Br. J. Pharmac. (1972), **46**, 260–269.

The nature of adrenergic mechanisms involved in anaphylatoxin activity in the guinea-pig

R. HICKS AND A. C. SACKEYFIO

Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford, Yorkshire BD7 1DP

Summary

1. Evidence is presented to elucidate the nature of the adrenergic mechanisms involved in responses of the guinea-pig to anaphylatoxin (AT).

2. Investigation by means of adrenalectomy, adrenergic neurone blockade, α- and β- adrenoceptor blockade and exclusion of autonomic reflexes, revealed that the adrenergic mechanisms provoked included catecholamine release from the adrenal medulla, sympathetic reflex activity, stimulation of adrenergic neurones and α- and β-adrenoceptor activity.

3. The cardiovascular effects of AT, mediated by histamine release, were largely attributable to adrenal medullary and adrenergic neuronal mechanisms. These mechanisms also exerted a restraint on the predominantly histamine mediated bronchoconstrictor effect of AT.

4. The cardiovascular effects of AT activity, not attributable to histamine release, were also probably associated with catecholamine release. However, the bronchoconstrictor component of this AT activity was not significantly affected by guanethidine, and it would, therefore, appear that neuronal bronchodilator mechanisms did not exert a restraint upon this aspect of AT activity.

5. These findings are generally compatible with previous work showing that adrenergic mechanisms operate during AT-induced responses. In contrast to previous reports, however, the adrenergic activity was predominantly associated with the effects of released histamine.

Introduction

It has been known for many years that intravenous administration of histamine provokes the release of catecholamines in various species (von Euler, 1966; Vane, 1969). More recently, Piper, Collier & Vane (1967) demonstrated that mediators of anaphylaxis released catecholamines in the guinea-pig. In the same year McCulloch, Proctor & Rand (1967) reported the effects of a variety of bronchoconstrictor agents in the guinea-pig and indicated the existence of a sympathetic bronchodilator reflex, counteracting the effects of such drugs.

Furthermore, it has been shown that both histamine and catecholamines are involved in responses to anaphylatoxin (AT) in the guinea-pig (Bodammer & Vogt, 1967; Bernauer, Hahn, Beck & Kury, 1970). But it is not clear whether the AT-induced catecholamine release is entirely a consequence of histamine liberation, or partly due to a direct action of AT on catecholamine-containing tissue (Bodammer & Vogt, 1967; 1970).

The present investigation was undertaken to determine the source of the catecholamines and assess the nature of the adrenergic mechanisms involved in AT activity in the guinea-pig.

Methods

AT was prepared according to the method of Rothschild & Rocha e Silva (1954) by incubating fresh rat serum with inulin (for details see Hicks & Sackeyfio, 1972). Each sample of AT was standardized by comparing its potency with that of histamine on the isolated guinea-pig ileum. It was used only if 0·002 ml of the test sample was equipotent with 120 ± 20 ng histamine and the corresponding control had no effect at 25 times the dose of the test sample.

Male Dunkin Hartley guinea-pigs were used (400–550 body weight) throughout this investigation. The animals were anaesthetized with pentobarbitone sodium 60–80 mg/kg i.p. The trachea was cannulated and artificial ventilation was performed by means of a Starling Ideal pump, operating through a closed tube system to the tracheal cannula. Ventilation stroke volume was 1 cc/100 g body weight and the rate was 36 strokes/minute. Bronchoconstriction was measured in terms of resistance to positive pressure inflation by the constant volume method of Dixon & Brodie (1903), with slight modifications to replace the mechanical tambour with an air-filled pressure transducer type 4-327-L221. The latter was connected, through a side attachment, to the outlet arm of the ventilation system. Systemic blood pressure changes were also recorded by means of a similar (oil-filled) pressure transducer connected to a saline-filled cannula inserted into the left carotid artery. Both pressure transducers were connected to a Devices M2 recorder, registering simultaneously the bronchoconstrictor and arterial pressure responses. The degree of bronchoconstriction was evaluated according to the method of Hicks & Leach (1963) and the arterial pressure was registered in mmHg. Responses were expressed as percentages of the maximal bronchoconstriction in each particular animal, produced by total occlusion of the bronchi.

Intravenous administration of drugs and solutions was through a cannula in the right external jugular vein, washed in with 0·9% NaCl w/v (normal saline) in volumes not exceeding 0·2 ml.

