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Catecholamines released from the adrenal medulla exert a compensatory, protective effect at β_2-adrenoceptors against Paf-induced death in mice

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1 The effects of a number of drugs and experimental conditions, which inhibit or stimulate adrenergic function, were evaluated on platelet-activating factor (Paf)-induced death in conscious mice.

2 Adrenalectomy markedly potentiated Paf toxicity, while guanethidine and reserpine did not. However, reserpine, which produced a virtually complete depletion of cathecolamines (CA) in cardiac tissue, was not able to reduce adrenal CA by more than 58%. Drugs which release noradrenaline from the adrenergic nerve terminals, such as tyramine and amphetamine, did not protect mice from Paf toxicity, while drugs or conditions which favour the release of CA from the adrenal medulla, such as urethane and cold-induced stress, did.

3. β_2- and $\beta_1 + \beta_2$-adrenoceptor antagonists (ICI 118551, propranolol and nadolol), but not β_1-antagonists (atenolol, practolol, metoprolol and CGP 20712 A), potentiated Paf toxicity at low doses; β_2- and $\beta_1 + \beta_2$-agonists (salbutamol, fenoterol and isoprenaline), but not β_1-agonists (prenalterol and tazolol) were potent inhibitors of Paf toxicity. α_1- and α_2-adrenoceptor agonists and antagonists did not exert significant effects. Propranolol did not appear to enhance the hypotensive action of Paf in pentobarbitone-anaesthetized mice.

4 It is concluded that manipulation of the release of CA from the adrenal medulla, but not from adrenergic nerves, has profound effects on Paf toxicity in mice. A number of considerations support the hypothesis that bronchoconstriction is a major determinant of Paf-induced death in mice.

Introduction

Paf (1-O-alkyl-2-acetyl-sn-glyceryl-3-phosphoryl-choline)-induced death in mice was proposed as a convenient animal model of systemic anaphylaxis (Myers *et al.*, 1983). Due to the great importance given to Paf as a possible mediator of a number of pathophysiological events (Venuti, 1985), pharmaceutical research in this field is now very active and at least ten antagonists have already been described (Braquet & Godfroid, 1986). Thus Paf-induced death in mice may be a simple economical test for the *in vivo* screening of new potential antagonists of this phospholipid autacoid, although the mechanisms of Paf toxicity and of its pharmacological modulation are poorly understood.

We have recently shown that propranolol but not metoprolol potentiates this phenomenon, suggesting that β_2- but not β_1-adrenoceptors are involved in its modulation (Criscuoli & Subissi, 1987). Moreover, in anaesthetized mice it is possible to elicit a bronchoconstrictor response with Paf, and this could be a major contributory factor to Paf-induced death. In order to substantiate our previous findings and to gain a deeper insight into this animal model, we assessed the effects of a number of drugs and experimental conditions which inhibit or stimulate adrenergic function.

Methods

The method used to assess Paf-induced lethality was described in detail in our previous paper (Criscuoli & Subissi, 1987). Briefly, conscious male Swiss mice (Nossan, Corezzana, VA, Italy) weighing 20–30 g were injected intravenously with doses of synthetic Paf (C18, Bachem), which induced death in 10–20% (LD_{20}: 10–20 $\mu g\,kg^{-1}$) or 80–90% (LD_{80}: 30–

50 $\mu g\,kg^{-1}$) of the animals. Drugs were dissolved in saline, with the exception of reserpine which was dissolved in dilute acetic acid, and administered i.p. unless otherwise stated. The protective effects of cold-induced stress were evaluated by maintaining the animals at +4°C for 30 min, immediately after the injection of Paf. The 24 h mortality rates of treated groups were compared with those of matched control groups receiving saline, by the χ^2 test.

