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Antagonism by fenamates and like-acting drugs of bronchoconstriction induced by bradykinin or antigen in the guinea-pig

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1. Nine non-steroidal anti-inflammatory drugs were tested for antagonism of bradykinin-induced bronchoconstriction in guinea-pig lungs *in vivo*. Only one, benzydamine, was inactive.

2. The descending order of potency of the active anti-inflammatory drugs was meclofenamate = Scha 306 > Scha 87/2 > indoxole > Mi85 > indomethacin > glafenine > ibufenac.

3. Of eight other drugs tested, chlorpromazine, phenoxybenzamine and four others were inactive, whereas phenelzine and mebanazine possessed activity.

4. In tests at two dose-levels of meclofenamate, the dose-ratio of bradykinin increased proportionately with the dose of meclofenamate.

5. The anti-bradykinin effect of meclofenamate was still observed after destruction of the brain and spinal cord, after bilateral adrenalectomy or after blockade of β-receptors for adrenaline.

6. Meclofenamate did not release catecholamines from the adrenal medulla or prevent such a release by bradykinin given intra-arterially.

7. The fenamates and like-acting drugs reduced the intensity of anaphylactic bronchoconstriction in guinea-pigs treated with mepyramine or propranolol. The descending order of potency was meclofenamate > flufenamate > mefenamate.

8. Dose/response curves for the antagonism of anaphylactic bronchoconstriction by the fenamates, in the presence of propranolol, turned downwards at high doses.

9. These findings suggest that the fenamates may find a useful place in the treatment of bronchial asthma or other conditions involving allergic ventilatory impairment.

Experiments reported some years ago suggested that drugs with antipyretic, analgesic and anti-inflammatory properties, of the general group to which aspirin, amidopyrine and phenylbutazone belong, characteristically antagonize bronchoconstriction induced by bradykinin but not that induced by acetylcholine or histamine in the guinea-pig (Collier & Shorley, 1960). Since then, the development of new drugs of this type has given further opportunities of testing this rule. That

some of these new drugs, such as fenamates, indomethacin and ibufenac, also possess this type of antibradykinin activity has already been reported (Collier & Shorley, 1963; Berry & Collier, 1964; Collier & James, 1967; Simke, Graeme & Sigg, 1967). We describe tests of the antagonism by still newer non-steroidal anti-inflammatory agents of bradykinin-induced bronchoconstriction. Among these agents meclofenamate showed outstandingly high potency, in keeping with its performance in anti-inflammatory tests (Winder, Wax & Welford, 1965). We also describe fuller tests of some drugs of other types, previously reported to have antibradykinin activity in guinea-pig lungs (Collier, James & Piper, 1965; Simke *et al.*, 1967).

Because the possibility has been raised that non-steroidal anti-inflammatory drugs might antagonize bradykinin through an effect on the central nervous system (Aarsen, 1966), we tested the ability of meclofenamate to antagonize bradykinin-induced bronchoconstriction in guinea-pigs with brain and spinal cord destroyed. In view of the other possibility that some drugs might act by releasing catecholamines, we also tested the efficacy of meclofenamate after adrenalectomy and after blockade of β-receptors for adrenaline.

That meclofenamate inhibits a part of anaphylactic bronchoconstriction in the guinea-pig has already been reported (Collier & James, 1967). In experiments described later, we determined the anti-anaphylactic potency of fenamates and of other non-steroidal anti-inflammatory drugs. β-Receptor blockade intensifies anaphylactic bronchoconstriction (Collier & James, 1967), so we also determined the potency of fenamates and other non-steroidal anti-inflammatory drugs during blockade of β-receptors for adrenaline. As release of catecholamines may well play a part in moderating human bronchial asthma (NcNeill, 1964; Zaid & Beall, 1966), we also tested whether fenamates blocked catecholamine release induced by bradykinin. These studies were designed to explore the best conditions for clinical trial of fenamates against bronchial asthma in man.

