored 10 min after washing out tolazoline from the bath but not after phentolamine. As phentolamine remained in the tissue after washout the sensitivity of the strips treated with this drug and also with thymoxamine were tested using 5-HT as a marker. Both blocking drugs inhibited noradrenaline, isoprenaline and 5-HT contractions (Table 2).

Thymoxamine (5 μg/ml) caused a marked reduction in contraction heights of noradrenaline but not of the marker fenfluramine (Fig. 3). The 10% reduction in fenfluramine response in strip 10 probably indicated a decrease in sensitivity, as in another preparation 50 μg/ml thymoxamine did not affect the response (Table 3).

Incubation with phenoxybenzamine (1 μg/ml for 10 min after the control responses) caused total blockade of noradrenaline and isoprenaline responses but not fenfluramine responses (Table 3, Fig. 4).

β-Adrenoceptors

Six preparations were maximally contracted with noradrenaline and when the response reached a steady plateau an ED50 contracting dose of isoprenaline was added according to the method used by Guimarães & Osswald (1969). The selection of doses was determined from dose response curves. Isoprenaline caused a

TABLE 1. *Relative potencies of sympathomimetic amines*

	Relative potencies			
Strip	A	N	PE	I
1	4	1	0·08	0·007
2	5	1	0·1	0·007
3	4	1	0·04	0·003

Potencies of (−)-adrenaline (A), (−)-phenylephrine (PE) and (±)-isoprenaline (I) relative to (−)-noradrenaline (N) in causing contraction of human saphenous vein calculated from the ED50 molar concentrations of the free bases.

TABLE 2. *Blocking action of tolazoline, phentolamine and thymoxamine on noradrenaline (N), isoprenaline (I) and 5-HT contractions*

		% blockade of agonist ED50		
Strip	Antagonist	N	I	5-HT
	Tolazoline			
4	1 μg/ml	30	70	—
4	10 μg/ml	—	95	—
	Phentolamine			
5	1 μg/ml	100	45	—
6	5 μg/ml	100	90	95
5	10 μg/ml	100	85	—
	Thymoxamine			
7	1 μg/ml	75	55	0
8	1 μg/ml	65	30	50
8	5 μg/ml	70	40	70

The contractions produced by noradrenaline and isoprenaline were restored to their original heights 10 min after washing out tolazoline from the bath, but not after phentolamine (strip 5). Contractions produced by 5-HT as the marker agonist, as well as the noradrenaline and isoprenaline contractions, were blocked by phentolamine and thymoxamine. The responses were not restored by washing out the antagonists.
—, Not tested.

