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RESPONSE OF RAT LUNG TO HUMORAL MEDIATORS OF ANAPHYLAXIS AND ITS MODIFICATION BY DRUGS AND SENSITIZATION

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1 The effects of humoral mediators of anaphylaxis on the bronchial function of the pithed rat have been assessed and compared with their effects in the guinea-pig and man.

2 5-Hydroxtryptamine, acetylcholine and bradykinin were bronchoconstrictor. Slow reacting substance in anaphylaxis and prostaglandin $F_{2\alpha}$ caused only slight constriction at high doses and histamine was not active.

3 Bradykinin was less active in the rat than in the guinea-pig and its effects were not antagonized by anti-inflammatory analgesic drugs.

4 β-Adrenoceptor blockade potentiated the activity of bradykinin but not that of 5-hydroxytryptamine or acetylcholine.

5 Dexamethasone reduced the activity of bradykinin but not that of 5-hydroxytryptamine or acetylcholine.

6 Sensitization with *Nippostrongylus brasiliensis* did not increase the sensitivity of the rat lung to the effects of mediators.

Introduction

An animal model of bronchial asthma should parallel the human reaction in as many respects as possible. The two main animal models used, bronchial anaphylaxis in the rat and the guinea-pig, have aspects which both resemble and differ from those in man.

In the rat, allergic reactions are, as in man, initiated primarily by the combination of antigen with IgE antibody (Johansson, 1967; Ogilvie, 1967; Ishizaka & Ishizaka, 1968; Stechschulte, Orange & Austen, 1970). Systemic challenge results in an anaphylactic reaction which is characterized by hypotension and death following engorgement of the heart and small intestine, although respiratory distress has been reported (Sanyal & West, 1958; Ogilvie, 1967). Measurement of bronchial function in anaesthetized rats has revealed that anaphylactic bronchoconstriction is mediated mainly by 5-hydroxytryptamine (Church, Collier & James, 1972).

In the guinea-pig, combination of antigen with IgG antibody is primarily responsible for the initiation of the allergic response (Benacerraf, 1968). The ensuing anaphylactic reaction affects primarily the respiratory tract (Herxheimer, 1952) where histamine, slow reacting substance in anaphylaxis (SRS-A) and kinins are the main mediators (Collier & James 1966, 1967).

This paper describes the response of the rat lung to some of the mediators implicated in anaphylaxis and compares these responses with those reported for guinea-pig and human lung. Modification by specific antagonists, propranolol, dexamethasone and sensitization with *Nippostrongylus brasiliensis* of the rat bronchial responses to mediators is described.

Methods

Materials

The following substances were used: acetylcholine bromide, atropine sulphate, bradykinin (Nicolaides & De Wald, 1961), dexamethasone sodium phosphate, histamine acid phosphate, 5-hydroxytryptamine creatinine sulphate, indomethacin, meclofenamate sodium, mepyramine maleate, methysergide bimaleate, propranolol hydrochloride, prostaglandin $F_{2\alpha}$ and slow reacting substance in anaphylaxis (SRS-A) (charcoal purified material prepared from the perfusate of

isolated lungs of sensitized guinea pigs as described by Berry & Collier, 1964, and tested for freedom from histamine as described by Collier & James, 1967). All doses of all compounds were calculated in terms of free base.

Sensitization of animals

All experiments were performed on male Wistar rats weighing 200-300 g. Rats were sensitized by subcutaneous injection of 5000 larvae of *Nippostrongylus brasiliensis*. They were tested for bronchial reactivity to humoral mediators 5 weeks after sensitization when anaphylactic sensitivity is maximal (Church, 1975).

Administration of substances

Dexamethasone was injected intraperitoneally 24 h before testing. Indomethacin was injected intraperitoneally 30 min before testing. Other antagonists were administered intravenously through a jugular cannula 5 min before intravenous injection of agonists.