Methods

Materials

The following drugs were used: acetylcholine bromide, amidopyrine, γ-aminobutyric acid, aspirin and its calcium and sodium salts, benzydamine [1-benzyl-3-(3-dimethylaminopropoxy) (1H) indazole] hydrochloride (Silvestrini, Garau, Pozatti & Cioli, 1966), bradykinin (Nicolaides & De Wald, 1961), sodium 2-butyl-1H-pyrazolo [1,2-a] cinnoline-1,3 (2H)-dione (Scha 87/2; Wagner-Jauregg, Schatz & Jahn, 1965), chlorpromazine hydrochloride, 2,4-dinitrophenol, flufenamate sodium [sodium N-(α,α,α-trifluoro-*m*-tolyl)-anthranilate], glafenine [2,3-dihydroxypropyl N-(7-chloro-4-quinolyl) anthranilate] (Peterfalvi, Branceni, Azadian-Boulanger, Chifflot & Jequier, 1966), ibufenac [*p*-isobutylphenylacetic acid], indomethacin, indoxole (Glenn, Bowman, Kooyers, Koslowske & Myers, 1967), iproniazid phosphate, mebanazine oxalate, meclofenamate sodium [sodium N-(2,6-dichloro-*m*-tolyl) anthranilate], mefenamate sodium [sodium N-2,3-xylylanthranilate], mepyramine maleate, sodium 5-(dimethyl-amino)-9-methyl-1H-pyrazolo [1,2-a] [1,2,4] benzotriazine-1, 3 (2H)-dione (Mi 85; Molnar, Wagner-Jauregg, Jahn & Mixich, 1964), ovalbumen (B.D.H.), oxalate sodium, sodium 2-pentyl-1H-pyrazolo [1,2-a] cinnoline-1,3 (2H)-dione (Scha 306, Wagner-Jauregg *et al.*, 1965), phenazone, phenelzine sulphate, phenoxybenzamine hydrochloride, phenylbutazone sodium, propranolol

hydrochloride, slow-reacting substance in anaphylaxis (SRS-A ; Berry & Collier, 1964), tranylcypromine sulphate, zinc ovalbumen complex (Collier & James, 1967). Weights of salts are expressed as active acid or base.

Tests against bradykinin-induced bronchoconstriction

To test antagonism of bronchoconstriction induced by bradykinin or SRS-A, guinea-pigs were anaesthetized with urethane (1.25–3.0 g/kg intraperitoneally) or with pentobarbitone sodium (60–80 mg/kg intraperitoneally) and prepared by the method of Konzett & Rössler (1940) as modified by Collier, Holgate, Schachter & Shorley (1960) for recording air overflow volume. Drugs were sometimes tested by the procedure of Collier & Shorley (1960), in which a single dose of test drug was given to each animal and a dose of bradykinin was given before and after test drug, the dose after being double that before. Acetylcholine or histamine was used as a reference substance, not antagonized by aspirin or like-acting drugs. Drugs were also tested by a modification of this procedure, in which up to two intravenous doses of drug were tested in the same guinea-pig, the dose of test drug being increased in each animal by a factor of four between each test ; but the dose of bradykinin remained unchanged after test drug. In both procedures doses were given at 5 min intervals and a positive effect was taken to be a reduction of $>50\%$ of the increased volume of air overflow induced by bradykinin. The results obtained at each dose level were pooled, the minimal effective dose (MED) being taken as the least dose giving a positive effect in the majority of tests (see Table 2). In some tests SRS-A was used as challenge substance, instead of bradykinin. Drugs were administered intravenously, except propranolol (10 mg/kg), which was given subcutaneously 20 min before the first challenge.

The dose-ratio of bradykinin was determined, in guinea-pigs anaesthetized with urethane and pretreated with propranolol (which stabilized the response to bradykinin), by giving two intravenous doses of bradykinin, 0.125 and 1,25 μg/kg, respectively 20 min and 10 min before a single intravenous dose of meclofenamate. Thirty seconds after meclofenamate a response was obtained to a further intravenous dose of bradykinin. The dose-ratio was derived by comparing the response to bradykinin after meclofenamate with the dose-response line to bradykinin before meclofenamate.

In some experiments the brain and spinal cord were destroyed by pithing, 30 min before the first challenge. Bilateral adrenalectomy was performed after midline incision of the abdominal wall and ligation of the blood supply to the adrenal glands, 30 min before the first challenge.