In some experiments the adrenal glands were removed under light ether anaesthesia. After surgery the animals were treated with penicillin G 2000 iu s.c. and allowed tap water and chow *ad libitum*. There were very few deaths (5%) up to the fourth day, when they were challenged with Paf. Groups of sham-operated mice were also tested as controls. Some adrenalectomized groups received substitution therapy with corticosteroids: desoxycorticosterone enantate, 1 or 10 $mg\,kg^{-1}$ i.m. was injected once only, soon after surgery, while hydrocortisone sodium succinate 0.33 or 3.3 $mg\,kg^{-1}$ was injected s.c. twice daily for a total of six treatments, between 08 h 00 min and 09 h 00 min. and between 16 h 00 min and 17 h 00 min., the last dose being given approximately 6 h before the Paf challenge. The effects of these two subacute treatments with corticosteroids were also evaluated in intact mice.

In order to correlate the effects of reserpine with its depleting action on tissue catecholamine (CA) content, the levels of these amines were measured by the method of Anton & Sayre (1962) in the heart and adrenals, removed immediately after death or 2 h after the injection of Paf in survivors.

The effects of Paf (0.1–10 $\mu g\,kg^{-1}$ i.v.) were also tested on arterial blood pressure of older mice (body weight 35–48 g), in the absence and presence of propranolol. Briefly, the mice were anaesthetized with sodium pentobarbitone 60 $mg\,kg^{-1}$ i.v. plus 20 mg kg^{-1} s.c., a catheter was inserted into a carotid artery and connected to a Bentley Trantec 800 transducer; blood pressure was recorded on a Basile 7070 Gemini Recorder. After a short stabilization period (10–15 min) the animal received propranolol 1 $mg\,kg^{-1}$ i.v. or saline. After 5 min increasing doses of Paf were injected at intervals of at least 5 min.

Drugs used were: reserpine, (−)-phenylephrine hydrochloride, isoprenaline sulphate and yohimbine hydrochloride (Sigma), propranolol hydrochloride (Gianni), ICI 118551 (erythro-DL-1(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol), practolol and atenolol (ICI), CGP 20712 A (1-[2-((3-carbamoyl-4-hydroxy)phenoxy)ethylamino] - 3 - [4 - (1 - methyl - 4 trifluoromethyl - 2 - imidazolyl)phenoxy] - 2 - propanol methane-sulphonate), metoprolol tartrate and phentolamine hydrochloride (Ciba-Geigy), guanethidine sulphate (Merck Sharp & Dohme), clonidine hydrochloride and fenoterol hydrobromide (Boehringer Ingelheim), prazosin hydrochloride (Pfizer), (+)-amphetamine sulphate (Recordati), tyramine hydrochloride (BDH), nadolol and penicillin G potassium salt (Squibb), salbutamol sulphate (Glaxo), tazolol (Syntex), prenalterol hydrochloride (Hässle), methoxamine hydrochloride (Wellcome), midodrine hydrochloride (Chemie Linz), desoxycorticosterone enantate (Cortiron Depot, Schering), hydrocortisone sodium succinate (Lepetit), ethyl urethane (Carlo Erba), sodium pentobarbitone (Abbott). All doses refer to the salts.

Results

As shown in Table 1, adrenalectomy produced a notable potentiation of Paf toxicity, while both reserpine and guanethidine were devoid of effect. We also measured CA content in tissues of mice after treatment with reserpine or vehicle. In control animals CA levels were 9.27 ± 0.96 $\mu mol\,g^{-1}$ and 3.37 ± 0.29 $nmol\,g^{-1}$ of fresh tissue (mean values ± s.e.mean, $n = 10$) in adrenals and hearts, respectively. The adrenal values were reduced by reserpine 1 and 5 $mg\,kg^{-1}$ s.c. to 5.97 ± 0.83 and 3.86 ± 0.51 $\mu mol\,g^{-1}$ respectively, while in the heart the CA levels were undetectable, i.e. less than 0.5 $nmol\,g^{-1}$, after either dose of the alkaloid.

Drugs (urethane) and experimental conditions (cold-induced stress) favouring the release of CA from the adrenal medulla exerted a protective effect against an LD_{80} of Paf, while drugs releasing CA from the adrenergic nerve terminals, such as tyramine and amphetamine, did not. The protective action of urethane appears to be independent of anaesthesia, since pentobarbitone was inactive.

