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ROLE OF ENDOGENOUS CATECHOLAMINES IN THE ANTI-INFLAMMATORY ACTIVITY OF α-ADRENOCEPTOR BLOCKING AGENTS

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1 Drugs which release or modify the response to catecholamines were examined for their effect on the permeability of the mouse peritoneal vascular bed to circulating plasma albumin, labelled with Evans blue.

2 Phenoxybenzamine, phentolamine, piperoxane, yohimbine or cocaine reduced the extravasation of Evans blue into the peritoneum, an effect which was antagonized by β-adrenoceptor blocking drugs. The inhibitory effect of desipramine on the extravasation of Evans blue was less completely antagonized by β-adrenoceptor blockade.

3 Inhibition of catecholamine biosynthesis, ganglion blockade or adrenergic neurone blockade antagonized the reduction in dye extravasation by α-adrenoceptor blocking agents and cocaine, but had no significant effect on the response to desipramine. The inhibitory effects of α-adrenoceptor blocking agents on dye extravasation were not prevented by bilateral adrenalectomy.

4 Mice subjected to the procedure for estimation of vascular permeability excreted increased amounts of adrenaline and noradrenaline. Pretreatment with phenoxybenzamine, piperoxane or cocaine further increased catecholamine excretion, but desipramine caused only a small increase in catecholamine excretion which did not correlate with its effect on dye extravasation.

5 It is suggested that phenoxybenzamine, phentolamine, piperoxane and cocaine reduce vascular permeability in the mouse peritoneum by releasing and/or potentiating the effects of endogenous catecholamines on β-adrenoceptors. Endogenous catecholamines do not appear to be involved in the anti-inflammatory activity of desipramine.

Introduction

α-Adrenoceptor blocking agents suppress certain forms of acute inflammation although the mechanism of the effect has not been established (Northover, 1963; Kellet, 1965; Sim, 1965; Brown, Kissel & Lish, 1968a; Riesterer & Jaques, 1968). In preliminary experiments on the permeability of the mouse peritoneal vascular bed to plasma albumin, it was observed that the inhibitory effect of α-adrenoceptor blocking agents on dye extravasation was prevented by pretreatment with β-adrenoceptor antagonists. Since catecholamines inhibit vascular permeability in the mouse peritoneum by activation of β-adrenoceptors (Green, 1972), it seemed possible that the permeability effects of α-adrenoceptor blocking agents were mediated via endogenous catecholamines. This hypothesis was examined by studying the anti-inflammatory effects of α-adrenoceptor blocking agents and various other drugs in bilaterally adrenalectomized mice or mice treated with ganglion blocking agents, guanethidine or an inhibitor of catecholamine biosynthesis. α-Adrenoceptor blocking agents and various other drugs were also examined for their effect on the urinary excretion of adrenaline and noradrenaline, to establish whether there was any correlation between catecholamine excretion and changes in vascular permeability.

Methods

Estimation of vascular permeability

The permeability of the mouse peritoneal vascular bed to circulating plasma albumin was estimated as described by Green (1972). Mice were injected intravenously with 0.2 ml of a 0.5% solution of Evans blue in saline (0.9% w/v NaCl solution) and intraperitoneally with 4 ml 0.05% acetic acid in saline. The peritoneal fluid was collected 1 h later and the concentration of dye in the fluid measured colorimetrically. The total volume of fluid in the peritoneal cavity of mice 1 h after injection with

4 ml of acidic saline was 3.62 ± 0.26 ml and this value was not significantly affected by drug treatment.

Estimation of catecholamine excretion

Urine was collected from pairs of mice kept in plastic metabolism cages. Throughout the period of urine collection, the room was maintained at a temperature of 18-20°C and lit continuously by artificial light. Urine was collected in tubes containing 1 ml 0.05 N HCl and 10 mg sodium metabisulphite. At the end of the collection period the abdomen of each mouse was gently pressed to evacuate the bladder and the cages sprayed with distilled water to collect residual traces of urine.