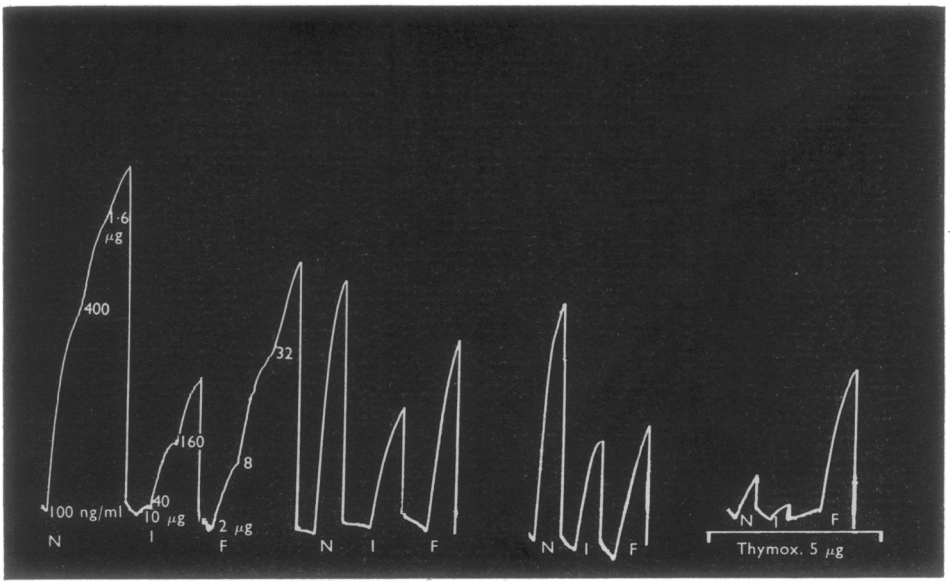

FIG. 3. Blockade by thymoxamine (5 μg/ml) of noradrenaline (N) and isoprenaline (I) but not fenfluramine (F) contractions (from strip 11). Cumulative doses of the agonists were added to the tissue bath to determine the approximate ED50s, which were 100 ng/ml N, 40 μg/ml I and 8 μg/ml F (N, I and F respectively). The ED50 doses of the agonists were added to the tissue bath for 3 min each. They were then added in the presence of thymoxamine (Thymox.). ED50 contractions were produced every 20 min.

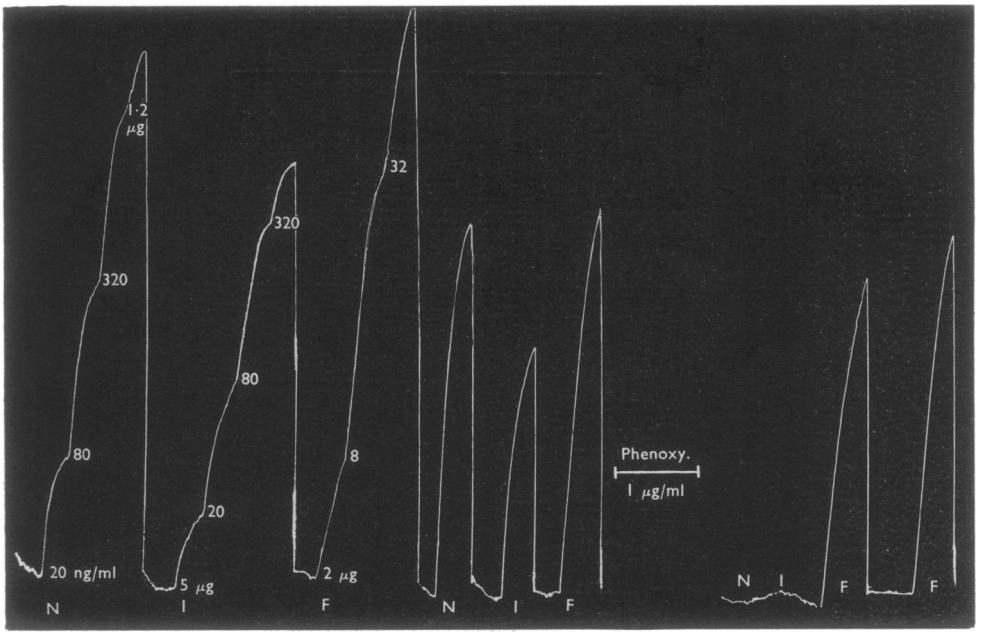

FIG. 4. Blockade by phenoxybenzamine (1 μg/ml incubated with the strip for 10 min) of noradrenaline (N) and isoprenaline (I) but not fenfluramine (F) contractions (from strip 14). Cumulative doses of the agonists were added to the tissue bath to determine the approximate ED50s, which were 80 ng/ml N, 20 μg/ml I, and 5 μg/ml F (N, I and F respectively). The ED50 doses of the agonists were added to the tissue bath for 3 min. They were then added after phenoxybenzamine pretreatment (Phenoxy.). ED50 contractions were produced every 20 min.

small relaxation in only one strip which was blocked by propranolol (5 μg/ml). Propranolol (10 μg/ml) incubated with the strip for 5 min before agonists were added did not affect the contraction heights produced by noradrenaline and isoprenaline (Fig. 5, Table 4).