As the adrenalectomy-dependent protection could be mediated by the depletion of either CA or corticosteroids, we evaluated the effects of the administration of corticosteroids in adrenalectomized mice. As shown in Table 2, neither of the two dosage regimens used was able to reverse significantly the dramatic increase in death following adrenalectomy, in spite of the inhibition of thymus weight increase that both treatments produced. In fact, the higher dose induced a lowering of thymus weight compared to intact animals, and also brought the body weight up to the basal values. The two corticosteroid regimens did not show any protective action *per se*, when administered to intact mice.

As shown in Table 3, neither α_1- (prazosin), α_2- (yohimbine) and $\alpha_1 + \alpha_2$- (phentolamine) antagonists nor α_1- (methoxamine and midodrine, a new agent with a long duration of action, Pittner *et al.*, 1976) and α_2- (clonidine) agonists protected against an LD_{80} of Paf. In contrast, another α_1-selective agonist, phenylephrine, exerted protective effects which were not, however, reversed by a high dose of prazosin. Also, the reputedly β_1-selective agonists, tazolol (Strosberg, 1976) and prenalterol (Weiner, 1985a), were devoid of

Table 1 Effects of drugs, or experimental conditions affecting adrenergic function' on Paf-induced death in mice

Treatment	*Dose* ($mg\,kg^{-1}$) *and route of administration*	*Pretreatment time* (h)	*Survivors / Tested*	*% survival*	*Significance* (χ^2)
Potentiating effects[a]					
Vehicle	—	—	63/70	90	—
Adrenalectomy	—	—	3/41	7	$P<0.001$
Sham-operation	—	—	23/25	92	NS
Reserpine	5, s.c.	24	25/25	100	NS
	1, s.c.	24	35/35	100	NS
Guanethidine	10 + 10, s.c.	24,2	15/15	100	NS
Inhibitory effects[b]					
Vehicle	—	—	7/60	12	—
Tyramine	10, i.p.	0.08	3/10	30	NS
		0.5	2/10	20	NS
Amphetamine	10, i.p.	0.5	4/20	20	NS
Urethane	1500, i.p.	0.5	10/10	100	$P<0.001$
Pentobarbitone	50, i.p.	0.5	0/10	0	NS
Cold-induced stress	—	—	14/20	70	$P<0.01$

[a] Test drugs were administered at different times before a dose of Paf lethal in 10–20% of the animals (10–20 $\mu g\,kg^{-1}$ i.v.). Adrenalectomy or sham-operation was performed 3 days before the test.
[b] Test drugs were administered at different times before a dose of Paf lethal in 80–90% of the animals (30–50 $\mu g\,kg^{-1}$ i.v.). In the cold-induced stress experiment the mice were kept at +4°C for 30 min immediately after the injection of Paf.

protective effects, while β_2- and non-selective β-agonists, salbutamol, fenoterol and isoprenaline, were potent inhibitors of Paf toxicity.

As shown in Table 4, of the β_1-antagonists, atenolol and metoprolol potentiated Paf toxicity only at $10\,mg\,kg^{-1}$, while practolol, and CGP 20712 A (a highly selective compound recently described by Dooley *et al.*, 1986) were inactive even at the highest doses. In contrast, non-selective (propranolol and nadolol) and β_2-antagonists (ICI 118551, O'Donnell & Wanstall, 1980) potentiated Paf-induced death at a dose of $0.1\,mg\,kg^{-1}$.

In pentobarbitone-anaesthetized mice, mean blood pressure was very similar or identical in saline- and propranolol-treated mice before each of the doses of Paf were administered. As shown in Table 5, the Paf-induced fall in blood pressure was generally superimposable both in intensity and in duration. The only significant difference was found with the lowest dose, but due to the very small absolute value of the pressure drop at this dose, this difference appears to be of negligible importance.