Anti-anaphylactic tests

Six-to-eight-week-old guinea-pigs were sensitized with ovalbumen and, 2 weeks later, treated with zinc ovalbumen complex, as described by Collier & James (1967). Five to eight days after injection of zinc ovalbumen, animals were anaesthetized and prepared, as above, for recording air overflow volume, except that in these experiments the lungs were forcibly re-inflated for 10 sec in each 30 sec at a pressure of 20 mm Hg, controlled by a water valve in the recording circuit, as described by Collier & James (1967). Test drugs were given by stomach tube 30 min before challenge. Mepyramine (2 mg/kg) was given by intrajugular cannula 5 min before

challenge with ovalbumen, 10 mg/kg intravenously. Propranolol was given intraperitoneally (10 mg/kg) 30 min and intravenously (5 mg/kg) 5 min before challenge with ovalbumen, 0.75 mg/kg intravenously. Tracings were measured with the D-Mac Pencil Follower and statistically analysed as previously described (Christianson, Dinneen, James & Perkins, 1967). To test the significance of drug effect at a given time, the percentage responses were subjected to an analysis of variance (Davies, 1954; Collier & James, 1967).

Tests on catecholamine release

To test whether a drug antagonized catecholamine release induced by bradykinin, or itself released catecholamines, guinea-pigs were anaesthetized with pentobarbitone sodium, 60–80 mg/kg intraperitoneally, and prepared for the blood-bathed organ technique as previously described (Piper, Collier & Vane, 1967). Catecholamines released into the circulation were detected and assayed by means of a combination of rat isolated stomach strip and chick isolated rectum. Bradykinin was administered through a catheter passed down the left carotid artery until its tip was in the arch of the aorta. By this route and in the doses used, bradykinin did not cause bronchoconstriction. Test drugs were given intravenously.

Results

Antagonism of bradykinin-induced bronchoconstriction

Seventeen drugs, nine of which had been developed as anti-inflammatory agents, were tested for ability to antagonize bradykinin-induced bronchoconstriction by the second procedure described above, in which two doses of test drug were given to each animal. Table 1 summarizes these tests. Of the nine anti-inflammatory drugs, only benzydamine failed to show activity. The most potent compounds were meclofenamate, Scha 87/2 and Scha 306.

TABLE 1. *Minimal effective doses of some new anti-inflammatory and other drugs against bradykinin-induced bronchoconstriction in the guinea-pig*

Drug		Total No. of tests	MED (mg/kg i.v.)
Class	Individual		
Anti-inflammatory	Benzydamine	22	>32
	Glafenine	25	4
	Ibufenac	26	8
	Indomethacin	29	2
	Indoxole	10	0·25
	Meclofenamate	21	0·06
	Mi 85	19	1
	Scha 87/2	20	0·125
	Scha 306	23	0·06
Other	γ-Aminobutyric acid	15	>64
	Chlorpromazine	18	>4
	2,4-Dinitrophenol	10	>8
	Iproniazid	23	>32
	Mebanazine	37	16
	Phenelzine	28	8
	Phenoxybenzamine	15	>32
	Tranylcypromine	17	>16

An effective dose is the intravenous dose of an antagonist that reduces the bronchoconstrictor effect of bradykinin given after the antagonist to less than half that of the same dose of bradykinin given before, without reducing the response to acetylcholine. A minimal effective dose (MED) is the least dose effective in a majority of tests. Up to two doses of drug were tested in each animal, the dose being increased by a factor of four between each test.

Of the other drugs in Table 1, mebanazine and phenelzine were active. Because the effectiveness of these drugs might have been attributed to potentiation of endogenous catecholamines released by bradykinin (Piper *et al.*, 1967), we tested phenelzine at its MED (8 mg/kg) in the presence of propranolol. In this situation phenelzine was active in five of nine guinea-pigs, compared with six of seven without propranolol. As mebanazine was given as the oxalate salt, we tested whether sodium oxalate antagonized bradykinin-induced bronchoconstriction. In intravenous doses up to 64 mg/kg, which was lethal, sodium oxalate did not antagonize bradykinin.

When the antibradykinin potency of meclofenamate was determined by the earlier procedure, as described by Collier & Shorley (1960), the MED value obtained in 22 animals was 0.125 mg/kg intravenously. Table 2 summarizes the tests used to determine the MED value of meclofenamate by each procedure and shows the dose/response relationships obtained. In ten animals, when SRS-A was used as challenge substance, the MED value of meclofenamate was the same as that in a corresponding test against bradykinin.