Free adrenaline and noradrenaline were extracted as described by Anton & Sayre (1962) and samples stored at −30°C until assayed. The average recovery of adrenaline added to urine was 74% and the average recovery of noradrenaline was 68%.

Assay of adrenaline

Extracts were assayed for adrenaline on the electrically stimulated rat uterus preparation of Harvey & Pennefather (1962), except that rats in dioestrous were used. The electrically-induced contractions of the uterus were inhibited by adrenaline in a final concentration of 100 pg/ml, and occasionally it was possible to detect concentrations as low as 0.1 pg/ml. The uterus was more than 10^4 times less sensitive to noradrenaline than to adrenaline. The inhibitory effects of the urine extracts on uterine contractions were abolished by sotalol (50 ng/ml) or by heating at pH 10, indicating that the inhibitory activity of the extracts was due to their adrenaline content.

Assay of noradrenaline

The noradrenaline in extracts of urine was estimated by a modification of the technique originally described by Shipley & Tilden (1947), using the blood pressure response of the pithed rat. The rat was treated with cocaine (10 mg/kg i.p.) and propranolol (1 mg/kg i.p.) to increase its sensitivity to catecholamines, and to convert the normally biphasic response to adrenaline into a larger and purely pressor one. The large initial pressor response caused by passing the pithing rod down the vertebral column was prevented by pretreating the rat with hexamethonium 10 mg/kg intraperitoneally. This procedure delayed the onset of pulmonary oedema and increased the time during which the preparation could be used.

Adrenaline had 1.5 times the pressor activity of noradrenaline. Pressor activity was absent after the rat had been given phenoxybenzamine (20 mg/kg i.v.) or after the urine extracts had been oxidized by heating at an alkaline pH.

Drugs

The following drugs and materials were used: (−)-adrenaline hydrogen tartrate (B.D.H.); (−)-noradrenaline bitartrate (Koch-Light); (±)-propranolol hydrochloride (I.C.I.); (±)-sotalol (Mead Johnson); phenoxybenzamine hydrochloride (Smith, Kline & French); phentolamine hydrochloride (CIBA); piperoxane hydrochloride (May & Baker); yohimbine hydrochloride (California Corp. for Biochemical Research); cocaine hydrochloride (Evans Medical); desipramine hydrochloride (Geigy); hexamethonium bromide (Koch-Light); mecamylamine hydrochloride (Merck, Sharp & Dohme); guanethidine sulphate (CIBA); DL α-methyl-*p*-tyrosine (Regis Chemical Co.); cortisone acetate (Organon); aluminium oxide (chromatographic, B.D.H.); Evans blue (Grübler-Farbstoff).

All doses and concentrations mentioned in the text refer to these salts, except for (−)-adrenaline and (−)-noradrenaline which are expressed in terms of the base.

Results

Phenoxybenzamine, phentolamine, piperoxane or yohimbine caused a dose-dependent reduction in the accumulation of Evans blue labelled plasma albumin in the peritoneal cavity, an effect which was antagonized by pretreatment with propranolol or sotalol (Table 1). Propranolol or sotalol alone did not affect the accumulation of Evans blue, confirming previous reports of the lack of activity of these drugs on the inflammatory response (Brown, Mackey, Riggilo & Schwartz, 1968b; Riesterer & Jaques, 1968). In the mouse peritoneum, β-adrenoceptor blocking agents antagonize only the anti-inflammatory activity of drugs which stimulate β-adrenoceptors (Green, 1972), and thus it seems likely that α-adrenoceptor blocking agents reduce the extravasation of dye either by direct stimulation of β-adrenoceptors, or by releasing and/or potentiating endogenous catecholamines.

One of the properties common to most α-adrenoceptor blocking agents is the ability to inhibit the neuronal uptake of noradrenaline (Axelrod, Hertting & Potter, 1962; Farrant, Harvey & Pennefather, 1964; Iversen, 1965). As a consequence of inhibiting noradrenaline uptake, α-adrenoceptor blocking agents might be expected

to potentiate the effects of catecholamines on β-adrenoceptors, so it was of interest to establish whether other inhibitors of noradrenaline uptake also reduced vascular permeability. Desipramine and cocaine, which are potent inhibitors of noradrenaline uptake (Iversen, 1965), were found to reduce the accumulation of dye in the peritoneal cavity, an effect which was antagonized by β-adrenoceptor blocking agents (Table 1).