Discussion

The notable potentiation of Paf-induced death in mice by propranolol suggested that CA released from adrenergic nerves and/or the adrenal medulla attenuate biological effects of Paf, which are important determinants of lethality (Criscuoli & Subissi, 1987). In fact we have shown here that adrenalectomy enhances Paf toxicity, apparently by a mechanism that does not involve adrenocortical steroids. Guanethidine and reserpine, in contrast, were devoid of effects. Guanethidine inhibits the response to stimulation of sympathetic nerves, but does not alter the concentration of CA in the adrenal medulla or their release therefrom, while reserpine depletes the stores of CA, including the adrenals, exerting its maximal effect after 24 h (Weiner, 1985b). However, depletion is less complete in adrenals than in other tissues: in fact we found a maximum reduction of 58% of adrenal levels of CA, in the same mice whose cardiac stores were virtually depleted. We therefore conclude that circulating CA, originating from the adrenal medulla, exert a protective action against the lethal effects of Paf in mice. This action is resistant to reserpine pretreatment, suggesting that its impairment may begin at levels below 40% of normal CA content in adrenal medulla. Other results are in agreement with this hypothesis: tyramine and amphetamine, sympathomimetic amines which act by releasing noradrenaline from adrenergic nerve terminals (Weiner, 1985a), do not protect from Paf lethality, while cold-induced stress and urethane do. Cold-induced stress releases corticosteroids as well as CA from the adrenals and it is known that these hormones protect mice from Paf

toxicity (Myers *et al.*, 1983). However the onset of their protective action requires more than two hours (Criscuoli & Subissi, 1987) and therefore we believe that they do not play a role in this model, in which Paf is injected just before exposing the animals to cold. As for urethane, this anaesthetic, at least when administered i.p. in various animal species, produces an activation of sympathetic structures at the CNS level leading to increased CA secretion from the adrenal medulla (Maggi & Meli, 1986).

Once we had obtained an insight into the origin of the biogenic amines (mainly adrenaline) involved in the compensatory protection from Paf lethality, we attempted to gain a better understanding of the receptors they act upon and, possibly, of the target organ(s). α-Adrenoceptor agonists and antagonists, whether subtype-selective or not, are unable to inhibit (or potentiate: clonidine 0.2 mg kg^{-1}, phentolamine 10 mg kg^{-1} and prazosin 20 mg kg^{-1}, data not shown) Paf toxicity, with the single exception of the classical α_1-agonist phenylephrine. It has, however, been reported that this drug possesses distinct β_2-adrenoceptor agonist properties (Lefevre *et al.*, 1977) and, as a high dose of prazosin was not able to counteract the protective effect of phenylephrine, it is likely that the latter is due to stimulation of β_2-adrenoceptors. In fact we previously suggested that Paf-induced death in mice can be modulated by β_2-adrenoceptors (Criscuoli & Subissi, 1987). Here we substantiated the previous findings, by assaying a number of antagonists endowed with similar potency but different selectivity for β-adrenoceptors (Weiner, 1985b): our results show that doses of β_1-selective antagonists at least 100 times higher than those of either β_2-selective or non-selective antagonists are needed to elicit comparably potentiation of Paf toxicity. Moreover, β_1-agonists do not exert protective effects up to 10 mg kg^{-1}, while β_2- and non-selective agonists are active at doses below 1 mg kg^{-1}. Taken together, these results suggest that β_2-adrenoceptors are profoundly involved in the modulation of Paf toxicity in mice, as well as in other receptor-mediated actions of the autacoid (Braquet & Godfroid, 1986), while β_1- and α-adrenoceptors do not seem to play a significant role. Moreover, as this simple model discriminates sharply between β_1- and β_2-agonists and antagonists, it could be used for the *in vivo* evaluation of the selectivity of β-adrenoceptor agents. Since β_2-adrenoceptors sensitive to Paf administration are stimulated by CA released from the adrenal medulla and not from adrenergic nerve terminals, as discussed above, it is likely that they are localized in tissues endowed with a high density of β_2-adrenoceptors, but with a scant adrenergic innervation. It is known from receptor binding studies that the density of β-adrenoceptors in lungs of several species is higher than in any other tissue (Nadel & Barnes, 1984), while, in contrast with their dense parasympathetic

Table 2 Effects of substitution therapy with corticosteroids on Paf-induced death in adrenalectomized (a) and intact (b) mice