The procedures employed for decerebration and pithing, bilateral vagotomy and adrenalectomy were as previously described (Hicks & Sackeyfio, 1972).

Stock solutions of (±)-propranolol hydrochloride 'Inderal' (I.C.I.), guanethidine sulphate 'Ismelin' (Ciba-Geigy) and mepyramine maleate 'Anthisan' (May & Baker) were made in glass-distilled water; phentolamine mesylate 'Rogitine' (Ciba-Geigy) in water for injection and (−)-adrenaline acid tartrate (Evans Medical) and (−)-noradrenaline hydrogen tartrate (Hoechst) in 0·001 N HCl. The stock solutions were kept at 6° C until they were required for use, when they were diluted in normal saline. All drugs administered were calculated as base.

Results

It was shown in an earlier investigation (Sackeyfio, 1971) that 0·5 ml/kg AT consistently elicited submaximal, approximately median bronchoconstrictor and cardiovascular responses in the guinea-pig. This dose of AT was also used throughout

the present investigation. The characteristics of the bronchoconstrictor and cardiovascular responses to AT in normal intact anaesthetized guinea-pigs were as represented in Figure 1. The systemic blood pressure changes induced by AT in every case consisted of a brief initial depressor phase followed by a pressor component. Two forms of bronchoconstrictor response could be identified. In approximately 50% of the animals the response was monophasic (Fig. 1A), but in the remainder a biphasic response (Fig. 1B) was seen. The secondary bronchoconstrictor response occurred consistently in those animals which exhibited an intense initial bronchoconstriction associated with only a moderate pressor response. Furthermore, the onset of this secondary bronchoconstrictor response coincided with the return of the pressor effect to a normal level. In contrast, an intense pressor response was always associated with a moderate monophasic bronchoconstrictor response. These results suggested a possible interaction between the mediators of these effects. Mechanisms associated with the pressor response may also have exerted a bronchodilator action accounting for the rapid and sometimes temporary recovery from the bronchoconstriction. Further elucidation of this interaction was sought by means of the adrenoreceptor blocking drugs propranolol and phentolamine and also by acute adrenalectomy.

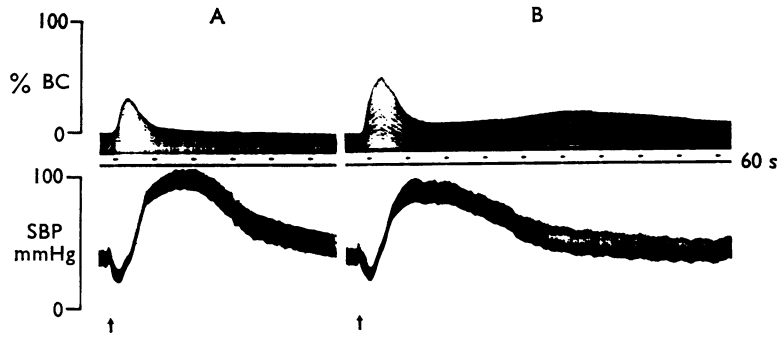

FIG. 1. Bronchoconstrictor (BC) and systemic blood pressure (SBP) responses to intravenous injections of anaphylatoxin (0·5 ml/kg at arrows) in pentobarbitone anaesthetized guinea-pigs. In approximately 50% of the animals investigated the bronchoconstrictor response was biphasic (B) and in the rest it was monophasic (A). Time marks every 60 seconds.