Measurement of bronchial function

Animals were lightly anaesthetized with ether and the brain and spinal cord destroyed by pithing. The trachea was cannulated and the rat ventilated with a Starling miniature respiratory pump of stroke volume 5-7 ml at 90 strokes/minute. The side arm of the tracheal cannula was connected to a non return water valve set at a pressure of 7.5 cm of water. Tracheal flow during lung inflation was measured with a pneumotachograph and recorded on a multichannel electronic recorder. Reduction of tracheal flow, indicative of bronchoconstriction, was estimated as a percentage of the maximum possible bronchoconstriction and was measured at the time of peak effect.

Results

Histamine

Histamine injected intravenously in doses of 10 μg-1 mg caused no significant bronchospasm in groups of two to five rats (Table 1). Activity was not enhanced by pretreatment with propranolol or by sensitization with *N. brasiliensis*.

5-Hydroxytryptamine

5-Hydroxytryptamine (5-HT) given intravenously in doses of 5-20 μg to a group of five rats caused a

Table 1 The response of the rat lung to mediators of anaphylaxis

Mediator	*Dose (μg)*	*No. of animals*	*Mean reduction in tracheal flow ± s.e. (%)*
Histamine	10	2	0 ± 0
	200	2	3 ± 3
	500	5	13 ± 2
	1000	5	8 ± 3
5-HT	5	5	41 ± 7
	10	5	63 ± 5
	20	5	86 ± 3
Acetylcholine	10	5	26 ± 2
	20	5	50 ± 5
	40	5	75 ± 4
Bradykinin	25	5	14 ± 2
	50	5	27 ± 4
	100	5	51 ± 6
SRS-A	1×10^3	5	0 ± 0
	2×10^3	4	1 ± 1
	5×10^3	5	2 ± 2
	10×10^3	4	3 ± 1
	20×10^3	4	7 ± 2
Prostaglandin $F_{2\alpha}$	50	5	8 ± 3
Saline	1 ml	6	0 ± 0

Rats were pithed and prepared for recording of tracheal flow. Responses were measured at the time of maximal effect after intravenous administration of mediator.

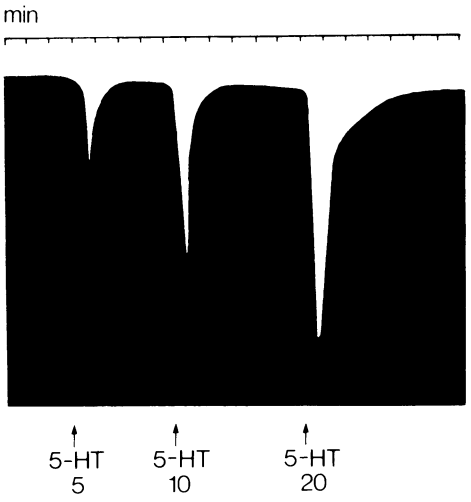

Figure 1 The effect of 5-hyrdoxytryptamine (5-HT) on the bronchial function of the rat. 5-HT, 5, 10 or 20 μg was injected intravenously into a pithed rat.

rapid but short lasting bronchoconstriction (Figure 1). This bronchospasm was linearly related to dose (Table 1). The dose of 5-HT calculated to produce a 50% constrictor response was 6.4 μg.

Methysergide, 0.1 mg/kg injected intravenously 5 min before 10 μg of 5-HT, caused a 93% reduction of response (Table 2). Atropine, 1 mg/kg, inhibited the response by 67%. Mepyramine, 2 mg/kg, and meclofenamate, 1 mg/kg, did not modify the response, The β-adrenoceptor blocking agent propranolol, 5 mg/kg, caused a small, but not significant, decrease in bronchoconstriction. Dexamethasone, 5 mg/kg injected intraperitoneally into a group of ten rats showed no effect in comparison with ten untreated animals. Sensitization 5 weeks previously with *N. brasiliensis* did not modify the response to 5-HT in a group of ten rats compared with ten unsensitized controls.

Acetylcholine

Acetylcholine injected intravenously in doses of 10-40 μg caused a marked but transient bronchospasm in a group of five rats. The response was linearly related to dose (Table 1). The dose calculated to produce a 50% constriction was 19.9 μg. Acetylcholine is, therefore, three times less spasmogenic in rat lung than is 5-HT.