The dose-ratio of bradykinin was determined at two intravenous doses of meclofenamate, each in nine guinea-pigs. The mean dose-ratios (with 95% fiducial limits) were: meclofenamate at 0.125 mg/kg, 13.3 (10.3–16.4); meclofenamate at 1.0 mg/kg, 129 (106–153). Thus, an eightfold increase in the dose of meclofenamate required an approximately tenfold increase in the dose of bradykinin to restore the response to its original level.

In three Konzett-Rössler preparations, in which the brain and spinal cord had been destroyed, meclofenamate (1 mg/kg intravenously) completely suppressed the response to five times the initial challenge dose of bradykinin, without reducing responses to histamine or acetylcholine (Fig. 1). In three adrenalectomized preparations, the same dose of meclofenamate also suppressed the response to bradykinin, but not that to histamine or acetylcholine (Fig. 1). When bradykinin was given after meclofenamate in an adrenalectomized guinea-pig a slight bronchodilatation was seen (Fig. 1). In six preparations pretreated with propranolol, the same dose of meclofenamate was also effective against bradykinin, but not against histamine or acetylcholine (Fig. 2).

Reduction of anaphylactic bronchoconstriction

Figure 3 gives the mean time/response curves of groups of twelve guinea-pigs, challenged intravenously with ovalbumen (10 mg/kg) after pretreatment with

TABLE 2. *Data giving the MED of meclofenamate against bradykinin-induced bronchoconstriction in the guinea-pig, using the present two-dose procedure and the earlier one-dose procedure*

Procedure	Drug	No. of animals	No. of tests	Proportion of tests positive at doses of drug (mg/kg) 0·03	0·06	0·125	0·25	0·5	1·0	2·0	4·0	8·0	MED (mg/kg i.v.)
Two-dose	Meclofenamate	11	21	0/3	3/5	6/6	5/5	2/2	—	—	—	—	0·06
One-dose	Meclofenamate	22	22	0/2	0/4	3/5	4/5	3/3	1/1	1/1	1/1	—	0·125
	Aspirin	42	42	—	—	0/1	—	0/4	5/16	13/14	5/5	2/2	2·0

For details of the two-dose procedure, see Table 1. In the one-dose procedure (Collier & Shorley, 1960), an effective dose of test drug is that which reduces the response to a dose of bradykinin that is twice the dose before test drug to less than half the response before test drug, without affecting the response to acetylcholine or histamine. The MED is the lowest dose effective in a majority of tests. All substances were administered intravenously at 5 min intervals; all doses are in mg/kg; —, not tested. Aspirin was given as the calcium or sodium salt.

FIG. 1. Antagonism by meclofenamate of bradykinin in the Konzett-Rössler preparation of guinea-pig lungs *in vivo*, after destruction of the central nervous system or after bilateral adrenalectomy. H, Histamine; B, bradykinin; A, acetylcholine; doses of each being in μg intravenously. M, 1 mg/kg intravenously of sodium meclofenamate. Time, 30 sec. Max, maximal air overflow volume in ml., obtained by clamping trachea. Doses were given at 5 min intervals, except that meclofenamate was given 30 sec before histamine. Left-hand tracing, guinea-pig (470 g) anaesthetized with urethane, 1.5 g/kg intraperitoneally. Brain and spinal cord destroyed 30 min before first challenge. Right-hand tracing, guinea-pig (410 g) anaesthetized with pentobarbitone sodium, 60 mg/kg intraperitoneally. Bilateral adrenalectomy 30 min before first challenge.

