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ACTIONS OF LOCALLY ADMINISTERED ADRENOCEPTOR AGONISTS ON INCREASED PLASMA PROTEIN EXTRAVASATION AND BLOOD FLOW IN GUINEA-PIG SKIN

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1 Bradykinin-induced increased plasma protein extravasation (IPPE) and blood flow have been assessed in guinea-pig skin by isotopic methods.

2 The effects of a range of adrenoceptor agonists on these parameters have been investigated.

3 α-Adrenoceptor agonists inhibited IPPE and reduced cutaneous blood flow. The potency of α-agonists as inhibitors of IPPE correlated with their vasoconstrictor effects. The actions of noradrenaline on both IPPE and blood flow were blocked by phentolamine but not by propranolol.

4 β-Adrenoceptor agonists inhibited IPPE at doses which either increased or caused little change in cutaneous blood flow. Isoprenaline inhibition of IPPE was reduced by propranolol but was unaffected by phentolamine.

5 The inhibitory action of α-agonists on IPPE can be explained by a reduction in blood flow to the affected site. Beta agonist inhibition is not due to effects on blood flow but is probably caused by a reduction in permeability of the microvessels.

Introduction

Locally applied adrenoceptor agonists inhibit mediator-induced extravasation of plasma protein in rat skin (Willoughby & Spector, 1964), mouse peritoneal cavity (Green, 1972), hamster cheek pouch (Svensjö, Persson & Arfors, 1976) and guinea-pig skin (Kenawy, Lewis & Williams, 1978; O'Donnell & Persson, 1978; Paul, Goodman & Morley, 1978).

Increased plasma protein extravasation (IPPE) in acute, dermal, inflammatory responses is the product of at least two variables: blood flow to the affected site and permeability of microvessels within the site (Williams & Peck, 1977). Prostaglandins, which cause no appreciable direct increase in extravasation, potentiate IPPE when injected together with bradykinin or histamine into the skin of guinea-pig (Williams & Morley, 1973) and rabbit (Williams, 1976). In the rabbit, the potency of a series of prostaglandins as potentiators of IPPE correlates with their potency as vasodilators (Williams, 1976); moreover, other vasodilators such as isoprenaline also potentiate the response, whereas vasoconstrictors such as noradrenaline cause inhibition (Williams, 1977). In contrast, isoprenaline (Kenawy *et al.*, 1978) and terbutaline (O'Donnell & Persson, 1978) inhibit IPPE in guinea-pig skin. The inhibitory action of isoprenaline occurs despite a concomitant increase in blood flow in some experiments (Kenawy *et al.*, 1978).

The present study describes the effects of a range of adrenoceptor agonists on both IPPE and blood flow in guinea-pig skin. A preliminary account of some of these results was presented at the 7th International Congress of Pharmacology (Paul *et al.*, 1978).

Methods

Increased plasma protein extravasation

Experiments were performed on conscious, male Dunkin-Hartley guinea-pigs (350 to 450 g). The dorsal and lateral skin was shaved at least 3 h before use.

Each animal was injected intravenously (dorsal foot vein) with 0.5 ml Evans blue dye (Searle Diagnostic; 2.5% w/v in phosphate buffered saline, PBS) containing 1 to 2 μCi ^{125}I-human serum albumin (Radiochemical Centre, Amersham). Twelve intradermal injections (each in 0.1 ml PBS) were made into marked sites (6 on each side of the spine) on each animal. Six treatments [PBS alone; bradykinin (0.5 μg); bradykinin (0.5 μg) plus four concentrations of drug under test] were allocated in duplicate to the sites on each of six animals, forming a balanced, complete Latin square. Forty minutes after the last injection each animal was killed by a blow on the head,

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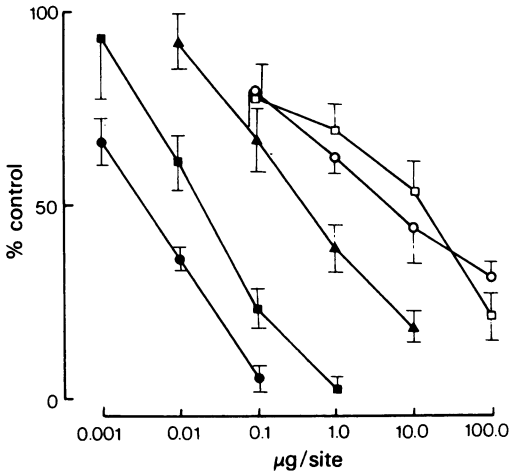