Atropine, 1 mg/kg, abolished the bronchoconstrictor response to 20 μg of acetylcholine (Table 2). Methysergide, 0.1 mg/kg and mepyramine, 2 mg/kg, did not modify the bronchoconstriction. Pretreatment with propranolol, 5 mg/kg, did not potentiate the response. Dexamethasone, 5 mg/kg, decreased the response to acetylcholine by 24% in a group of ten rats but this was not significantly different from untreated controls. Sensitization

Table 2 The modification by drugs and sensitization of 5-hydroxytryptamine, acetylcholine and bradykinin-induced bronchospasm in the rat

		Bronchoconstriction (%)					
	Dose	*5-HT*		*Acetylcholine*		*Bradykinin*	
Treatment	*(mg/kg)*	*Control*	*Treated*	*Control*	*Treated*	*Control*	*Treated*
Methysergide	0.1	58	4*	43	47	74	74
Atropine	1	67	22*	57	0*	–	–
Mepyramine	2	60	57	63	64	38	35
Meclofenamate	1	54	53	–	–	30	30
Propranolol	5	59	48	59	63	46	61*
Dexamethasone	5	63	60	50	38	51	28*
Sensitization	–	63	59	50	62	51	51

The results were obtained in groups of 3-10 rats pithed and prepared for recording of tracheal flow. Methysergide, atropine, mepyramine, meclofenamate and propranolol were given intravenously 5 min before constrictor agent. Dexamethasone was given intraperitoneally 24 h before test. Sensitization was with 5,000 *N. brasiliensis* larvae injected subcutaneously five weeks before test. 5-HT, 10 μg, acetylcholine, 20 μg, and bradykinin, 100 μg, were given intravenously. The bronchoconstriction was measured at the time of maximum effect. The levels of bronchoconstriction in bradykinin treated animals have been corrected for tachyphylaxis as described in the text.

* Significantly ($P < 0.001$) different from control. – Not tested.

with *N. brasiliensis* increased bronchospasm by 24% in a group of five rats. This increase was not statistically significant.

Bradykinin

Bradykinin injected intravenously in doses of 25-100 μg caused a more slowly developing bronchoconstriction in a group of five rats (Figure 2). The bronchospasm was linearly related to dose (Table 1). The dose calculated to produce a 25% response was 41 μg.

Two characteristics of the rat bronchoconstrictor response to bradykinin were observed. Firstly, tachyphylaxis to bradykinin developed with repeated doses (Figure 2). Secondly, after a constrictor response to bradykinin the lung did not reinflate to its original volume. Reinflation of the lung at a higher pressure restored the tracheal flow to near its original value but did not alter the tachyphylaxis (Figure 2, Table 3). When assessing the effects of drugs on the bradykinin response allowance for the developing tachyphylaxis was made. In these experiments each animal received five doses of bradykinin. The mean of the responses to the second and third doses was taken as the predosed level of bronchoconstriction and the mean of the responses to the fourth and fifth doses as the bronchoconstriction following treatment. Drug or saline (0.9% w/v NaCl solution) was given between the third and fourth doses. A correction factor, calculated in control animals (Table 3), was then applied to drug-treated animals to compensate for tachyphylaxis.

Methysergide, 0.1 mg/kg, mepyramine, 2 mg/kg, and meclofenamate, 1 mg/kg, did not inhibit the bronchoconstrictor response induced by 100 μg of bradykinin (Table 2). Propranolol, 5 mg/kg, potentiated the response by 33%. This increase was statistically significant ($P < 0.001$).