Group	Adrenalectomy	Treatment	Survivors / Tested	% survival	Significance vs group 1 or 5 (χ^2)	Body weight (g)	Thymus weight (mg)
		Paf 10 μg kg^{-1}[a]					
1	−	Vehicle	20/20	100	—	22.5 ± 0.69	61.2 ± 5.5
2	+	Vehicle	1/19	5	$P < 0.001$	18.1 ± 0.43**	74.8 ± 3.8*
3	+	Corticosteroids, low dose	4/19	21	$P < 0.001$	19.1 ± 0.45**	66.5 ± 4.7
4	+	Corticosteroids, high dose	7/20	35	$P < 0.001$	21.2 ± 0.56[§]	47.4 ± 3.5**[§]
		Paf 40 μg kg^{-1}[b]					
5	−	Vehicle	4/10	40	—	—	—
6	−	Corticosteroids, low dose	4/9	44	NS	—	—
7	−	Corticosteroids, high dose	4/10	40	NS	—	—

[a] Adrenalectomy was performed 3 days before the experiment. The mice received a single dose of desoxycorticosterone enantate 1 (low dose) or 10 (high dose) mg kg^{-1} i.m. and b.i.d. hydrocortisone sodium succinate 0.33 (low dose) or 3.3 (high dose) mg kg^{-1} s.c. for a total of 6 treatments. Approximately 6 h after the last treatment, the mice were challenged with Paf 10 μg kg^{-1} i.v. Mean values ± s.e.mean of body and thymus weight are also shown ($n = 13$–20). % survival in groups 3 and 4 was not significantly different from that in group 2 ($P > 0.05$). Significantly different (Student's t test) from group 1: * $P < 0.05$; ** $P < 0.001$; from group 2: [§] $P < 0.001$. [b] The possible protective effects of the treatment schedules presented above were tested also in intact mice against a higher dose of Paf (40 μg kg^{-1} i.v.).

Table 3 Inhibitory effects of adrenoceptor agonists and antagonists on Paf-induced death in mice

Treatment	*Dose* (mg kg^{-1} i.p.)	*Survivors* / *Tested*	*%* *survival*	*Significance* (χ^2)
Vehicle	—	47/296	16	—
Phenylephrine[a]	5	16/20	80	$P<0.001$
Methoxamine	5	10/30	33	NS
Midrodrine	20	2/18	11	NS
Clonidine	1	0/10	0	NS
	0.2	1/10	10	NS
Phentolamine	10	3/10	30	NS
Prazosin	20	4/10	40	NS
	5	3/10	30	NS
Yohimbine	20	1/10	10	NS
	5	0/10	0	NS
Prenalterol	10	2/20	10	NS
	1	0/10	0	NS
Tazolol	10	1/10	10	NS
	1	3/10	30	NS
Isoprenaline	1	19/20	95	$P<0.001$
	0.3	7/10	70	$P<0.001$
	0.1	3/20	15	NS
Salbutamol	1	19/20	95	$P<0.001$
	0.1	14/20	70	$P<0.001$
	0.03	11/30	37	$P<0.05$
Fenoterol	1	10/10	100	$P<0.001$
	0.3	19/20	95	$P<0.001$
	0.1	10/20	50	$P<0.01$

Test drugs were administered i.p. 30 min (5 min in the case of phenylephrine) before a dose of Paf lethal in 80–90% of the animals (30–50 μg kg^{-1} i.v.).

[a] The effects of phenylephrine were unchanged in the presence of prazosin 20 mg kg^{-1} i.p., given 30 min before Paf (7/10, 70%, $P<0.001$).

Table 4 Effects of β_1-(practolol, atenolol, metoprolol and CGP 20712 A), $\beta_1 + \beta_2$-(propranolol and nadolol) and β_2-(ICI 118551) adrenoceptor antagonists on Paf-induced death in mice

Treatment	*Dose* (mg kg^{-1} i.p.)	*Survivors* / *Tested*	*%* *survival*	*Significance* (χ^2)
Vehicle	—	229/280	82	—
Practolol	10	6/10	60	NS
	1	9/10	90	NS
Atenolol	10	7/20	35	$P<0.001$
	1	9/10	90	NS
Metoprolol	10	5/20	25	$P<0.05$
	1	9/10	90	NS
CGP 20712 A	30	12/20	60	NS
	10	16/20	80	NS
	1	17/21	81	NS
Propranolol	1	4/30	13	$P<0.001$
	0.1	14/40	35	$P<0.001$
	0.01	27/30	90	NS
Nadolol	1	1/10	10	$P<0.001$
	0.1	16/30	53	$P<0.05$
	0.01	8/10	80	NS
ICI 118551	1	0/10	0	$P<0.001$
	0.1	16/50	32	$P<0.001$
	0.01	26/39	67	NS

Test drugs were administered i.p. 30 min before a dose of Paf lethal in 10–20% of the animals (10–20 μg kg^{-1} i.v.).