Figure 1. Inhibition of bradykinin-induced increased plasma protein extravasation by (●) (−)-adrenaline, (■) (−)-noradrenaline, (▲) (−)-phenylephrine, (○) amidephrine, and (□) methoxamine. Results are expressed as a % of the control (i.e. bradykinin 0.5 μg alone) response. Each point is the mean from duplicate sites in $n = 6$ animals; vertical lines show s.e. mean.

bled (1 ml of blood was collected) and then skinned. Discs of skin containing the whole of each lesion were removed with a metal wad punch and counted in a gamma spectrometer (Nuclear Enterprises). Extravasation in each lesion was expressed as μl whole blood equivalents (counts in lesion/counts in 1 ml of blood) × 1000. For each animal the mean extravasation due to PBS alone was subtracted from the mean extravasation for each of the 5 remaining treatments. The mean ± s.e. mean ($n = 6$) values were then calculated for each treatment. The mean response to bradykinin alone was taken as 100% and all other responses (±s.e. mean) expressed as percentages of this value.

Blood flow

Experiments were performed on male Dunkin-Hartley guinea-pigs (450 to 600 g) anaesthetized with pentobarbitone sodium (Sagatal; May & Baker Ltd.).

Cutaneous blood flow was measured indirectly by following the rate of clearance of ^{133}Xe (Radiochemical Centre, Amersham) following intradermal injection. Three (and, in some instances, four) sites were marked on the shaved flank of an animal and each site was injected with 0.1 ml containing 50 μCi ^{133}Xe diluted in PBS alone or PBS containing a variable amount of drug under test. Treatments were allocated randomly to sites and the radioactivity (C_t) in each was monitored at intervals up to 1 h following the initial count (C_0) using a manual gamma probe (Nuclear Enterprises Ltd., Gamma probe GP6). The log of the ratio C_t/C_0 was plotted against time and the half-life ($T_{1/2}$ min) of clearance of the gas was obtained by interpolation (or by extrapolation in a few instances). A decrease in $T_{1/2}$ was taken to denote vasodilatation and an increase to denote vasoconstriction as described by Dick, Grennan & Zeitlin (1976). Each drug, or drug combination, was tested in at least 3 animals.

Drugs

The drugs used were: (−)-adrenaline bitartrate (Sigma); amidephrine mesylate (Mead Johnson); synthetic bradykinin (Sandoz); fenoterol hydrobromide (Boehringer Ingelheim); (±)-isoprenaline sulphate, (+)-isoprenaline-D-bitartrate, (−)-isoprenaline-D-bitartrate (Sigma); methoxamine hydrochloride (Burroughs Wellcome); NAB 365CL (Boehringer Ingelheim); (−)-noradrenaline hydrogen tartrate (Sigma); orciprenaline sulphate (Boehringer Ingelheim); phentolamine mesylate (Ciba); (−)-phenylephrine hydrochloride (Sigma); (±)-salbutamol sulphate (Allen & Hanbury) and terbutaline sulphate (Astra). All drugs were dissolved in PBS immediately before use; doses refer to the base.

Results

Increased plasma protein extravasation (IPPE)

The dose of bradykinin (0.5 μg/0.1 ml) chosen for use in all experiments was known to produce a submaximal response. In twenty, 6-animal experiments the mean coefficient of variation (CV [$\bar{x}$] = [s.e. mean/mean] × 100%) of the response to bradykinin alone was 7.0% (range 3.4 to 10.5%).

α-Adrenoceptor agonists Intradermal injection of the highest dose of α-adrenoceptor agonists did not produce a significant increase in plasma protein exudation compared to sites injected with saline. Extravasation tended to be less than in saline injection sites.

All 5 compounds caused dose-related reductions in bradykinin-induced IPPE (Figure 1). Adrenaline (0.1 μg) and noradrenaline (1 μg) completely suppressed the increase in extravasation; amidephrine, methoxamine and phenylephrine reduced the response by 70 to 80% at the highest doses (Figure 1). Increasing the dose of phenylephrine to 100 μg/site led to virtually complete suppression. The potency order as inhibitors was adrenaline > noradrenaline > phenylephrine > amidephrine ≥ methoxamine. The potency of phenylephrine (ID_{50}) relative to noradrenaline (ID_{50}) estimated from Figure 1 was 20.5, which was comparable

