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ANTAGONISM BY MEFENAMIC AND FLUFENAMIC ACIDS OF THE BRONCHOCONSTRICTOR ACTION OF KININS IN THE GUINEA-PIG

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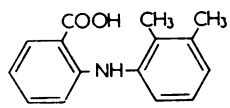
In the guinea-pig, *N*-(2,3-xylyl)anthranilic acid (mefenamic acid) and *N*-(α,α,α-trifluoro-*m*-tolyl)anthranilic acid (flufenamic acid), two new anti-inflammatory agents, antagonize bronchoconstriction, but not hypotension, produced by kinins. They do not reduce bronchoconstrictor responses to acetylcholine, histamine or 5-hydroxytryptamine. The antibradykinin potencies of mefenamic and flufenamic acids approximately equal that of acetylsalicylic acid when given intravenously and of phenylbutazone when given into the duodenum. After administration of mefenamic and flufenamic acids, the bronchoconstrictor response can be restored by higher doses of bradykinin. The quantitative relationship between the intravenous dose of sodium mefenamate or flufenamate and the dose of bradykinin needed to surmount either antagonist in bronchial muscle fulfils the requirements for competitive antagonism. Antagonism by calcium acetylsalicylate can also be surmounted with higher doses of bradykinin, but in this instance the relationship of antagonist to agonist fulfils requirements for competitive antagonism only at the lower part of the dose range.

Among non-steroidal anti-inflammatory agents a correlation has been observed between potency in delaying skin erythema of guinea-pigs after exposure to ultraviolet irradiation and activity against rheumatism in man (Winder, Wax, Burr, Been & Rosiere, 1958). More recently, potency in delaying erythema due to ultra-violet irradiation has been correlated with that in antagonizing bradykinin-induced bronchoconstriction in the guinea-pig (Collier & Shorley, 1960). We have therefore studied the antibradykinin action of two arylanthranilic acids recently reported to delay the onset of erythema due to ultra-violet irradiation of guinea-pig skin. These are *N*-(2,3-xylyl)anthranilic acid (mefenamic acid, CI-473, formula I), first described by Winder, Wax, Scotti, Scherrer, Jones & Short (1962), and *N*-(α,α,α-trifluoro-*m*-tolyl)anthranilic acid (flufenamic acid, CI-440, formula II), described by Winder, Wax, Serrano, Jones & McPhee (1963). Since acetylsalicylate also antagonizes the decapeptide kallidin-10 or -II in guinea-pig lung (Bhoola, Collier, Schachter & Shorley, 1962), we tested mefenamic and flufenamic acids as antagonists of this peptide as well as of bradykinin, using synthetic preparations of both kinins. This

paper describes the nature of the antagonism observed and compares it with that of acetylsalicylic acid.



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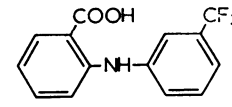

II

METHODS

Guinea-pigs weighing 300 to 800 g were prepared as previously described (Collier, Holgate, Schachter & Shorley, 1960) for recording resistance of the lungs to inflation *in vivo* by the method of Konzett & Rössler (1940). Arterial blood pressure was recorded with a Condon manometer in other guinea-pigs anaesthetized with intraperitoneal sodium phenobarbitone (60 mg/kg) and urethane (500 mg/kg). The minimal effective dose (MED) against intravenous bradykinin or kallidin-10 of a drug administered intravenously was determined by the procedure of Collier & Shorley (1960), giving doses of histamine, to test specificity, alternately with kinin at 5 to 10 min intervals. Drugs administered into the duodenum were tested in the same way against intravenous doses of bradykinin, alternately with histamine, at 10 to 15 min intervals. Substances were given intravenously or into the duodenum in saline, mefenamic and flufenamic acids being dissolved in 1 equiv. of NaOH, or suspended in 5% gum acacia before administration. Sodium acetylsalicylate was prepared by dissolving in saline a tablet containing 324 mg of acetylsalicylic acid, 975 mg of citric acid and 1,625 mg of sodium bicarbonate (" Alka-Seltzer "). Calcium acetylsalicylate was prepared as previously described (Collier & Shorley, 1960). Other substances used were synthetic bradykinin (Nicolaides & DeWald, 1961), synthetic kallidin-10 (Nicolaides, DeWald & McCarthy, 1961), acetylcholine chloride, acetylsalicylic acid, hexamethonium bromide, histamine acid phosphate, 5-hydroxy-

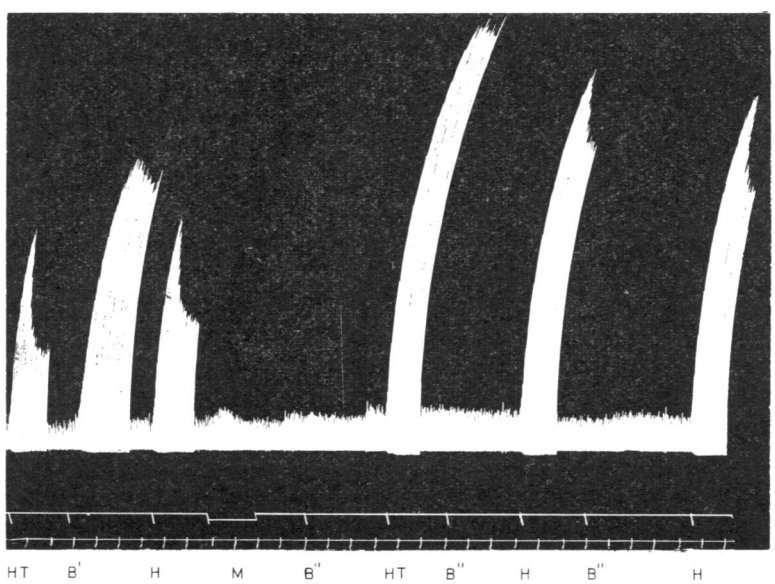

Fig. 1. Resistance to inflation of guinea-pig lungs *in vivo*. Specificity of antagonism of bradykinin by mefenamate. HT, 4 μg of 5-hydroxytryptamine ; B′, 1 μg, and B″, 2 μg of bradykinin ; H, 2 μg of histamine ; M, 16 mg of sodium mefenamate. Doses are given per kg and were administered intravenously at 5 min intervals. Time, 30 sec.

tryptamine creatinine sulphate, and sodium phenylbutazone. Weights of salts are expressed either as acid or as base.