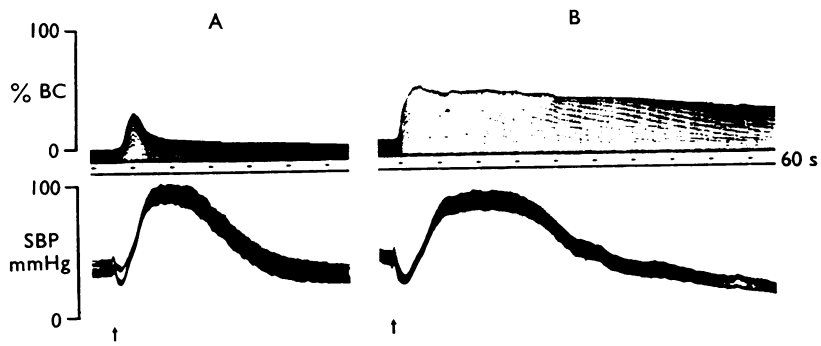

FIG. 2. Bronchoconstrictor (BC) and systemic blood pressure (SBP) responses to anaphylatoxin (0·5 ml/kg injected intravenously at arrow) in a pentobarbitone anaesthetized guinea-pig showing the effect of propranolol (total dose: 15 mg/kg divided into 10 mg/kg i.p. and 5 mg/kg i.v. injected 30 min and 5 min respectively before anaphylatoxin) (B). Effects compared with the control responses to the same dose of anaphylatoxin obtained in a different guinea-pig (A) which had no propranolol pretreatment. Time marks every 60 seconds.

The influence of adrenoceptor blocking agents on anaphylatoxin activity

Propranolol was administered in divided doses as follows: 10 mg/kg i.p. 30 min before and 5 mg/kg i.v. 5 min before injection of AT. As shown in Fig. 2B this treatment had no significant effect upon the cardiovascular responses to AT, but the AT-induced bronchoconstriction was markedly potentiated both in magnitude and duration. It therefore appears that the dilator β-adrenoceptors on the bronchiolar smooth muscle had been effectively blocked by propranolol.

Phentolamine 1 mg/kg i.v. abolished the pressor responses to both adrenaline and noradrenaline 4 μg/kg (Fig. 3 (i)). However, at doses ranging from 1–4 mg/kg it significantly reduced, but did not abolish the pressor response to AT (Fig. 3 (ii)). The bronchoconstrictor response was also slightly reduced. The depressor response was almost abolished, possibly as a consequence of the phentolamine-induced hypotension.

Effect of acute adrenalectomy

Groups of guinea-pigs were anaesthetized and prepared for removal of one or both adrenal glands. Fifteen to 30 min after the operation, when the blood pressure had stabilized at a lowered resting level, AT (0·5 ml/kg) was intra-

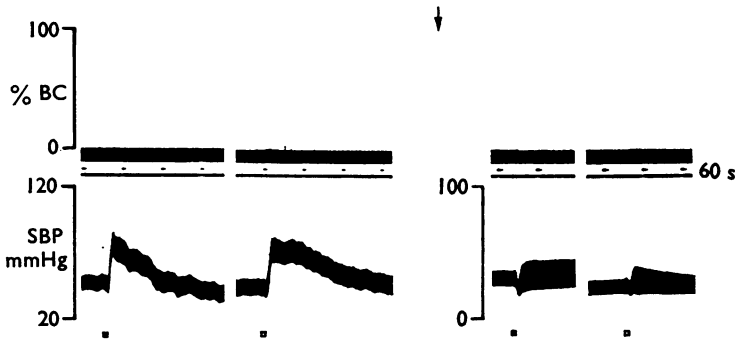

FIG. 3 (i). Systemic blood pressure (SBP) changes in response to adrenaline (0·5 μg/kg i.v. ■) and noradrenaline (0·5 μg/kg i.v. □) in a guinea-pig anaesthetized with pentobarbitone, showing the inhibitory effect of phentolamine (1 mg/kg i.v. injected at arrow). Top panel shows the absence of bronchoconstriction (BC). Time marks every 60 seconds.

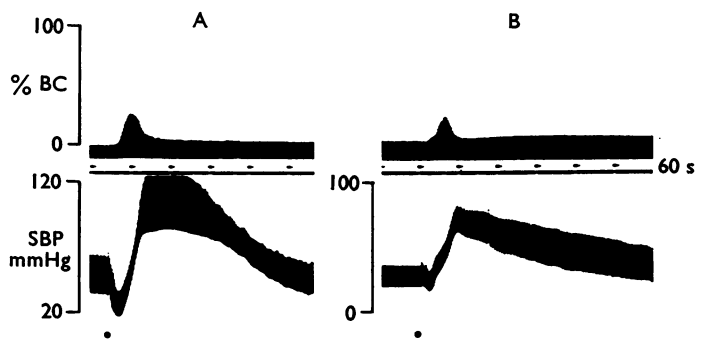

FIG. 3 (ii). Bronchoconstrictor (BC) and systemic blood pressure (SBP) responses to AT (0·5 ml/kg i.v. injected at ●). Panel A shows control responses to AT in a normal guinea-pig which had no pretreatment and panel B shows responses in a guinea-pig pretreated with phentolamine 4 mg/kg before AT administration. Note change in SBP scale on Panel B. Time marks every 60 seconds.