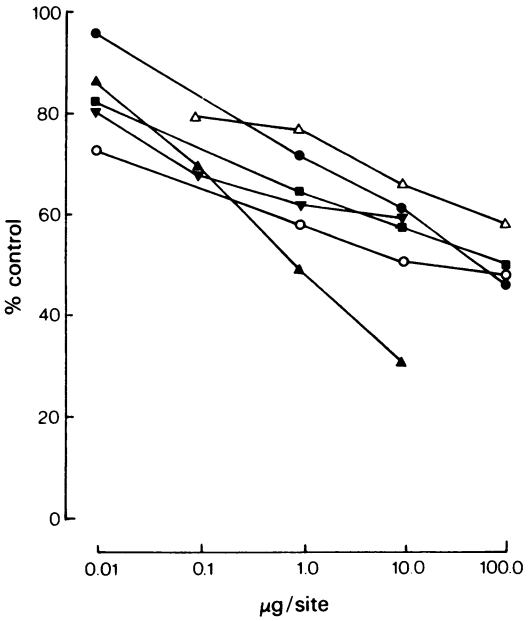

Figure 2 Inhibition of bradykinin-induced increased plasma protein extravasation by (▲) (±)-isoprenaline, (○) salbutamol, (▼) NAB 365, (■) fenoterol, (●) terbutaline and (△) orciprenaline. Results are expressed as a % of the control (i.e. bradykinin 0.5 μg alone) response. Each point is the mean from duplicate sites in $n = 6$ animals.

to the value of 19.4 obtained in a within animals (2 + 2) assay ($n = 6$).

β-Adrenoceptor agonists The highest doses of β-adrenoceptor agonists did not produce a significant increase in exudation of plasma protein.

All 6 compounds reduced bradykinin-induced IPPE, although the dose-response regression was flatter than for α-agonists (Figure 2). (±)-Isoprenaline was the most potent inhibitor and reduced the response by up to 70%, whereas the β_2 specific compounds caused, at most, a 40 to 55% reduction. (−)-Isoprenaline was more than 100 times as potent as (+)-isoprenaline (Results not shown). Because of the flat dose-response curves, no attempt was made to assess the relative potencies of the β_2 agonists.

Antagonists At doses of 1 and 10 μg/site, neither phentolamine nor propranolol had a significant effect on bradykinin-induced IPPE.

The inhibitory action of noradrenaline was reversed by 1 μg of phentolamine added to the injection mixture but was unaffected by the same dose of propranolol; in contrast, isoprenaline inhibition was reduced by 10 μg propranolol but not by phentolamine (Table 1).

Cutaneous blood flow

Though there was considerable individual variation in the basal ^{133}Xe clearance in the skin of different guinea-pigs (see Tables 2, 3a, 4a) no significant inter-site differences were found within animals ($n = 4$).

α-Adrenoceptor agonists All compounds reduced blood flow in guinea-pig skin demonstrated by a slower rate of clearance of ^{133}Xe. Noradrenaline caused dose-related reductions in flow at doses which suppressed bradykinin-induced IPPE (Figure 3). The potency order in reducing flow was adrenaline > noradrenaline > phenylephrine > amidephrine (Table 2). No attempt was made to quantitate relative potencies.

Table 1 Effects of phentolamine and propranolol on noradrenaline and isoprenaline inhibition of bradykinin (0.5 μg)-induced increased plasma protein extravasation (IPPE)

Adrenoceptor agonist	*Antagonist*	*% control response**	P (*t* test)
None	None	100 ± 6.4	<0.001†
(−)-Noradrenaline (30 ng)	None	49.0 ± 6.4	—
(−)-Noradrenaline (30 ng)	Phentolamine (1 μg)	91.3 ± 8.6	<0.01†
(−)-Noradrenaline (30 ng)	(±)-Propranolol (1 μg)	51.5 ± 5.2	NS†
None	None	100 ± 7.2	<0.001‡
(±)-Isoprenaline (2 μg)	None	54.9 ± 6.4	—
(±)-Isoprenaline (2 μg)	Phentolamine (10 μg)	53.9 ± 4.1	NS‡
(±)-Isoprenaline (2 μg)	(±)-Propranolol (10 μg)	79.8 ± 3.4	<0.01‡

*Each value is the mean (±s.e. mean) of 6 duplicate skin sites. NS: not significantly different.
†Compared with the response in the presence of noradrenaline alone; ‡Compared with the response in the presence of isoprenaline alone.