Table 5 Hypotensive effects of Paf in pentobarbitone-anaesthetized mice, in the absence and presence of propranolol

		Blood pressure fall (mmHg) *and, in parentheses, duration of hypotensive effects* (min)				
		Doses of Paf (μg kg^{-1} i.v.)				
Pretreatment	*Basal blood pressure* (mmHg)	*0.1*	*0.3*	*1*	*3*	*10*
Saline	74 ± 6	1.5 ± 0.6 (0.4 ± 0.2)	2.7 ± 0.9 (1.2 ± 0.6)	9.1 ± 4.6 (6.5 ± 3.0)	28 ± 4.4(a) (10 ± 2.5)(b)	50 ± 7.2(b)
Propranolol	79 ± 6	3.0 ± 0.3* (0.9 ± 0.3)	4.7 ± 0.5 (1.7 ± 0.5)	9.4 ± 1.2 (3.3 ± 0.7)	30 ± 4.9 (11.4 ± 2.5)(b)	59 ± 8.1(b)

Paf was injected in increasing doses 5 min after pretreatment with saline or propranolol (1 mg kg^{-1} i.v.) Each dose was administered when the effects of the preceding one had subsided, with time intervals of at least 5 min; where the hypotensive effects lasted for more than 15 min, the next dose was not administered. Mean values ± s.e.mean are presented (n = 8; (a) n = 7; (b) n = 5). Significantly different (Student's t test) from controls: *$P < 0.05$.

nerve supply to airways, most species of laboratory animals have very few if any adrenergic neurones supplying their bronchial tree (Ind & Dollery, 1983). It is therefore tempting to speculate that β_2-adrenoceptors modulating Paf toxicity are located on the mouse airways and that lethality largely depends on the bronchoconstriction which we have shown following Paf administration (Criscuoli & Subissi, 1987). A major role of hypotension as a cause of Paf-induced death is, in our opinion, excluded by the observation that propranolol, while exacerbating the lethal effects in conscious mice, does not potentiate the hypotensive effect of Paf in anaesthetized mice.

If we consider the biological properties of Paf (Venuti, 1985), other possible causes of its immediate toxicity are thrombosis, pulmonary and coronary vasoconstriction and cardiac depression. Death caused by Paf in mice does not appear to be thrombotic in nature because mouse platelets are refractory to Paf (Namm *et al.*, 1982) and platelet aggregates were not observed in the lung microvasculature of mice administered lethal doses of this autacoid (Criscuoli & Subissi, 1987). Although, to our knowledge, very little is known about the physiology of pulmonary and coronary vessels in the mouse, vasoconstriction at these levels does not seem likely to be a major factor in Paf toxicity because these vessels are generally (a) regulated by their dense adrenergic innervation, while Paf toxicity is enhanced by suppressing the adrenals, but not the adrenergic neurones; (b) highly dependent on extracellular Ca^{2+} for contraction, but high doses of calcium antagonists do not protect from Paf lethality (Myers et al., 1983; Young *et al.*, 1985). There are also arguments against a major involvement of cardiac depression as a primary cause of Paf toxicity. In fact the mammalian heart contains mainly β_1-adrenoceptors (Minneman *et al.*, 1979), mediating an increase in the force of contraction, but we have shown that neither the activation nor the blockade of these receptors is capable of interfering with the lethal effects of Paf, contrary to the potent modulating action by β_2-adrenoceptors, whose presence in the heart is scant.

In conclusion, although we have at the moment no direct proof, the present work provides further evidence suggesting that Paf-induced lethality in mice may be attributed to bronchoconstriction.