venously administered and the bronchoconstrictor and pressor responses were recorded. Similar records were obtained for sham-operated and normal intact animals. A comparison of these responses revealed no significant difference between the sham-operated and normal intact animals. However, as shown in Fig. 4A extirpation of one adrenal gland resulted in increased bronchoconstriction with a corresponding diminution in the pressor response, compared with the responses in the experiment of Fig. 1. Removal of both adrenal glands caused a further reduction in the pressor response and the bronchoconstriction was further potentiated (Fig. 4B). It was consistently noted that although the pressor response was very much reduced in the bilaterally adrenalectomized animals it was not competely abolished. This indicated the involvement of a mechanism other than release of catecholamines from the adrenal medullae in the elicitation of the pressor response.

The influence of pithing and bilateral vagotomy

In order to assess the influence of autonomic reflexes, two groups of ten and six guinea-pigs respectively, were either pithed or vagotomized bilaterally. Bronchoconstrictor and systemic blood pressure responses to AT were compared with those of a control group of 15 normal guinea-pigs. The results are summarized in Table 1. In the pithed animals there was a marked enhancement of the bronchoconstriction ($P<0{\cdot}001$). However, both the depressor and pressor responses were significantly ($P<0{\cdot}05$) reduced. In the bilaterally vagotomized animals also the depressor response was significantly ($P<0{\cdot}01$) reduced, but neither the bronchoconstrictor nor the pressor response was appreciably affected. These results suggested that the adrenergic mechanisms contributing to both the pressor response and a bronchodilator effect were, in part, reflex in nature.

The influence of guanethidine in (i) normal, and (ii) mepyramine-treated guinea-pigs

Six guinea-pigs were treated with guanethidine (4 mg/kg i.v.) 30–40 min before AT administration. The results obtained in these animals, as presented in Table 2 (i), show that guanethidine potentiated the bronchoconstrictor response to AT

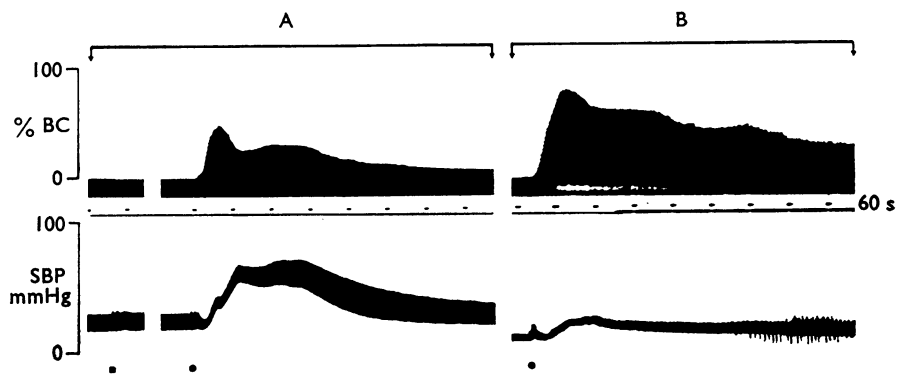

FIG. 4. Bronchoconstrictor (BC) and systemic blood pressure (SBP) responses to AT (0·5 ml/kg i.v. injected at ●) in a unilaterally adrenalectomized guinea-pig (A) and a bilaterally adrenalectomized guinea-pig (B). Normal saline 0·2 ml/kg ■ had no effect in the unilaterally adrenalectomized animal. Effects compared with the control responses illustrated in Fig. 1 (A).