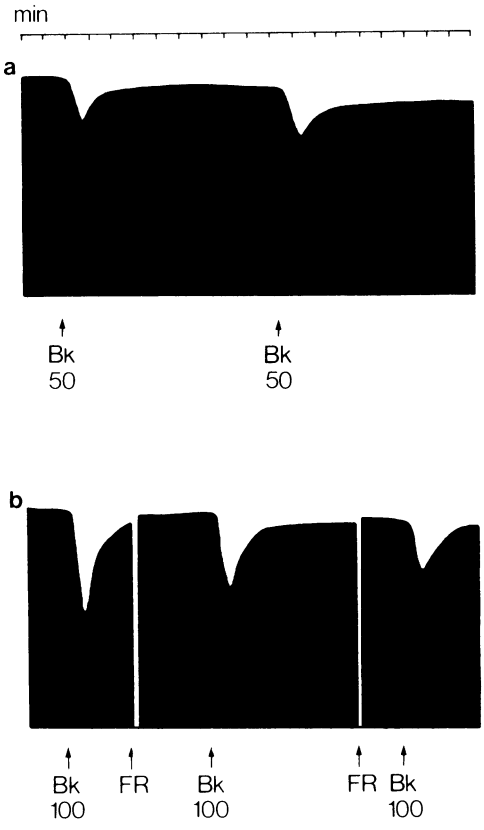

Figure 2 The effect of repeated doses of bradykinin on the bronchial function of the rat. (a) The effect of repeated injections of bradykinin (Bk) 50 μg, given intravenously to a pithed rat. (b) The effect of repeated injections of bradykinin, 100 μg, given intravenously to a pithed rat in which the lungs were forcibly reinflated (FR) between injections.

Table 3 Tachyphylaxis of rat bronchoconstriction to bradykinin

	Dose No.				
	1	*2*	*3*	*4*	*5*
Decrease in tracheal flow (%)	50	52	40	32	31
Mean			46		31
Correction factor for tachyphylaxis			$^{46}/_{31} = 1.48$		

Rats were pithed and prepared for recording of tracheal flow. Responses to a constant dose of 100 μg bradykinin given every 4 min, were measured at the time of maximum effect. Each response is the mean of those obtained in four animals.

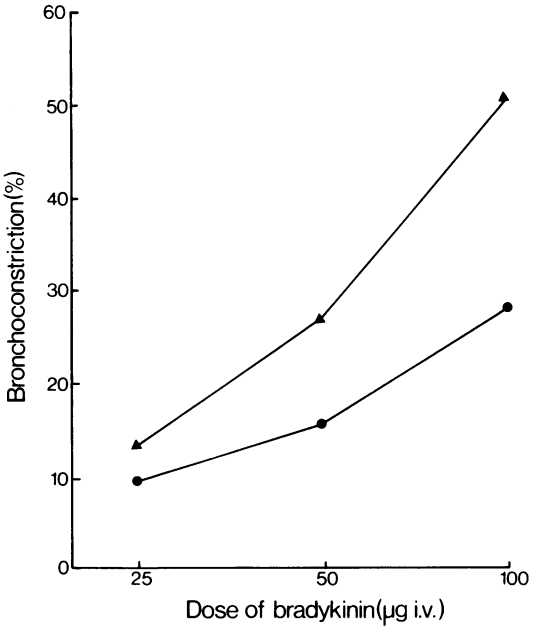

Figure 3 The effect of dexamethasone on bradykinin-induced bronchoconstriction in the rat. Dexamethasone, 5 mg/kg, (●) or saline, control (▲) was injected intraperitoneally 24 h before the rats were pithed and prepared for recording of tracheal flow. Each point is the mean of results obtained in ten rats.

Dexamethasone, 5 mg/kg, injected intraperitoneally into a group of ten rats 24 h previously inhibited the spasmogenic effects of bradykinin compared with undosed controls. Dexamethasone was more active against higher doses of bradykinin than against smaller doses (Figure 3). Sensitization with *N. brasiliensis* 5 weeks previously did not modify bradykinin-induced bronchoconstriction.

Slow reacting substance in anaphylaxis (SRS-A)

SRS-A injected intravenously in doses of 1-20 mg produced little effect on the rat lung. Only the highest dose used, 20 mg, produced a significant ($P < 0.05$) bronchospasm (Table 1). The response to SRS-A reached a maximum intensity 30 s after injection and was over by 1.5 minutes.

Propranolol, 5 mg/kg, increased the bronchospasm induced by 20 mg of SRS-A from 7% to 15%. Propranolol also extended the time course of the reaction, the maximum intensity occurring 1-2 min after dosage and being over by 4-5 minutes. Meclofenamate, 1 mg/kg, administered to three propranolol pretreated rats, abolished the SRS-A response in one animal but only slightly reduced the response in the other two rats. Because of the weak activity of SRS-A, the validity of the results obtained with meclofenamate may be questioned. No further experiments with antagonists were performed due to the relative ineffectiveness of SRS-A in this preparation.