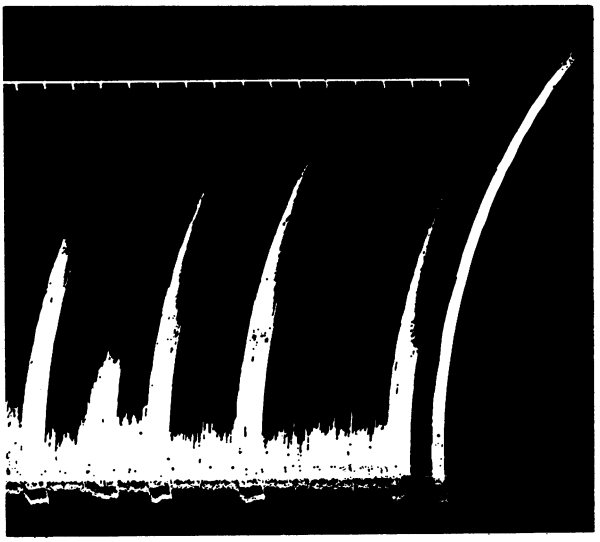

FIG. 2. Antagonism by meclofenamate of bradykinin in the Konzett–Rössler preparation of guinea-pig lungs *in vivo* during blockade of β-receptors for adrenaline. Guinea-pig (580 g) anaesthetized with urethane, 1.7 g/kg intraperitoneally. H, histamine; B, bradykinin; A, acetylcholine; doses of each being in μg intravenously. M, meclofenamate sodium, 1 mg/kg intravenously. Propranolol (10 mg/kg) was given subcutaneously 25 min before the first dose of histamine. Doses were given at 5 min intervals, except that meclofenamate was given 30 sec before the second dose of histamine. Time scale, 30 sec. Max, maximal air overflow volume in ml., obtained by clamping the trachea.

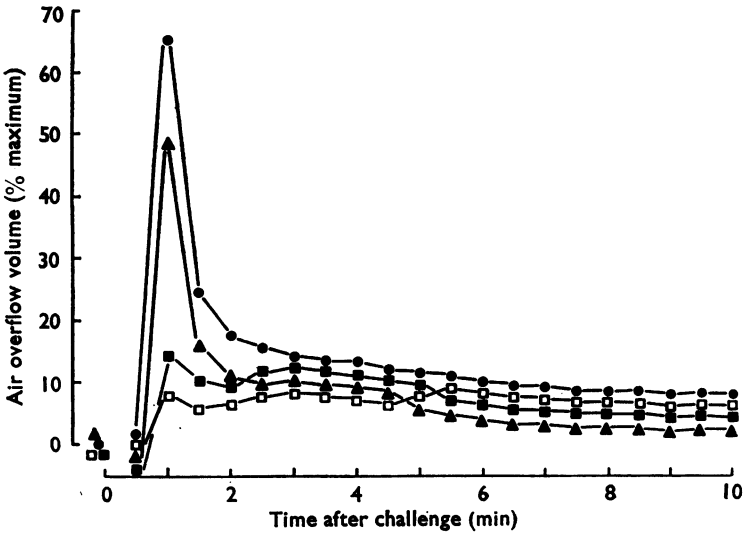

FIG. 3. Time/response curves of meclofenamate against anaphylactic bronchoconstriction in the guinea-pig during blockade of histamine receptors. Each curve is the mean of results in twelve guinea-pigs, anaesthetized with urethane (1.25–3.0 g/kg intraperitoneally) and prepared for recording air overflow volume by the method of Konzett & Rössler (1940). Mepyramine (2 mg/kg) was given intravenously 5 min before intravenous challenge with ovalbumen (10 mg/kg). ●——●, 0.9% w/v NaCl in water; ▲——▲, 0.25 mg/kg meclofenamate; ■——■, 1.0 mg/kg meclofenamate; □——□, 4.0 mg/kg meclofenamate. Meclofenamate was given as the sodium salt by stomach tube 30 min before challenge with antigen.

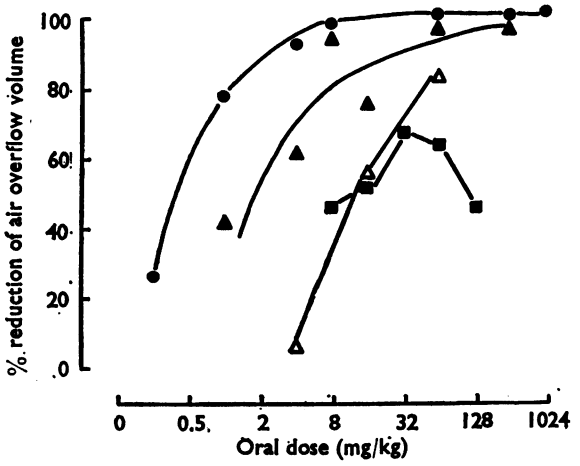

FIG. 4. Dose/response curves of the fenamates and aspirin against anaphylactic bronchoconstriction in the guinea-pig during blockade of histamine receptors. Pretreatment and preparation of animals as in Fig. 3. Each point is the mean of results in twelve animals. The effect of the test drug is determined 1 min after intravenous challenge with ovalbumen (10 mg/kg). Test drugs were given as the sodium salt by stomach tube 30 min before challenge with ovalbumen. ●——●, Meclofenamate; ▲——▲, flufenamate; △——△, aspirin; ■——■, mefenamate.