TABLE 1. *A comparison of the bronchoconstrictor, depressor and pressor responses to anaphylatoxin (0·5 ml/kg i.v.) in normal intact, pithed and bilaterally vagotomized guinea-pigs*

	Normal			Pithed			Bilaterally vagotomized		
	% Broncho-constriction	Depressor response	Pressor response	% Broncho-constriction	Depressor response	Pressor response	% Broncho-constriction	Depressor response	Pressor response
Mean	35·5	13·6	56·5	52·5	2·5	46·4	30·3	5·5	60·7
±S.E.M.	2·5	1·3	3·1	3·1	0·2	3·1	6·3	0·8	3·6
t				4·1	6·6	2·2	0·9	3·6	0·7
P<				0·001	0·001	0·05		0·01	
(*n*)		(15)			(10)			(6)	

Values presented are means ± standard error of means (S.E.M.). Values are quoted for *t* in Student's *t* test and for the probability level (*P*) where significant difference occurs between the pithed or bilaterally vagotomized group and the normal group. (*n*) Denotes numbers of animals in the group.

but did not appreciably affect the depressor or pressor responses. This was a further indication that the activity of AT in the guinea-pig involved an adrenergic neurone function that inhibited the AT-induced bronchoconstriction.

To test whether this adrenergic neurone stimulating activity was mediated by released histamine or by factors independent of histamine release, the following experiments were performed.

Four guinea-pigs were treated with mepyramine (2 mg/kg i.v.). Fifteen min later the guinea-pigs received guanethidine (4 mg/kg i.v.). Then 30 min after the guanethidine injection AT (0·5 ml/kg) was administered. The bronchoconstrictor and cardiovascular responses to the injection of AT in these animals were compared with those of a control group that received only mepyramine pretreatment. The results are given in Table 2 (ii) and show that in the mepyramine-treated guinea-pigs guanethidine did not significantly affect the bronchoconstrictor or depressor responses to AT but markedly potentiated the pressor response.

TABLE 2 (i). *The influence of guanethidine (4 mg/kg, i.v.) on the responses to anaphylatoxin (0·5 mg/kg, i.v.) in normal guinea-pigs*

	Control group (no pretreatment)			Test group (pretreated with guanethidine 4 mg/kg, i.v.)		
	% Broncho-constriction	Depressor response	Pressor response	% Broncho-constriction	Depressor response	Pressor response
Mean	35·5	13·6	56·5	60·0	11·2	66·8
±S.E.M.	2·5	1·3	3·1	5·0	0·5	2·1
t				4·4	0·9	1·8
P<				0·001		
(*n*)		(15)			(6)	

Values presented are the means ± standard error of means (S.E.M.). Values are quoted for *t* in Student's *t* test and for the probability level (*P*) where significant difference occurs between the guanethidine-treated group and the control group. (*n*) Denotes number of animals in the group.

TABLE 2 (ii). *The effect of guanethidine (4 mg/kg, i.v.) on the responses to anaphylatoxin (0·5 ml/kg, i.v. in guinea-pigs pretreated with mepyramine (2 mg/kg, i.v.)*

	Control group (pretreated with mepyramine 2 mg/kg i.v.)			Test group (pretreated with mepyramine 2 mg/kg i.v. and guanethidine 4 mg/kg i.v.)		
	% Broncho-constriction	Depressor response	Pressor response	% Broncho-constriction	Depressor response	Pressor response
Mean	11·3	12·6	17·9	14·1	12·5	41·0
±S.E.M.	0·6	1·2	1·2	1·3	0·5	4·2
t				0·7	0·3	5·3
P<						0·001
(*n*)		(6)			(4)	

Values presented are means ± standard error of means (S.E.M.). Values are given for *t* in Student's *t* test and for the probability level (*P*) where significant difference occurs between the mepyramine treated group and the group which received combined mepyramine and guanethidine pretreatment. (*n*) Denotes numbers of animals in each group.

Discussion

The results of this investigation are consistent with the view that adrenergic mechanisms are involved in AT effects in the guinea-pig, and have helped to elucidate the nature of the adrenergic mechanisms.

The interpretation of the results depends upon an understanding of the complex interaction of several different effects of AT. On the basis of previous investigations it is well established that the components to be considered are:

1. A direct smooth muscle stimulating activity contributing to the bronchoconstrictor effect (Bodammer & Vogt, 1967 and 1970 ; Sackeyfio, 1971).

2. Release of histamine (Bodammer & Vogt, 1970) accounting for the major part of the bronchoconstrictor responses and other smooth muscle stimulation, and also leading to a major influence on the cardiovascular system (Bodammer & Vogt, 1967 ; Sackeyfio, 1971).