Effect of adrenalectomy

Mice which had been bilaterally adrenalectomized were maintained on 0.9% saline drinking fluid and then subjected to the test for vascular permeability 14 days after surgery. Such mice accumulated about 50% less dye in their peritoneal cavities than unoperated or sham-adrenalectomized mice, although the concentration of dye in the plasma of adrenalectomized mice did not differ significantly from that of control mice. Cortisone (40 mg/kg i.m.) administered 24 h before the estimation of vascular permeability increased the accumulation of Evans blue in the peritoneal cavity of adrenalectomized mice to values equal to those of unoperated or sham-adrenalectomized mice. In contrast, the accumulation of Evans blue in the peritoneal cavity of normal or sham-adrenalectomized mice was unaffected by cortisone pretreatment (Table 2). Thus, when assessing the effect of adrenalectomy on the permeability-reducing activity of drugs, both adrenalectomized and sham-adrenalectomized mice were routinely pretreated with a single dose of cortisone 24 h before the experiment.

Adrenaline or noradrenaline administered intraperitoneally reduced dye extravasation to a similar extent in both adrenalectomized and sham-adrenalectomized mice. The inhibitory effects of piperoxane or desipramine on dye extravasation were not antagonized by adrenalectomy (Table 3), suggesting that the permeability effects of these drugs were not due to the release of catecholamines from the adrenal medulla.

Effect of reducing sympathetic nervous activity

Pretreatment with hexamethonium, mecamylamine or guanethidine caused a reduction in dye extravasation. In interpreting the results, the accumulation of dye in the peritoneal cavities of mice treated with a combination of drugs was thus expressed as a percentage of the dye accumulating in mice treated with ganglion blocking agent or

Table 1 Effect of β-adrenoceptor blocking agents on the inhibition of dye extravasation by phenoxybenzamine, phentolamine, piperoxane, yohimbine, cocaine and desipramine

Group	*Treatment*	*Concentration of dye in peritoneal fluid (μg/ml ± s.e. mean)*	P *compared with*
(a)	No drug treatment	10.32 ± 0.89	–
(b)	Sotalol	10.38 ± 0.95	(a) NS
(c)	Propranolol	9.76 ± 0.88	(a) NS
(d)	Phenoxybenzamine	5.65 ± 0.51	(a) <0.001
(e)	Phenoxybenzamine + sotalol	7.81 ± 0.72	(d) <0.05
(f)	Phentolamine	4.64 ± 0.51	(a) <0.001
(g)	Phentolamine + sotalol	7.80 ± 0.71	(f) <0.002
(h)	Phentolamine + propranolol	8.96 ± 0.72	(f) <0.001
(i)	Piperoxane	6.21 ± 0.61	(a) <0.001
(j)	Piperoxane + sotalol	8.22 ± 0.69	(i) <0.05
(k)	Piperoxane + propranolol	9.59 ± 0.81	(i) <0.01
(l)	Yohimbine	5.98 ± 0.50	(a) <0.001
(m)	Yohimbine + sotalol	9.11 ± 0.84	(l) <0.01
(n)	Cocaine	6.48 ± 0.59	(a) <0.002
(o)	Cocaine + sotalol	8.42 ± 0.71	(n) <0.05
(p)	Cocaine + propranolol	9.21 ± 0.81	(n) <0.02
(q)	Desipramine	5.30 ± 0.48	(a) <0.001
(r)	Desipramine + sotalol	6.19 ± 0.64	(q) NS
(s)	Desipramine + propranolol	7.20 ± 0.62	(q) <0.05

Propranolol (10 mg/kg), sotalol (5 mg/kg), phentolamine (10 mg/kg), piperoxane (10 mg/kg) or yohimbine (10 mg/kg) were injected subcutaneously 30 min before the experiment and phenoxybenzamine (10 mg/kg) injected intravenously immediately before the injection of Evans blue. Each value is the mean of at least 24 determinations ± s.e. mean. NS, not significant.

guanethidine alone. Pretreatment with a ganglion blocking agent or guanethidine antagonized the inhibitory effects of piperoxane, phentolamine and cocaine on dye extravasation (Table 4), suggesting that the permeability effect of the latter three drugs depended upon the availability of autonomic nervous pathways. However, the inhibitory effect of desipramine on dye extravasation was not significantly antagonized ($P > 0.05$) by ganglion or adrenergic neurone blockade.