mepyramine and with various oral doses of meclofenamate. After mepyramine pretreatment, anaphylactic bronchoconstriction reached a sharp peak 1 min after challenge, at which time the effect of test drugs was assessed. Meclofenamate (4 mg/kg) greatly reduced, but did not abolish, this peak. Smaller doses reduced the peak proportionately (Fig. 3). The dose/response relationship obtained in this experiment and similar relationships obtained in experiments with other fenamates and aspirin are plotted in Fig. 4.

Figure 5 gives the mean time/response curves of groups of twelve guinea-pigs, challenged intravenously with ovalbumen (0.75 mg/kg) after pretreatment with propranolol and with various oral doses of meclofenamate. After propranolol pretreatment, anaphylactic bronchoconstriction reached a plateau a few minutes after challenge, and the effect of test drugs was therefore assessed over the period from 0–10 min after challenge. Meclofenamate at 16 mg/kg was the only dose that significantly reduced the response to antigen. Usually, after propranolol pretreatment, the dose/response curves turned downwards at high doses and only one dose of test drug on a geometric scale was significantly effective.

Table 3 gives the minimal dose of fenamates or other like-acting drugs that significantly ($P<0.05$) reduced anaphylactic bronchoconstriction after pretreatment with either mepyramine or propranolol. The orders of potency were: after mepyramine, meclofenamate>flufenamate>mefenamate>aspirin; after propranolol, meclofenamate>amidopyrine>flufenamate = phenazone = phenylbutazone>aspirin = indomethacin>mefenamate.

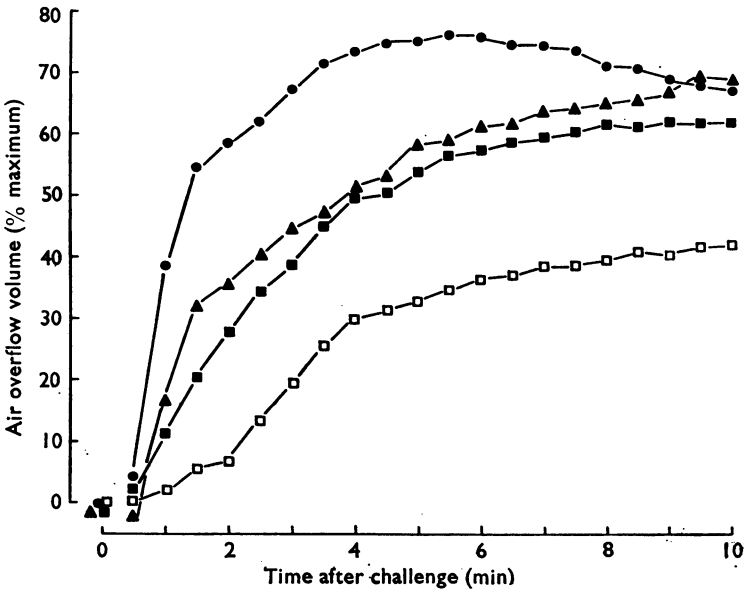

FIG. 5. Time/response curves of meclofenamate against anaphylactic bronchoconstriction in the guinea-pig during blockade of β-receptors for adrenaline. Each curve is the mean of results in twelve guinea-pigs, anaesthetized with pentobarbitone sodium (60–90 mg/kg intraperitoneally) and prepared as in Fig. 3. Propranolol (10 mg/kg) was given intraperitoneally 30 min and intravenously (5 mg/kg) 5 min before intravenous challenge with ovalbumen (0.75 mg/kg). ●——●, 0.9% w/v NaCl in water; ▲——▲, meclofenamate 1 mg/kg; ■——■, meclofenamate 4 mg/kg; □——□, meclofenamate 16 mg/kg. Meclofenamate was given as the sodium salt by stomach tube 30 min before challenge with antigen.