Cocaine and tyramine

(*a*) The relative potencies and ED50 concentrations of isoprenaline were compared when measured in the presence and in the absence of cocaine (5 μg/ml) in the

TABLE 3. *Effect of thymoxamine and phenoxybenzamine on noradrenaline (N), isoprenaline (I) and fenfluramine (F) contractions*

Strip	Antagonist	% blockade of agonist ED50		
		N	I	F
	Thymoxamine			
9	5 μg/ml	—	—	0
9	50 μg/ml	—	—	0
10	5 μg/ml	60	60	10
11	5 μg/ml	85	85	0
12	5 μg/ml	70	80	0
	Phenoxybenzamine			
13	5 μg/ml	100	100	15
14	1 μg/ml	100	100	0
15	1 μg/ml	100	100	0
12	1 μg/ml	100	100	10

Doses of 5 and 50 μg/ml of thymoxamine did not block fenfluramine contractions. Thymoxamine caused a small increase in tone in strips 10 and 12. The incubations with phenoxybenzamine for 10 min, however, did not affect tone.
—, Not tested.

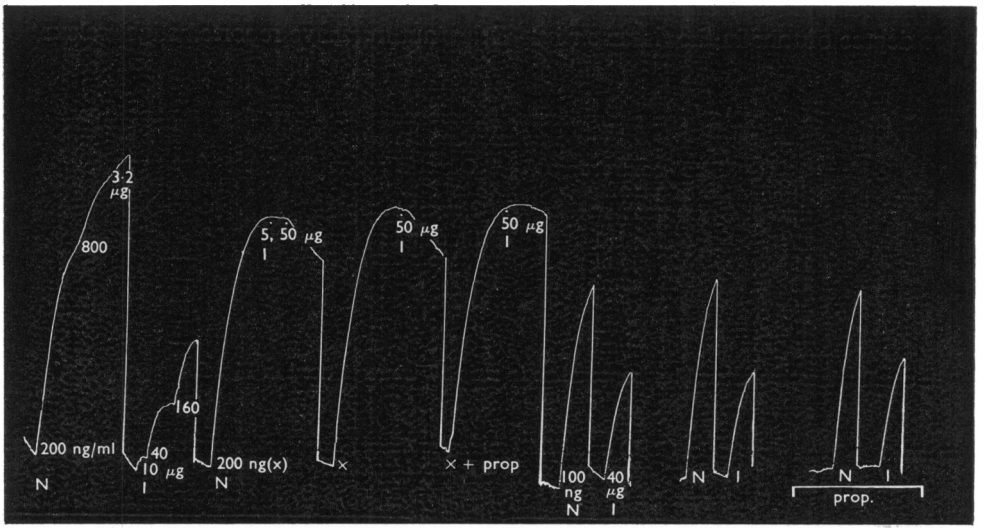

FIG. 5. Relaxation caused by an approximately ED50 contracting dose of isoprenaline (I) (50 μg/ml) on a noradrenaline-contracted strip. The contracting dose of noradrenaline [200 ng/ml (x)] was added to the bath for 7 min before isoprenaline was added for a further 5 min. Propranolol (5 μg/ml for 5 min) blocked the relaxing effect of isoprenaline. Approximately ED50 contracting doses of noradrenaline (N) (100 ng/ml) and isoprenaline (I) (40 μg/ml) were unaffected by propranolol (10 μg/ml for 5 min). The ED50 contracting doses were determined at the start of the experiment by adding cumulative doses at 3 min intervals. The contracting doses of N and I were added to the bath at 20 min intervals (strip number 17).

Krebs solution to determine whether a noradrenaline-releasing action of isoprenaline was involved. Both sets of values were similar (Table 5).

(*b*) Tyramine, which releases stores of noradrenaline from sympathetically innervated tissues (Burn & Rand, 1958), caused only small contractions. On one strip contractions caused by tyramine (10 μg/ml) were not antagonized by cocaine (5 μg/ml), but on a second strip cocaine blocked contractions induced by tyramine (80 μg/ml) by 35%. Noradrenaline-induced contractions (ED50s) were not affected by cocaine on either strip.