To assess further the role of endogenous catecholamines in the mediation of anti-inflammatory activity, experiments were performed on animals depleted of catecholamines by pretreatment with the tyrosine hydroxylase inhibitor, α-methyl-*p*-tyrosine (Spector, Sjoerdsma & Udenfriend, 1965). α-Methyl-*p*-tyrosine (100 mg/kg) was administered by stomach tube every 4 h and the mice used for experiment 7, 14 or 21 h after the first dose. Control groups of mice were given water by stomach tube every 4 hours.

Pretreatment with α-methyl-*p*-tyrosine caused a dose-dependent reduction in dye extravasation. Pretreatment with α-methyl-*p*-tyrosine for 7 h before estimation of vascular permeability antagonized the inhibitory effect of piperoxane and phentoolamine on dye extravasation, but had no appreciable effect on the response to desipramine or to locally injected catecholamines (Table 5).

Table 2 Effect of cortisone on vascular permeability in adrenalectomized and sham-adrenalectomized mice

Treatment	*Concentration of dye in peritoneal fluid (μg/ml ± s.e. mean)*	*Permeability as a % of control*
Control	10.05 ± 0.79	–
Sham-adrenalectomized	9.81 ± 0.74	98 ± 7
Sham-adrenalectomized + cortisone	9.98 ± 0.86	99 ± 9
Adrenalectomized	4.43 ± 0.56	44 ± 6
Adrenalectomized + cortisone	9.14 ± 0.73	91 ± 7

Mice were bilaterally adrenalectomized 14 days previously and maintained on 0.9% sodium chloride drinking fluid. Sham-adrenalectomized mice were allowed access to tap water. Cortisone (40 mg/kg i.m.) was injected 24 h before the measurement of vascular permeability. Pretreatment with cortisone significantly increased the accumulation of dye in the peritoneum of adrenalectomized mice ($P < 0.001$). Each value is the mean of at least 18 determinations.

Table 3 Effect of adrenaline, piperoxane, phentolamine or desipramine on vascular permeability in adrenalectomized and sham-adrenalectomized mice

Treatment	*Concentration of dye in peritoneal fluid (μg/ml ± s.e. mean)*	*Permeability as a % of corresponding control*
Control (sham-adrenalectomized)	9.78 ± 0.62	–
Control (adrenalectomized)	8.41 ± 0.59	–
Adrenaline	4.10 ± 0.49	42 ± 4
Adrenalectomized + adrenaline	3.92 ± 0.53	47 ± 6
Piperoxane	4.50 ± 0.40	46 ± 4
Adrenalectomized + piperoxane	4.69 ± 0.65	56 ± 8
Phentolamine	4.10 ± 0.49	42 ± 5
Adrenalectomized + phentolamine	4.22 ± 0.51	50 ± 6
Desipramine	4.30 ± 0.44	44 ± 4
Adrenalectomized + desipramine	4.11 ± 0.59	49 ± 7

Both adrenalectomized and sham-adrenalectomized mice were pretreated with cortisone (40 mg/kg). Adrenaline (1 μg/ml) or piperoxane (50 μg/ml) were injected intraperitoneally in 4 ml of acidic saline. Phentolamine (5 mg/kg) or desipramine (10 mg/kg) were injected subcutaneously 30 min before injection of acidic saline and Evans blue. Each value is the mean of at least 18 determinations.