Catecholamine release

When tested by means of the blood-bathed organ technique, intravenous doses of fenamates and of aspirin, which exceeded the antibradykinin MED values, failed to block the release of adrenaline, induced by bradykinin given intra-arterially (Table 4). The maximal doses of aspirin and meclofenamate used did not themselves release adrenaline; but those of flufenamate and mefenamate did so. With both drugs, release of adrenaline was associated with bronchoconstriction, which occurred at a lower dose of mefenamate than of flufenamate.

TABLE 3. *Comparative anti-anaphylactic doses of the fenamates and some like-acting drugs*

Test compound	Minimal anti-anaphylactic dose	
	Mepyramine pretreatment	Propranolol pretreatment
Amidopyrine	N.T.	32
Aspirin	64	128
Flufenamate	4	64
Indomethacin	N.T.	128
Meclofenamate	1	16
Mefenamate	32	>128
Phenazone	N.T.	64
Phenylbutazone	N.T.	64

The minimal anti-anaphylactic dose is the least dose on a geometric scale that significantly ($P<0.05$) reduces the intensity of anaphylactic bronchoconstriction recorded in the Konzett–Rössler preparation. Where mepyramine pretreatment was used (details in Fig. 3) the statistical analysis was performed on responses obtained 1 min after challenge. Where propranolol pretreatment was used (details in Fig. 5) the statistical analysis was performed on the responses obtained over the period of 0–10 min after challenge. Doses are in mg/kg given by stomach tube 30 min before challenge. N.T., test not carried out.

TABLE 4. *Effect of fenamates and aspirin on catecholamine release, detected by means of the blood-bathed organ technique*

Drug	Dose range (mg/kg i.v.)	No. of animals	Block of adrenaline release induced by bradykinin	Release of adrenaline by test drug	Broncho-constriction
Aspirin	0·05–32	8	0	0	0
Flufenamate	0·2–16	3	0	+	+
Meclofenamate	0·03–5·0	5	0	0	0
Mefenamate	1·0–16	3	0	+	+

+, Effect obtained at or near maximal dose. 0, No effect at maximal dose used.

TABLE 5. *Subacute lethality of fenamates and therapeutic ratios against anaphylactic bronchoconstriction*

Test compound	LD50 (95% limits)	Therapeutic ratio	
		Mepyramine pretreatment	Propranolol pretreatment
Flufenamate	294 (220–396)	73·5	4·6
Meclofenamate	127 (70–176)	127	7·9
Mefenamate	249 (199–294)	7·8	<1·9

Sodium salts of the fenamates were administered by stomach tube at several dose-levels daily for 5 days to groups of ten guinea-pigs, and deaths were counted 14 days after the beginning of treatment. The median lethal dose (LD50) was determined from the proportions of deaths; it is expressed in mg/kg/day with 95% fiducial limits. The therapeutic ratio has been defined as the ratio of the LD50 to the minimal anti-anaphylactic dose in Table 3.

Subacute toxicity

Each of the three fenamates was administered by stomach tube daily for 5 days to several groups of ten guinea-pigs and the median lethal dose of each drug was determined from the proportions of survivors at each dose-level 14 days after the beginning of treatment. Table 5 gives the values obtained for the subacute median lethal dose and the ratios of these values to the minimal anti-anaphylactic doses in Table 3.

Discussion

All the anti-inflammatory drugs in Table 1 antagonized bradykinin-induced bronchoconstriction, except benzydamine, which Silvestrini *et al.* (1966) found to have anti-inflammatory activity in four tests in the mouse or rat (inhibition of foot oedema, of granuloma, of peritonitis and of pneumonitis). Benzydamine, however, differs from typical non-steroidal anti-inflammatory drugs in being inactive against erythema of guinea-pig skin induced by ultraviolet irradiation (C. V. Winder, personal communication) and in being basic. Benzydamine therefore seems to belong to a different category from the other anti-inflammatory drugs in Table 1, and to be the first member we have tested of a sub-group of anti-inflammatory drugs not antagonizing bradykinin-induced bronchconstriction in the guinea-pig.