Table 2 Relative potencies of α-adrenoceptor agonists on cutaneous blood flow

Animal	*Saline*	^{133}Xe *clearance:* $T_{1/2}$ *(min)* (−)-*Adrenaline* (*0.3 nmol*)	(−)-*Noradrenaline* (*0.3 nmol*)
1	6	35	30
2	10	37	24
3	27	145	65
Average	14	72	40

Animal	^{133}Xe *clearance:* $T_{1/2}$ *(min)* (−)-*Noradrenaline* (*0.3 nmol*)	(−)-*Phenylephrine* (*1 nmol*)	(−)-*Amidephrine* (*1 nmol*)
4	20	14	9
5	43	26	18
6	88	29	19
Average	50	23	15

$T_{1/2}$ (min) values (to the nearest minute) were obtained from individual ^{133}Xe clearance curves by interpolation (or extrapolation).

β-Adrenoceptor agonists The effects of isoprenaline (100 ng, 1 μg) and fenoterol (100 ng, 10 μg) on blood flow were variable in that they had little effect on flow or caused a modest increase (Table 3a). While 10 μg isoprenaline reduced blood flow, this reduction was less than that produced by a dose of noradrenaline (50 ng), which had an equivalent effect on IPPE (Table 3b).

Table 3 (a) Effect of β-agonists on cutaneous blood flow

Animal	*Saline*	^{133}Xe *clearance:* $T_{1/2}$ *(min)* (±)-*Isoprenaline* (100 ng)	(±)-*Isoprenaline* (1 μg)	*Animal*	*Saline*	^{133}Xe *clearance:* $T_{1/2}$ *(min)* *Fenoterol* (100 ng)	*Fenoterol* (10 μg)
1	8	3	7	4	13	10	7
2	5	6	4	5	11	14	11
3	11	9	12	6	31	27	20
Average:	8	6	8	Average:	18	17	13

(b) Blood flow comparison of isoprenaline and noradrenaline at doses having equivalent effects on increased plasma protein extravasation

Animal	*Saline*	^{133}Xe *clearance:* $T_{1/2}$ *(min)* (±)-*Isoprenaline* (10 μg)	(−)-*Noradrenaline* (50 ng)
7	12	18	32
8	14	25	49
9	16	28	78
Average:	14	24	53

$T_{1/2}$ (min) values (to the nearest minute) were obtained from individual ^{133}Xe clearance curves by interpolation (or extrapolation).

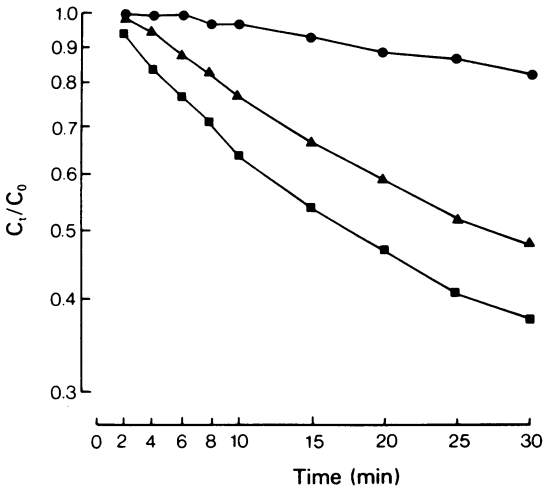

Figure 3 Time course of cutaneous clearance of ^{133}Xe (50 μCi) injected in the presence of (■) saline, (▲) noradrenaline 10 ng and (●) noradrenaline 100 ng. The change in residual radioactivity is shown as counts at time t/counts at time zero (C_t/C_0, log scale) with time in a single representative experiment.

Antagonists The effect of noradrenaline on blood flow was reversed by phentolamine but was intensified (or, in some instances, unaffected) by propranolol (Table 4a). Because of the uncertainty of producing a reasonable vasodilator response with isoprenaline alone, we studied the effect of isoprenaline on the vasoconstriction induced by noradrenaline (50 ng). Under these conditions, isoprenaline (1 μg) consistently increased ^{133}Xe clearance and this effect was reversed by propranolol (Table 4b). The effect of phentolamine on this vasodilator response to isoprenaline could not be assessed because the α-antagonist would have blocked the vasoconstrictor action of noradrenaline.

Used in the same doses isoprenaline did not reverse noradrenaline-induced inhibition of the IPPE response to bradykinin (results not shown).

Discussion

Noradrenaline inhibits histamine-induced increased plasma protein extravasation (IPPE) in guinea-pig and rabbit skin and this effect is blocked by phenoxybenzamine, suggesting that the reduction in exudation is attributable to the vasoconstrictor action of noradrenaline (Kenawy *et al.*, 1978). The present experiments are consistent with this viewpoint since they demonstrate that, in guinea-pig skin, (a) 5 α-adrenoceptor agonists, including noradrenaline, inhibit bradykinin-induced IPPE, (b) their inhibitory potencies correlate with their ability to reduce cutaneous blood flow and (c) the actions of noradrenaline on both IPPE and blood flow are blocked by an α-blocker, phentolamine, but not by propranolol, a β-blocker. In contrast, α-receptors appear to play little part in inhibition of exudation in the mouse peritoneal cavity since adrenaline-induced inhibition of bradykinin-induced dye leakage was unaffected by phenoxybenzamine (Green, 1972). However, it should be noted that the combination of propranolol and phenoxybenzamine had a greater blocking effect than propranolol alone (Green, 1972).