Table 4 Effect of ganglion or adrenergic neurone blockade on the inhibition of dye accumulation by phentolamine, piperoxane, cocaine or desipramine

Group	*Treatment*	*Concentration of dye in peritoneal fluid (μg/ml ± s.e. mean)*	*Permeability as a % of corresponding control group*	P *compared with*
(a)	No drug treatment	10.09 ± 0.88	–	–
(b)	Hexamethonium	6.86 ± 0.81	(a) 68 ± 8	(a) <0.02
(c)	Mecamylamine	8.17 ± 0.78	(a) 81 ± 8	(a) NS
(d)	Guanethidine	8.27 ± 0.84	(a) 82 ± 8	(a) NS
(e)	Phentolamine	4.54 ± 0.46	(a) 45 ± 5	(a) <0.001
(f)	Hexamethonium + phentolamine	4.29 ± 0.45	(b) 63 ± 7	(e) <0.05
(g)	Guanethidine + phentolamine	6.05 ± 0.72	(d) 73 ± 9	(e) <0.02
(h)	Piperoxane	4.07 ± 0.41	(a) 40 ± 4	(a) <0.001
(i)	Mecamylamine + piperoxane	5.15 ± 0.52	(c) 63 ± 6	(h) <0.01
(j)	Guanethidine + piperoxane	5.96 ± 0.69	(d) 72 ± 8	(h) <0.01
(k)	Cocaine	4.85 ± 0.46	(a) 49 ± 5	(a) <0.001
(l)	Mecamylamine + cocaine	5.65 ± 0.59	(c) 69 ± 7	(k) <0.05
(m)	Guanethidine + cocaine	5.45 ± 0.56	(d) 66 ± 7	(k) <0.05
(n)	Desipramine	4.44 ± 0.49	(a) 44 ± 5	(a) <0.001
(o)	Mecamylamine + desipramine	4.14 ± 0.44	(c) 51 ± 5	(n) NS
(p)	Guanethidine + desipramine	4.15 ± 0.48	(d) 50 ± 6	(n) NS

Hexamethonium (20 mg/kg) or mecamylamine (5 mg/kg) were injected subcutaneously 30 min before injection of acidic saline and Evans blue. Guanethidine (10 mg/kg) was injected subcutaneously 12 h beforehand and phentolamine (5 mg/kg), piperoxane (10 mg/kg), cocaine (20 mg/kg) or desipramine (20 mg/kg) were administered subcutaneously 30 min before the acidic saline and Evans blue. Each value is the mean of at least 15 determinations. NS, not significant.

Table 5 Effect of pretreatment with α-methyl-*p*-tyrosine (α-MPT) on the inhibition of vascular permeability by adrenaline, piperoxane, phentolamine or desipramine

Group	*Treatment*	*Concentration of dye in peritoneal fluid (μg/ml ± s.e. mean)*	*Permeability as a % of corresponding control group*	P *compared with*
(a)	No drug treatment	9.97 ± 0.82	–	–
(b)	α-MPT (7 h pretreatment)	7.78 ± 0.66	(a) 78 ± 7	(a) <0.05
(c)	α-MPT (14 h pretreatment)	5.84 ± 0.55	(a) 58 ± 6	(a) <0.001
(d)	α-MPT (21 h pretreatment)	4.29 ± 0.47	(a) 43 ± 5	(a) <0.001
(e)	Adrenaline	4.31 ± 0.48	(a) 43 ± 5	(a) <0.001
(f)	αMPT (7 h pretreatment) + adrenaline	3.77 ± 0.45	(b) 48 ± 6	(e) NS
(g)	Piperoxane	3.10 ± 0.41	(a) 31 ± 4	(a) <0.001
(h)	α-MPT (7 h pretreatment) + piperoxane	5.92 ± 0.62	(b) 75 ± 8	(g) <0.001
(i)	Phentolamine	4.49 ± 0.48	(a) 45 ± 5	(a) <0.001
(j)	α-MPT (7 h pretreatment) + phentolamine	5.51 ± 0.59	(b) 70 ± 8	(i) <0.01
(k)	Desipramine	4.08 ± 0.39	(a) 41 ± 4	(a) <0.001
(l)	α-MPT (7 h pretreatment) + desipramine	3.73 ± 0.43	(b) 48 ± 6	(k) NS