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References

ANTON, A.H. & SAYRE, D.F. (1962). A study of the factors affecting the aluminum oxide-trihydroxyindole procedure for the analysis of cathecholamines. *J. Pharmacol. Exp. Ther.*, **138**, 360–375.

BRAQUET, P. & GODFROID, J.J. (1986). Paf-acether specific binding sites: 2. Design of specific antagonists. *Trends Pharmacol. Sci.*, **7**, 397–403.

CRISCUOLI, M. & SUBISSI, A. (1987). Paf-acether-induced death in mice: involvement of arachidonate metabolites and β-adrenoceptors. *Br. J. Pharmacol.*, **90**, 203–209.

DOOLEY, D.J., BITTIGER, H. & REYMANN, N.C. (1986). CGP 20712 A: a useful tool for quantitating β_1- and β_2-adrenoceptors. *Eur. J. Pharmacol.*, **130**, 137–139.

IND, P.W. & DOLLERY, C.T. (1983). Pulmonary adrenoceptors and asthma. In *Pharmacology of Asthma*, ed. Morley, J. & Rainsford, K.D., pp. 213–244. Basel: Birkhauser Verlag.

LEFEVRE, F., FENARD, S. & CAVERO, I. (1977). Vascular β-adrenoceptor stimulating properties of phenylephrine. *Eur. J. Pharmacol.*, **43**, 85–88.

MAGGI, C.A. & MELI, A. (1986). Suitability of urethane anaesthesia for physiopharmacological investigations in various systems. Part 1: General considerations. *Experientia*, **42**, 109–114.

MINNEMAN, K.P., HEGSTRAND, L.R. & MOLINOFF, P.B. (1979). Simultaneous determination of Beta-1 and Beta-2 adrenergic receptors in tissues containing both receptor subtypes. *Mol. Pharmacol.*, **16**, 34–46.

MYERS, A.K., RAMEY, E. & RAMWELL, P. (1983). Glucocorticoid protection against Paf-acether toxicity in mice. *Br. J. Pharmacol.*, **79**, 595–598.

NADEL, J.A. & BARNES, P.J. (1984). Autonomic regulation of the airways. *A. Rev. Med.*, **35**, 451–467.

NAMM, D.H., TADEPALLI, A.S. & HIGH, J.A. (1982). Species specificity of the platelet responses to 1-0-alkyl-2-acetyl-sn-glycero-3-phosphorylcholine. *Thromb. Res.*, **25**, 341–350.

O'DONNELL, S.R. & WANSTALL, J.C. (1980). Evidence that ICI 118,551 is a potent, highly beta2-selective adrenoceptor antagonist and can be used to characterize beta-adrenoceptor populations in tissues. *Life Sci.*, **27**, 671–677.

PITTNER, H., STORMAN, H. & ENZERHOFER, R. (1976). Pharmacodynamic actions of Midodrine, a new α-adrenergic stimulating agent, and its main metabolite, ST 1059. *Arzneim. Forsch.*, **26**, 2145–2154.

STROSBERG, A.M. (1976). The cardiovascular pharmacology and hemodynamic activity of tazolol, a selective myocardial β-stimulant. *Arch. Int. Pharmacodyn.*, **222**, 200–215.

VENUTI, M.C. (1985). Platelet-activating factor: multifaceted biochemical and physiological mediator. *A. Rep. Med. Chem.*, **20**, 193–202.

WEINER, N. (1985a). Norepinephrine, epinephrine and the sympathomimetic amines. In *The Pharmacological Basis of Therapeutics* ed. Gilman, A.G., Goodman, L.S., Rall, T.W. & Murad, F. pp. 145–180. New York: Macmillan Publishing Company.

WEINER, N. (1985b). Drugs that inhibit adrenergic nerves and block adrenergic receptors. In *The Pharmacological Basis of Therapeutics*. ed. Gilman, A.G., Goodman, L.S., Rall, T.W. & Murad, F. pp. 181–214. New York: Macmillan Publishing Company.

YOUNG, J.M., MALONEY, P.J., JUBB, S.N & CLARK, J.S. (1985). Pharmacological investigation of the mechanisms of platelet-activating factor induced mortality in the mouse. *Prostaglandins*, **30**, 545–551.

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