While the effects of α-agonists are qualitatively the same in both guinea-pig and rabbit, this does not apply to all potential vasoconstrictors since angiotensin II inhibits IPPE in rabbit skin (Williams, 1977) but is without effect on exudation in guinea-pig skin (Paul, unpublished observations).

Potent vasodilators (e.g. E-type prostaglandins) potentiate IPPE in guinea-pig skin (Williams & Morley, 1973). β-Adrenoceptor agonists cause negligible to modest direct increases in cutaneous blood flow in the guinea-pig, though they produce consistent vasodilator responses in the presence of a vasoconstrictor such as noradrenaline (Tables 3a and 4b). On the basis of their effects on blood flow, β-agonists might be expected to cause slight potentiation of mediator-induced IPPE and to reverse the inhibitory effect of noradrenaline on these responses. However, β-agonists actually inhibited mediator-induced IPPE (Figure 2) and did not reverse the inhibitory effect of noradrenaline. These results show that isoprenaline and other β-agonists have a vascular action, distinct from their effects on blood flow, which opposes the ability of inflammatory mediators to increase plasma protein exudation. A likely explanation is that these compounds reduce the permeability of vascular endothelium to plasma proteins. This conclusion is strengthened by results obtained by other workers (Green, 1972; Svensjö *et al.*, 1976; Kenawy *et al.*, 1978; O'Donnell & Persson, 1978).

The opposite actions of isoprenaline on IPPE in guinea-pig and rabbit skin may reflect the fact that β-agonists are more powerful cutaneous vasodilators in the latter (Kenawy *et al.*, 1978) and this outweighs effects on endothelial permeability. In the guinea-pig the modest vasodilatation apparently does not completely counteract the permeability reducing action of β-agonists but may account for the flattened dose-response curves.

Isoprenaline has a greater maximum inhibitory effect on IPPE relative to the β_2 specific agonists; this is possibly a consequence of the vasoconstrictor effect seen with the high doses of isoprenaline (Table 3b). High doses of isoprenaline, but not fenoterol or terbutaline, constrict the isolated, perfused, femoral artery of the guinea-pig (O'Donnell & Wanstall, 1974).

Table 4 (a) Effect of α and β-adrenoceptor antagonists on noradrenaline-induced reduction of blood flow

Animal	^{133}Xe *clearance:* $T_{1/2}$ *(min)*			
	Saline	*Phentolamine* (1 μg)	(−)-*Noradrenaline* (50 ng)	*Noradrenaline plus phentolamine*
1	6	7	22	4
2	9	10	48	9
3	17	16	42	24
Average:	11	11	37	12
Animal	^{133}Xe *clearance:* $T_{1/2}$ *(min)*			
	Saline	(±)-*Propranolol* (1 μg)	(−)-*Noradrenaline* (50 ng)	(−)-*Noradrenaline plus* (±)-*propranolol*
4	13	15	33	49
5	14	21	110	102
6	16	7	29	51
Average:	14	14	57	67

(b) Effect of propranolol on isoprenaline-induced increase of blood flow

Animal	^{133}Xe *clearance:* $T_{1/2}$ *(min)*			
	(−)-*Noradrenaline* (50 ng)	(−)-*Noradrenaline plus* (±)-*isoprenaline* (1 μg)	(−)-*Noradrenaline plus* (±)-*propranolol* (10 μg)	(−)-*Noradrenaline plus* (±)-*isoprenaline plus* (±)-*propranolol*
7	67	28	80	47
8	43	19	37	45
9	35	20	56	64
Average:	48	22	58	52

$T_{1/2}$ (min) values (to the nearest min) were obtained from individual ^{133}Xe clearance curves by interpolation (or extrapolation).

In conclusion, bradykinin-induced IPPE in guinea-pig skin is inhibited by adrenoceptor agonists acting via either α- or β-receptors. This is in contrast to the mouse peritoneum where inhibition is mediated predominantly by β-receptors (Green, 1972) and the rabbit skin where only α-agonists are inhibitory (Kenawy *et al.*, 1978).

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