α-Methyl-*p*-tyrosine (100 mg/kg) was given orally every 4 h and the mice used for experiment 7 h (2 x 100 mg/kg), 14 h (4 x 100 mg/kg) or 21 h (6 x 100 mg/kg) after the first dose. Mice not treated with α-methyl-*p*-tyrosine were given water by stomach tube 7 h and 3 h before the experiment. Adrenaline (1 μg/ml) and piperoxane (50 μg/ml) were injected intraperitoneally in 4 ml of acidic saline. Phentolamine (5 mg/kg) and desipramine (10 mg/kg) were injected subcutaneously 30 min before experiment. Each value is the mean of at least 18 determinations. NS, not significant.

Effect of drugs on catecholamine excretion

The results presented so far indicate that cocaine and a number of α-adrenoceptor blocking agents reduce vascular permeability by potentiating and/or releasing endogenous catecholamines. It was anticipated that these drugs would increase plasma levels of catecholamines and consequently increase catecholamine excretion.

Catecholamine excretion was estimated in normal untreated mice after maintaining the mice on a milk diet for 2 days in order to increase the volume of urine excreted. These mice excreted adrenaline (0.12 ± 0.02 μg/kg body weight) and noradrenaline (0.52 ± 0.07 μg/kg) over the 4 h period of collection. Mice subjected to the regime for estimating the permeability of the peritoneal vascular bed excreted approximately 2-3 times more adrenaline and noradrenaline than untreated mice kept under the same conditions (Figure 1). Since the greatest increase in plasma levels of catecholamines might be expected to occur within the first hour of injecting Evans blue and acidic saline, the observed 2-3 fold increase in catecholamine excretion is probably an under-estimate of the change in plasma levels of catecholamines which occurred during the 1 h period in which vascular permeability was measured. Pretreatment with cocaine, piperoxane or phenoxybenzamine further increased the excretion of adrenaline and noradrenaline by mice subjected to the test for estimation of vascular permeability and the increase in catecholamine excretion showed good correlation with the effect of these drugs on vascular permeability (Table 6, Figure 2). However, desipramine caused only a modest increase in catecholamine excretion.

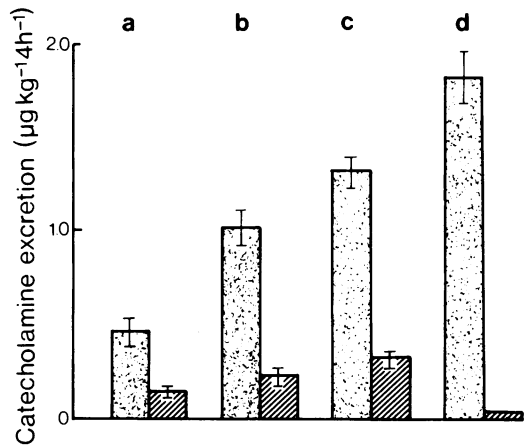

Fig. 1 Urinary excretion of noradrenaline and adrenaline from mice subjected to various stresses. Each extract was prepared from the pooled urine of 4 mice collected over a period of 4 hours. Catecholamine excretion is expressed as μg/kg body weight during 4 hours. Bars indicate s.e. mean ($n = 8$). Stippled columns, noradrenaline; hatched columns, adrenaline.

(a) Mice maintained on a milk diet for 2 days to increase urine volume. No further treatment received.

(b) Mice maintained on a normal diet and injected i.p. with 4 ml of 0.9% saline.

(c) Mice injected i.p. with 4 ml of 0.9% saline containing 0.05% acetic acid, and i.v. with 0.2 ml of 0.5% Evans blue in 0.9% saline (i.e. as in the test used for measuring vascular permeability).

(d) Treated as 'group c', but the mice were bilaterally adrenalectomized 14 days before and maintained on 0.9% saline drinking fluid. Cortisone (40 mg/kg i.m.) was injected 24 h before the collection of urine.