When the MED against bradykinin-induced bronchoconstriction was determined by the two-dose procedure, meclofenamate appeared to be about twice as active as it was by the older one-dose procedure (Table 2). Such a difference might be expected because, in the newer procedure, the dose of bradykinin is not doubled after that of test drug. Possibly, for the same reason, Mi 85 and Scha 306 were somewhat more active in our hands than in those of Jahn & Wagner-Jauregg (1968).

We were unable to confirm the claim of Simke *et al.* (1967) that chlorpromazine and phenoxybenzamine antagonize bradykinin-induced bronchoconstriction in guinea-pig lungs *in vivo* (Table 1). Phenelzine and mebanazine proved to be the first drugs, which are not anti-inflammatory agents, that we have found to antagonize bradykinin in this test. The failure of propranolol to block the activity of phenelzine seems to rule out the possibility that phenelzine acts in this situation by potentiating endogenous adrenaline or noradrenaline.

After propranolol, responses to bradykinin were remarkably consistent between animals, although between guinea-pigs not treated with propranolol responses to bradykinin varied much. The figures for the dose-ratio of bradykinin at each dose of meclofenamate were also very consistent. The finding that an eightfold increase in the dose of meclofenamate required an approximately tenfold increase in bradykinin to restore the response to its original level accords with a previous finding with two fenamates and with aspirin (Collier & Shorley, 1963). The finding with meclofenamate, however, does not accord with that of Aarsen (1966), who reported that the dose-ratio of bradykinin did not increase proportionately with an increased dose of aspirin.

That meclofenamate fully suppressed the response to a large intravenous dose of bradykinin after destruction of the brain and spinal cord (Fig. 1) does not accord with the conclusion of Aarsen (1966) that " analgesics (of the aspirin type) inhibit the effect of bradykinin on guinea-pig lungs *in vivo*, by an effect on the central nervous system." Our finding with meclofenamate, however, is consistent with

previous experiments with aspirin and bradykinin in pithed guinea-pigs (Berry & Collier, 1964; Collier, James & Schneider, 1966).

Three types of experiment reported show that meclofenamate does not antagonize bradykinin by releasing catecholamines. Meclofenamate was effective after adrenalectomy or after propranolol and it did not release adrenaline when tested by the blood-bathed organ technique (Table 4). The small bronchodilator effect of bradykinin in an adrenalectomized guinea-pig after treatment with meclofenamate (Fig. 1) could be explained by a release of catecholamine from sympathetic nerves, induced by bradykinin (Lewis & Reit, 1965).

The ability of fenamates to reduce anaphylactic bronchoconstriction suggests their clinical trial in conditions of bronchial allergy. The high activity of meclofenamate and flufenamate in the presence of an antihistamine drug (Fig. 4) and their high therapeutic ratios (Table 5) in this situation suggest their clinical trial in association with antihistamine therapy. In the more intense anaphylaxis occurring after propranolol pretreatment, in which the natural protective effects of catecholamines were eliminated, meclofenamate showed significant activity at a lower dose than did the other drugs tested. That none of the three fenamates blocked bradykinin-induced release of adrenaline (Table 4) indicates that they are unlikely to interfere with the natural adrenergic protective mechanism in clinical asthma. None the less, the ability of fenamates to cause bronchoconstriction at high intravenous doses (Table 4) and the turning downwards of the dose-response curves with drugs of this type in the more severe anti-anaphylactic test (using propranolol) warn against the use of excessive doses in clinical trials against bronchial asthma, especially because bronchoconstriction with high doses of fenamates has occasionally been observed clinically (Brocks, 1967).

Although an anti-asthmatic effect has been reported with aspirin (Cook, 1947; Pearson, 1963), amidopyrine and phenazone (Herxheimer & Streseman, 1961), phenylbutazone (von Rechenberg, 1962) and with mefanamic acid (Jackson, Raymer & Etter, 1968), this is relatively slight or rare. Moreover, Wilson, Bhoola & McNicol (1967) and J. C. Batten (unpublished communication) failed to obtain a significant effect of flufenamate in a few cases of severe chronic asthma of long standing, also treated with corticosteroids. These findings and those reported above suggest that fenamates might be useful in the treatment of appropriate cases of asthma at carefully chosen doses and especially in the presence of an antihistamine. Preliminary tests show that meclofenamate exerts a protective effect in experimental anaphylaxis of cattle which involves the respiratory system (J. Sanford, personal communication).

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