Prostaglandin $F_{2\alpha}$

In an experiment using five rats, intravenous injection of 50 μg of prostaglandin $F_{2\alpha}$ produced only a weak but significant ($P < 0.05$) bronchoconstriction (Table 1). Because of the lack of efficacy of prostaglandin $F_{2\alpha}$ no experiments with antagonists were performed.

Discussion

Comparison of the activity of mediators of anaphylaxis on rat, guinea-pig and human bronchial function shows that the rat responds in a manner both qualitatively and quantitatively differently from the other two species.

The major qualitative difference is in the reactivity to histamine. In man and the guinea-pig, histamine is a potent bronchoconstrictor substance (Curry, 1946; Herxheimer, 1951; Berry & Collier, 1964). In the rat, however, histamine in doses up to 1 mg intravenously failed to cause bronchospasm. Similar findings have been reported *in vivo* by Bhoola, Collier, Schachter & Shorley (1962) and *in vitro* by Brocklehurst (1958) although Foggie (1937) found weak constriction in isolated perfused lung with large doses of histamine. Lack of reactivity of the bronchial tree to histamine has also been observed in the cat (Maengwyn-Davies, 1968) and the sheep (Eyre, 1969).

In the rat, 5-HT was the most potent bronchoconstrictor substance examined, giving an ED_{50} of 6.4 μg. 5-HT is also spasmogenic in the guinea-pig where the bronchial tree appears to be more sensitive than that of the rat (Berry & Collier, 1964). In man, 5-HT, injected intravenously or given by aerosol, causes no bronchospasm in normal subjects (Brocklehurst, 1958) and only slight bronchospasm in asthmatics (Michelson, Holander & Lowell 1958).

The spasmogenic activity of 5-HT in the rat was antagonized by methysergide. In acute anaphylaxis, methysergide antagonizes bronchoconstriction by nearly 70% (Church *et al.*, 1972). The sensitivity of the rat lung to 5-HT and the blockade of 5-HT and antigen-induced bronchospasm by methysergide implicates this amine as

the major mediator of rat anaphylactic bronchoconstriction.

The partial inhibition of 5-HT-induced bronchospasm by atropine is not seen in the guinea-pig (Holgate & Warner, 1960) and was not expected in the rat. Two possible explanations for this may be put forward. Firstly, that the inhibition by atropine was due to a non-specific antagonism of amines (Vane, 1957). Secondly, that the rat lung contains neurotropic or M receptors for 5-HT, similar to those found in guinea-pig ileum and calf tracheal muscle, which excite cholinergic neurones rather than exert a direct effect on smooth muscle (Gaddum & Picarelli, 1957; Offermeier & Ariens 1966).

Acetylcholine causes contraction of bronchial muscle in most species including guinea-pig, rat and man (Brocklehurst, 1958; Bhoola *et al.*, 1962). Its ED_{50} in the rat was 20 μg intravenously which shows that the guinea-pig is more sensitive than the rat to acetylcholine. Although atropine blocks acetylcholine-induced bronchospasm in the rat, its failure to block anaphylactic bronchoconstriction (Church *et al.*, 1972) suggests that acetylcholine is not a primary mediator of allergic bronchospasm in this species. Similarly, atropine does not block anaphylactic bronchoconstriction in the guinea-pig (Collier & James, 1967) and is relatively ineffective in relieving the symptoms of asthma (Altounyan, 1969).

The sensitivity of rat lung to bradykinin was lower than that of guinea-pig and man. In the rat the ED_{25} of bradykinin was 41 μg whilst in the guinea-pig severe bronchospasm may be induced with 0.25-4 μg of bradykinin (Bhoola *et al.*, 1962; Collier, James & Piper, 1968). Bradykinin-induced bronchoconstriction in the rat is similar to that of the guinea-pig (Collier, Holgate, Schachter & Shorley, 1960) in that it is slower than the response to 5-HT. Recovery, however, is faster in the rat. A similar action in both species is suggested by the finding that the lung does not readily return to its predosed state and that tachyphylaxis develops with repeated dosing.