Discussion

The inhibitory effects of phenoxybenzamine, phentolamine, piperoxane, yohimbine or cocaine on dye extravasation were antagonized by β-adrenoceptor blocking agents or by pretreatment with drugs which reduce sympathetic nervous activity. Furthermore, α-adrenoceptor blocking agents were shown to increase catecholamine excretion, an effect which showed good correlation with their effect in reducing dye extravasation. This study thus provides evidence that certain drugs inhibit increased vascular permeability in the mouse peritoneum by releasing and/or potentiating the effects of endogenous catecholamines on β-adrenoceptors. The catecholamines involved in the anti-inflammatory effect appeared to be extra-adrenal, since the inhibitory effects of α-adrenoceptor blocking agents on dye extravasation were not prevented by bilateral adrenalectomy.

The ability of desipramine to inhibit vascular permeability has been reported by Rocha e Silva (1964), Brown *et al.* (1968a) and by Arrigoni Martelli, Toth, Segre & Corsico (1967) who obtained evidence that the anti-inflammatory activity of desipramine was mediated via endogenous catecholamines. However, the results presented here suggest that the effects of desipramine on dye extravasation are largely independent of catecholamines. Thus, the permeability effects of desipramine were only partially antagonized by β-adrenoceptor blocking agents and were not significantly ($P > 0.05$) antagonized by adrenalectomy or by pretreatment with guanethidine or α-methyl-*p*-tyrosine. Also, desipramine caused only a modest increase in

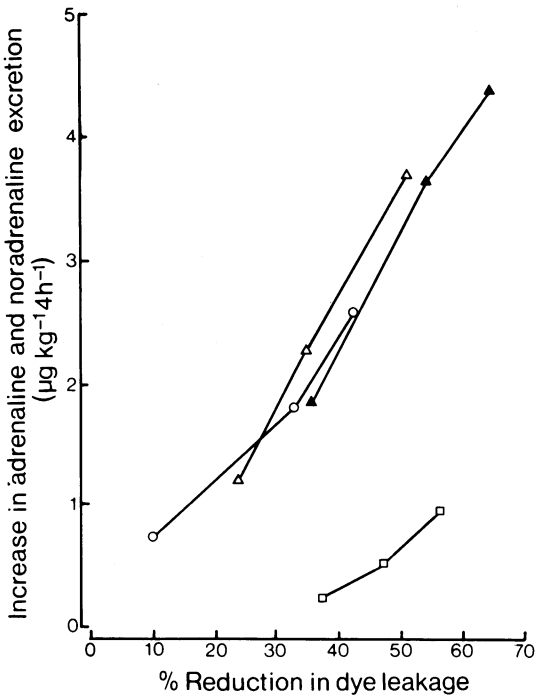

Fig. 2 Relationship between the vascular permeability-inhibiting effect of cocaine (○), desipramine (□), phenoxybenzamine (▲) or piperoxane (△) and their effect on adrenaline and noradrenaline excretion.

adrenaline and noradrenaline excretion. Since desipramine is a potent inhibitor of the neuronal uptake of noradrenaline (Iversen, 1965), it would appear that this property alone is not responsible for the inhibitory effects of α-adrenoceptor blocking agents on dye extravasation, and that besides potentiating the effects of catecholamines on β-adrenoceptors they cause the release of endogenous catecholamines. α-Adrenoceptor blocking agents probably reflexly increase sympathetic nervous activity by lowering arterial blood pressure and some appear to stimulate sympathetic nervous activity directly (Goldenburg, Snyder & Aranow, 1947; Gershon & Lang, 1962; Ingram & Domino, 1963).

Since endogenous catecholamines appear to exert a restraining influence on inflammation it would be expected that inhibition of sympathetic nervous activity would potentiate the inflammatory response. It seems paradoxical, therefore, that drugs which block ganglia, adrenergic neurones or catecholamine biosynthesis have an anti-inflammatory effect in the mouse peritoneum and other models of inflammation (Arrigoni Martelli *et al.*, 1967; Brown *et al.*, 1968a; Arntzen & Briseid, 1973). Various explanations have been put forward to account for a reduced inflammatory response in animals with low sympathetic activity. Garattini, Jori, Bernardi, Carrara, Paglialunga & Segre (1965) proposed that hypotension inhibited the development of oedema, but Jaques (1965)