Discussion

Isoprenaline contracts isolated human saphenous vein provided that the tissue strips contract to noradrenaline. The order of potency of sympathomimetic amines in causing contraction is (−)-adrenaline>(−)-noradrenaline>(−)-phenylephrine>(±)-isoprenaline, which is characteristic of an α-adrenoceptor population (Ahlquist, 1948; Furchgott, 1967). The blockade of both noradrenaline and isoprenaline contractions by tolazoline, phentolamine, thymoxamine and phenoxybenzamine shows the α-adrenoceptor stimulant nature of the isoprenaline response. These results are consistent with those of Sutter (1965) for the rabbit isolated anterior mesenteric vein where phentolamine (1 μg/ml) and dihydroergotamine (1 μg/ml) blocked isoprenaline-induced contractions. Clement, Vanhoutte & Leusen (1969) also found that phentolamine (20 μg/ml) completely blocked isoprenaline induced contractions of the dog isolated lateral saphenous and mesenteric veins (by recording isobaric volume changes). Guimarães & Osswald (1969) have shown that phenoxybenzamine (370 ng/ml–7·4 μg/ml) and ergotamine (7·5 ng/ml–680 ng/ml) not only caused blockade of isoprenaline contractions of the dog isolated lateral saphenous vein but also caused reversal of the response (relaxation) according to the degree of tone in the tissue strips. Other authors have reported slight relaxation

TABLE 4. *Effect of ED50 contracting doses of isoprenaline on preparations maximally contracted with noradrenaline*

Strips	ED50 contracting dose of isoprenaline	Direct effect on maximally contracted strips	Effect of propranolol (5 μg/ml for 5 min)
17	5 μg/ml	Small relaxation	90% block
15	20 μg/ml	No effect	—
18	20 μg/ml	No effect	—
16	40 μg/ml	No effect	—
12	40 μg/ml	No effect	—
19	40 μg/ml	No effect	—

The small relaxation caused by isoprenaline in strip 17 is reproduced in Fig. 5.

TABLE 5. *Relative potencies (R.P.) and ED50 concentrations of isoprenaline measured in the presence and absence of cocaine hydrochloride (5 μg/ml)*

With cocaine			Without cocaine		
Strip	R.P.	ED50 conc.	Strip	R.P.	ED50 conc.
1	0·007	$3{\cdot}8\times10^{-5}$	14	0·006	$6{\cdot}3\times10^{-5}$
2	0·007	$5{\cdot}6\times10^{-5}$	20	0·004	$8{\cdot}0\times10^{-5}$
3	0·003	$4{\cdot}2\times10^{-4}$	21	0·01	$6{\cdot}3\times10^{-5}$

The potencies of isoprenaline are expressed as the molar concentration causing 50% contractions and relative to noradrenaline.
(Noradrenaline=1·0.)

or no effect after adding isoprenaline to the tissue bath. O'Mahony (1963), again using the dog isolated lateral saphenous vein, could only show isoprenaline-induced relaxation if tone was first induced by noradrenaline or acetylcholine. The femoral, external iliac, inferior vena cava and superior vena cava veins did not respond to isoprenaline. Sutter (1965) found that the external jugular vein of the rabbit was not contracted by isoprenaline but that low doses (below 10 μg/ml) caused relaxation of the anterior mesenteric vein (isoprenaline caused contraction above 10 μg/ml). Hughes & Vane (1967) have shown that isoprenaline (10 ng/ml–1 μg/ml) relaxes the rabbit isolated portal vein, but Hughes (1967) could not demonstrate that isoprenaline in doses up to 10 μg/ml contracted human isolated saphenous vein. The effect of isoprenaline is dependent, therefore, on the type of vein studied and on the concentration of isoprenaline added to the tissue bath. Low doses of isoprenaline (below 10 μg/ml) in the experiments reported here did not cause relaxation nor did higher doses cause relaxation after α-adrenoceptor blockade, indicating that β-adrenoceptors mediating relaxation were not present in the tissue. However, the response to β-adrenoceptor stimulation might be masked in this situation by lack of tone and might be detectable by inducing tone and then adding isoprenaline. To test this proposition an approximately maximum contracting dose of noradrenaline was added to six strips, but in only one strip did isoprenaline then cause relaxation which was blocked by propranolol. The small β-adrenoceptor population present in this strip could not be detected using potentiation as a pharmacological measurement. If a significant number of β-adrenoceptors were present then it would have been expected that propranolol, by abolishing the β-adrenoceptor mediated relaxation, would potentiate the isoprenaline contractions. Potentiation, however, is an indirect method of measurement and it might be expected that a direct method would be more sensitive. The method of inducing tone with noradrenaline has the added advantage that the majority of α-adrenoceptors are occupied so that the direct (relaxant) effect of isoprenaline on β-adrenoceptors is most easily detected. Human saphenous vein, therefore, differs from dog saphenous, where isoprenaline causes large relaxations when the tissue is contracted with noradrenaline (O'Mahony, 1963 ; Guimarães & Osswald, 1969).