In the guinea-pig, but not in the rat bradykinin-induced bronchospasm may be blocked by meclofenamate and indomethacin (Collier, *et al.*, 1968). Piper & Vane (1969) and Palmer, Piper & Vane (1973) postulate that these compounds act in the guinea-pig by inhibiting the release of rabbit aorta contracting substance (RCS) by bradykinin. The failure of meclofenamate and indomethacin to block bradykinin-induced bronchospasm in the rat and the finding of Piper (personal communication) that histamine-like, SRS-A-like and prostaglandin-like, but no RCS-like activity, could be demonstrated in the perfusate from rat lung during anaphylaxis, suggest that RCS may not be involved in anaphylactic bronchoconstriction in the rat. In man, bradykinin aerosol induces a mild bronchospasm only in asthmatic patients, which is not inhibited by anti-inflammatory analgesic compounds (Streseman, 1963). In these respects man resembles the rat more closely than the guinea-pig.

SRS-A and prostaglandin $F_{2\alpha}$ cause bronchoconstriction in the guinea-pig and man (Herxheimer & Stresemann, 1963; Berry & Collier, 1964; Sweatman & Collier, 1968; James. 1969; Mathé, Hedqvist, Holmgren & Svanborg, 1973). In the rat, guinea-pig SRS-A, pharmacologically indistinguishable from rat SRS-A (Orange, Valentine & Austen, 1968), and prostaglandin $F_{2\alpha}$ both caused only slight constriction at unphysiologically high doses. Thus, the rat appears to differ from the guinea-pig and man in its sensitivity to these mediators.

Mediators of anaphylaxis release sympathomimetic amines (Feldberg & Lewis, 1964; Staszewska-Barczak & Vane, 1965. 1967; Piper, Collier & Vane, 1967) and blockade of β-adrenoceptors potentiated anaphylaxis and the effects of mediators, especially bradykinin (Collier, James & Piper, 1965; Collier & James, 1967). β-blockade also exacerbates human asthma (McNeill, 1964). Responses of the rat lung to bradykinin and SRS-A were potentiated by β-blockade less than were those of the guinea-pig (Collier *et al.*, 1965) whilst responses to 5-HT, acetylcholine and antigen (Church *et al.*, 1965) were not potentiated. This suggests that sympathomimetic amines are less effective on rat lung than on guinea-pig lung.

Although guinea-pig anaphylaxis is not inhibited by corticosteroids (Hicks, 1969), the activity of these drugs against rat anaphylactic bronchoconstriction (Church & James 1969, Church *et al.*, 1972) accords with their efficacy against human bronchial asthma. Rat bronchospasm induced by 5-HT or acetylcholine was not inhibited by dexamethasone, although Blythe (personal communication) found inhibition of the 5-HT response 24 h after intraperitoneal administration of 5 mg/kg of betamethasone. The inhibition of bradykinin bronchospasm by dexamethasone may be due to either a direct modulation of the systemic actions of kinins (Suddick 1966) or a potentiation by steroid of the actions of catecholamines (Logsdon, Middleton & Coffey, 1972) released by bradykinin. From this evidence it would seem unlikely that the anti-anaphylactic action of dexamethasone is due to its inhibition of the activity of mediators.

Human asthmatics are more sensitive to inhaled histmaine and prostaglandin $F_{2\alpha}$ than are

non-asthmatics (Curry, 1946; Mathé *et al.*, 1973). In rats, sensitization increases systemic toxicity to histamine (Keller & Beeger, 1971). In my experiments, however, sensitization did not increase the sensitivity of rat lung to 5-HT, acetylcholine, histamine or bradykinin.

Rat anaphylactic bronchoconstriction resembles human asthma more closely than does guinea-pig anaphylaxis in its immunological triggering and its responsiveness to drugs that alleviate human asthma, namely disodium cromoglycate and corticosteroids (Church *et al.*, 1972). However, the qualitative differences in the responses to histamine and 5-HT and the quantitative differences in the responses to bradykinin, SRS-A and prostaglandin $F_{2\alpha}$ indicate that the reactivity of rat lung to mediators of anaphylaxis does not resemble that of human lung as closely as does that of the guinea-pig.

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