Table 6 Effect of piperoxane, phenoxybenzamine, cocaine or desipramine on the urinary excretion of adrenaline and noradrenaline

Treatment	*Catecholamine excretion (μg/kg body weight during 4 h ± s.e. mean)*	
	Adrenaline	*Noradrenaline*
Control	0.31 ± 0.03	1.30 ± 0.08
Piperoxane (1.5 mg/kg)	0.51 ± 0.24	2.29 ± 0.24
Piperoxane (4 mg/kg)	0.63 ± 0.28	3.26 ± 0.31
Piperoxane (12 mg/kg)	0.99 ± 0.28	4.30 ± 0.66
Phenoxybenzamine (4 mg/kg)	0.73 ± 0.18	2.70 ± 0.59
Phenoxybenzamine (8 mg/kg)	0.83 ± 0.16	4.38 ± 0.78
Phenoxybenzamine (16 mg/kg)	1.29 ± 0.17	4.64 ± 0.43
Cocaine (5 mg/kg)	0.63 ± 0.23	1.78 ± 0.27
Cocaine (15 mg/kg)	0.73 ± 0.31	2.66 ± 0.44
Cocaine (40 mg/kg)	0.79 ± 0.29	3.58 ± 0.42
Desipramine (5 mg/kg)	0.25 ± 0.17	1.62 ± 0.42
Desipramine (15 mg/kg)	0.35 ± 0.18	1.84 ± 0.39
Desipramine (30 mg/kg)	0.59 ± 0.20	1.98 ± 0.41

Mice were injected intraperitoneally with 4 ml of saline containing 0.05% acetic acid, intravenously with 0.2 ml of 0.5% Evans blue and urine collected over the next 4 hours. Piperoxane, cocaine or desipramine were injected subcutaneously 30 min before the experiment and phenoxybenzamine intravenously 30 min before the experiment. Values for the excretion of adrenaline and noradrenaline are not corrected for losses in recovery. Each value is the mean of at least 8 determinations.

found no correlation between the two effects. Likewise, the suggestion by Rocha e Silva (1964) that catecholamines activate kinins is not supported by more recent evidence (Arntzen & Briseid, 1973; Green, 1973). In the present experiments, drugs antagonizing sympathetic nervous activity did not appear to reduce dye extravasation by increasing plasma volume, or by increasing the activity of catecholamines in reducing vascular permeability. The finding that bilaterally adrenalectomized mice which have not been treated with corticosteroids have a reduced inflammatory response to irritants confirms a previous observation of Aschheim & Zweifach (1961), who attributed the effect to poor vascular tone. The tendency for noradrenaline excretion to be increased following bilateral adrenalectomy has been reported previously (De Schaepdryver, Preziosi & van der Stricht, 1959; Harrison & Seaton, 1966), and may reflect a compensatory response of the sympathetic nervous system to the loss of adrenal medullary hormones.

Various forms of stress increase catecholamine excretion and thus it would appear that the method used to estimate vascular permeability subjects the mice to considerable stress. Some of the increased excretion of catecholamines may also be due to their release by bradykinin, histamine and other mediators of the inflammatory response (Feldberg & Lewis, 1964; Staszewska-Barczak & Vane, 1965; Piper, Collier & Vane, 1967; McCulloch, Proctor & Rand, 1967). The finding that β-adrenoceptor blocking agents do not significantly increase the permeability of the inflamed mouse peritoneal vascular bed, or potentiate other forms of inflammation (Brown *et al.*, 1968b; Riesterer & Jaques, 1968), suggests that endogenous catecholamines do not normally play an important role in reducing the inflammatory response. Nevertheless, it would appear that screening tests for anti-inflammatory activity which are particularly stressful are liable to give variable results depending upon whether the substance potentiates or depresses sympathetic nervous tone. When substances are screened for potential anti-inflammatory activity, false positive results due to the potentiation or release of endogenous catecholamines by these substances could be prevented by pretreatment of the animals with β-adrenoceptor blocking agents.

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