3. Release of catecholamines resulting in profound changes in cardiovascular functions, and interacting with released histamine to produce complex effects upon smooth muscles (Bernauer *et al.*, 1970). The sources of the catecholamines and the mode of provocation of release are several, and may contribute both adrenaline and noradrenaline to interact in different ways with the other components of the AT effects. Adrenaline release may be brought about by: (*a*), direct action on the adrenal medullae ; (*b*), reflex action on the adrenal medullae ; (*c*), action of released histamine causing release from the adrenal medullae. Noradrenaline release would most probably arise from either direct or reflex action of AT or released histamine on sympathetic neurones.

In normal intact animals, it was noted that the AT effects which elicited the more severe pressor responses were associated with relatively minor bronchoconstrictor responses ; and conversely that AT induced more severe bronchoconstrictor effects when the pressor component was small. Furthermore, the reduction of the pressor component after removal of the adrenal glands was associated with marked potentiation of the bronchoconstrictor effects of AT. The pressor effect of AT is probably mediated, substantially, by adrenaline released from the adrenal medullae. The depression of the bronchoconstrictor effects of AT could thus be explained largely in terms of a bronchodilator action of the adrenaline antagonistic to the direct effects of AT and of released histamine.

Adrenalectomy did not, however, completely abolish the AT-induced pressor response, and this residual activity was thus independent of adrenal function. In pithed animals, AT-induced bronchoconstrictor responses were again potentiated while the pressor effects were significantly reduced. It is probable, therefore, that the pressor effect may be the result of activation of both the adrenal medullae and sympathetic neurones, both systems also contributing to an adrenergic bronchodilator component. Catecholamine release from the adrenal medullae and from adrenergic neurones may be due to both reflex nerve stimulation and to a direct effect of released histamine on catecholamine stores. Bilateral vagotomy had no appreciable effect upon either the pressor or bronchoconstrictor responses to AT, thus precluding the likelihood of a parasympathetic involvement in the system.

Consistent with these conclusions were the findings that the bronchoconstrictor action of AT was enhanced by the β-adrenoceptor blocking agent, propranolol. The adrenergic nature of the pressor response was confirmed by the fact that it was reduced significantly by the α-adrenoceptor blocking agent, phentolamine, which, on the other hand, did not influence the bronchoconstrictor response. The observation that phentolamine did not completely abolish the pressor response is consistent with the additional involvement of a sympathetic neuronal component,

since phentolamine is known to be more effective as an antagonist to circulating pressor catecholamines than against the responses to sympathetic nerve stimulation (Nickerson, 1959).

Guanethidine, an inhibitor of adrenergic neuronal transmission, which has no effect upon the chromaffin cells of the adrenal medulla (Hertting, Axelrod & Whitby, 1961) was shown to potentiate the bronchoconstrictor response to AT without a significant effect upon the cardiovascular responses. This also would be consistent with the involvement of a sympathetic neuronal bronchodilator component.

Pretreatment of the guinea-pigs with mepyramine resulted in a marked inhibition of both the pressor and bronchoconstrictor effects of AT. It may, therefore, be considered that both these effects are mediated, to a large extent, by released histamine. There was, however, a significant residual effect in each case which could not be eliminated even though mepyramine doses in excess of 1 mg/kg were administered (Sackeyfio, 1971). These residual bronchoconstrictor and pressor effects could be attributed to a direct action of AT or at least to a component that is independent of histamine release. It was found that guanethidine had no effect upon this residual bronchoconstrictor response but markedly potentiated the corresponding residual pressor effect. These results would suggest that the residual bronchoconstrictor response was not subject to any sympathetic neuronal restraint. It would, therefore, follow that the sympathetic neuronal bronchodilator component could be activated only as a result of the AT-induced histamine release.

In an animal not subjected to mepyramine pretreatment, it would be expected that any sympathetic neuronal contribution to the AT-induced pressor response would be inhibited by guanethidine, but no such inhibitory effect was observed. On the other hand, the residual pressor effect in mepyramine-treated animals was found to be potentiated by this drug. It may, therefore, be deduced that the lack of observable inhibitory effects of guanethidine upon the AT-induced pressor response as a whole was only apparent. This could have been due to the potentiating effect of the drug on the mepyramine-resistant component counteracting a sympathetic neuronal blocking activity of guanethidine on the mepyramine-sensitive component. The mepyramine-resistant residual pressor effect could have been due to a direct action of AT on the adrenal medulla causing catecholamine release, and the potentiation by guanethidine could have been due to its known ability to enhance the effects of circulating catecholamines (Hertting, Axelrod & Whitby, 1961). It has not yet been shown whether this component exerts any significant restraint upon the bronchoconstrictor effect, but the contribution, if any, must be small.