The possibility that isoprenaline might release tissue-bound stores of noradrenaline is excluded by the experiments with tyramine and cocaine (Table 5). Cocaine blocks the amine uptake system of sympathetic nerve endings (Iversen, 1965) and would lower the relative potency of isoprenaline if release were involved. A concentration of 5 μg/ml of cocaine was selected as it fell between the concentration range used by others. For example, Hughes (1967) found that 2 μg/ml cocaine caused a large relative potentiation of noradrenaline contractions of the rabbit isolated portal vein and that this concentration also potentiated the contractor effect of noradrenaline on human isolated gastric vein by 20–30%. The concentration used by Furchgott (1967) for the determination of relative potencies of sympathomimetic amines on isolated animal tissues was 10 μg/ml cocaine, but there is no information showing that these concentrations cause total blockade of noradrenaline uptake in venous smooth muscle. Iversen (1967) has shown that 5 μg/ml cocaine only causes approximately 70% inhibition of noradrenaline uptake in the isolated rat heart.

The results with tyramine are not convincing since only two strips were used. The effects of tyramine in the presence and absence of cocaine need to be repeated

on more specimens before assumptions can be made about the amounts of releasable noradrenaline that may be present in strips of human saphenous vein. However, a noradrenaline-releasing action of isoprenaline was not pursued since isoprenaline is not concentrated by sympathetic nerve terminals in the isolated rabbit heart (Anden, Corrodi, Ettles, Gustafsson & Persson, 1964).

The doses of 5-HT added as a marker were blocked by phentolamine and thymoxamine. It was for this reason that fenfluramine was selected as the marker agonist since its action is not blocked by α-adrenoceptor blocking drugs (Coupar, Hedges, Metcalfe & Turner, 1969). Phentolamine and phenoxybenzamine block the action of 5-HT on the dog lateral saphenous, jugular and mesenteric veins and it has been suggested that 5-HT stimulates α-adrenoceptors in these veins (Clement & Vanhoutte, 1967). Thymoxamine is said to be a more " specific " α-adrenoceptor blocking drug, having a weak antihistamine action and no anti-5-HT action (Birmingham & Szolcsányi, 1965 ; Birmingham, Akube & Szolcsányi, 1967 ; Birmingham, Ernest & Newcombe, 1969). 5-HT contractions of human vein were antagonized by thymoxamine, however, although not to the same extent as noradrenaline contractions, which might support the idea that α-adrenoceptors and 5-HT-receptors in venous smooth muscle are similar or closely related. However, high doses of propranolol (10 μg/ml) selectively blocked 5-HT contractions (which could be reversed by washing out the antagonist) but did not affect noradrenaline contractions. Propranolol blocks both noradrenaline and 5-HT contractions on the isolated rabbit thoracic aorta (Gulati, Gokhale, Parikh & Udwadia, 1969).

The results of the experiments reported here indicate that human saphenous vein contains a dominant α-adrenoceptor population with few β-adrenoceptors. Isoprenaline stimulates the α-adrenoceptors to cause contraction, but only in high doses. It is difficult to relate these findings to the results of other authors because of the lower doses used in *in vivo* experiments and the differences in reactivity of veins both between and within the same species.

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