The initial vasodepressor response was not modified by pretreatment with either mepyramine or guanethidine, and may be attributable to a direct effect of AT (Sackeyfio, 1971). It is, therefore, unlikely that this response is involved in the proposed interactions between the adrenergic mechanisms and the histamine-mediated effects.

It may be concluded, therefore, that the AT-induced bronchoconstriction is due mainly to the release of histamine. This effect would normally be susceptible to inhibition by concomitant histamine-induced release of bronchodilator catecholamines from both the adrenal medulla and sympathetic neurones. The small bronchoconstrictor effect of AT, not attributable to histamine release, was not

subject to a sympathetic neurone-mediated restraint. These findings are generally compatible with the suggestion by Bernauer *et al.* (1970) that adrenergic mechanisms operate during AT activity. They are not, however, consistent with the suggestion by these authors that the adrenergic mechanisms preferentially inhibit those components that are not mediated by histamine release.

The authors wish to express their gratitude to Messrs. D. Greatorex, D. Goundry and A. Sykes, for valuable technical assistance. One of us (A.C.S.) is indebted to the Government of Ghana for financial support during the performance of this work.

REFERENCES

BERNAUER, W., HAHN, F., BECK, E. & KURY, H. (1970). The role of histamine and catecholamines in anaphylatoxin shock as compared to the anaphylactic shock. *Naunyn-Schmiedebergs Arch. Exp. Path. Pharmak.*, **266**, 208–222.

BODAMMER, G. & VOGT, W. (1967). Actions of anaphylatoxin on circulation and respiration of the guinea-pig. *Int. Archs Allergy*, **32**, 417–428.

BODAMMER, G. & VOGT, W. (1970). Contraction of the guinea-pig ileum induced by anaphylatoxin independent of histamine release. *Int. Archs. Allergy*, **39**, 648–657.

DIXON, W. E. & BRODIE, T. G. (1903). Contributions to the physiology of the lungs. Part 1. The bronchial muscles, their innervation and the action of drugs upon them. *J. Physiol., Lond.*, **29**, 97–173.

EULER, U. S. v. (1966). Relationship between histamine and the autonomous nervous system. In: *Handbuch der Expt. Pharmak.*, Vol. XVIII/1, 318–333.

HERTTING, G., AXELROD, J. & WHITBY, L. G. (1961). Effect of drugs on the uptake and metabolism of H^3-noradrenaline. *J. Pharmac. exp. Ther.*, **134**, 146–153.

HICKS, R. & LEACH, G. D. H. (1963). Quantitative evaluation of guinea-pig anaphylaxis *in vivo*. *Br. J. Pharmac. Chemother.*, **21**, 441–449.

HICKS, R. & SACKEYFIO, A. C. (1972). An interaction between exogenously administered anaphylatoxin and histamine in the guinea-pig. *Archs Int. Pharmacodyn. Thér.* (in press).

MCCULLOCH, M. W., PROCTOR, C. & RAND, M. J. (1967). Evidence for an adrenergic homeostatic bronchodilator reflex mechanism. *Eur. J. Pharmac.*, **2**, 214–223.

NICKERSON, M. (1959). Blockade of the action of adrenaline and noradrenaline. *Pharmac. Rev.*, **11**, 443–461.

PIPER, P. J., COLLIER, H. O. J. & VANE, J. R. (1967). Release of catecholamines in the guinea-pig by substances involved in anaphylaxis. *Nature, Lond.*, **213**, 838–840.

ROTHSCHILD, A. & ROCHA E SILVA, M. (1954). Activation of a histamine-releasing agent (anaphylatoxin) in normal rat plasma. *Br. J. Exp. Path.*, **35**, 507–518.

SACKEYFIO, A. C. (1971). Definition of the histaminic component of the bronchoconstrictor and cardiovascular effects of anaphylatoxin in the guinea-pig. *Br. J. Pharmac.*, **43**, 424–425*P*.

VANE, J. R. (1969). The release and fate of vaso-active hormones in the circulation. *Br. J. Pharmac.*, **35**, 209–242.

(*Received April* 4, 1972)