RESULTS

Bronchoconstriction

Mefenamic and flufenamic acids suppressed the bronchoconstriction produced by bradykinin or kallidin-10, without reducing the responses to acetylcholine, histamine or 5-hydroxytryptamine. This effect is shown in Fig. 1, in which a large intravenous dose of sodium mefenamate (16 mg/kg) abolished the response to bradykinin, but enhanced those to histamine and to 5-hydroxytryptamine. Atropine (10 mg/kg, intravenously) and hexamethonium (10 mg/kg, intravenously) did not modify this antibradykinin action of mefenamate.

Table 1 gives some of the minimal effective doses of mefenamic and flufenamic acids and their sodium salts as antagonists of the bronchoconstriction caused by bradykinin and kallidin-10. This table shows that mefenamate and flufenamate

TABLE 1

MINIMAL EFFECTIVE DOSES (MED) OF MEFENAMATE, FLUFENAMATE, ACETYLSALICYLATES AND PHENYLBUTAZONE IN REDUCING BRONCHOCONSTRICTION PRODUCED BY BRADYKININ AND KALLIDIN-10

The MED is the minimal dose of an antagonist, on the scale 0·5, 1, 2, 4, 8 mg/kg., etc., which reduces the response to a dose of agonist, which is twice the preceding dose, to less than half the preceding response, without reducing that to histamine. I.V., intravenous ; I.D., intraduodenal; —, not tested. * Collier & Shorley (1960). † Bhoola *et al.* (1962)

Antagonist	Salt or acid	Route	MED (mg/kg) against Bradykinin	MED (mg/kg) against Kallidin-10
Mefenamate	Sodium	I.V.	1	0·5
		I.D.	32	—
	Acid	I.D.	32	—
Flufenamate	Sodium	I.V.	1	0·5
		I.D.	16	—
	Acid	I.D.	16	—
Acetylsalicylate	Sodium	I.V.	2	—
		I.D.	128	—
	Calcium	I.V.	2*	2†
	Acid	I.V.	2	—
		I.D.	64*	—
Phenylbutazone	Sodium	I.V.	4*	—
	Acid	I.D.	16*	—

are of approximately the same potency as acetylsalicylate when given intravenously and as phenylbutazone when given into the duodenum. Table 1 also shows that the intravenous MED of acetylsalicylate is the same whether it is given as the calcium or sodium salt or as the free acid.

The durations of action of sodium mefenamate and flufenamate were compared with those of calcium acetylsalicylate and sodium phenylbutazone, by observing the time interval between intravenous administration of twice an MED and the return of a normal response to bradykinin. This response returned 15 to 25 min after flufenamate (Fig. 2), 15 to 45 min after mefenamate and 25 to 45 min after phenylbutazone. After calcium acetylsalicylate, however, a normal response to bradykinin did not return within 5 hr.

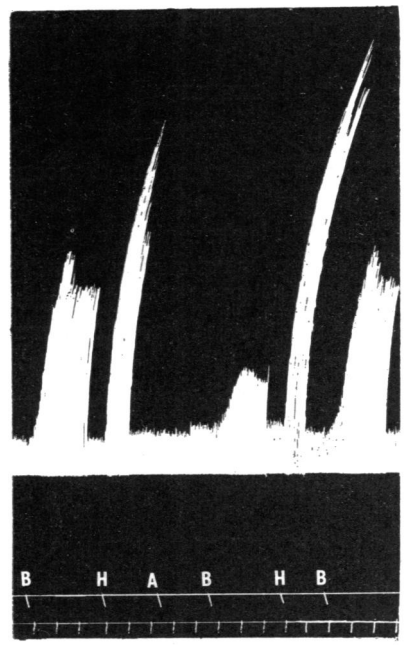

Fig. 2. Resistance to inflation of guinea-pig lungs *in vivo*. Duration of antibradykinin action of flufenamate. B, 4 μg of bradykinin; H, 4 μg of histamine; A, 2 mg of sodium flufenamate. Doses are given per kg and were administered intravenously at 5 min intervals. Time, 30 sec.

TABLE 2

ABILITY OF BRADYKININ TO SURMOUNT ANTAGONISM OF MEFENAMATE, FLUFENAMATE AND ACETYLSALICYLATE IN GUINEA-PIG LUNG *IN VIVO*

After obtaining a standard response to 1 μg/kg of bradykinin, a dose of antagonist was administered and the dose of bradykinin giving a response comparable to the standard was then determined. All drugs were given intravenously

Antagonist		Bradykinin which restored response	
Name	Dose (mg/kg)	Dose (μg/kg)	95% fiducial limits
Sodium mefenamate	1	4·7	1·6– 14
	2	11·8	6·3– 22
	4	56	26 –122
	8	67	41 –108
	16	138	58 –330
Sodium flufenamate	1	2·1	1·1– 4
	2	13·5	7·2– 25
	4	16	10·5– 24
	8	50	24 –103
	16	53	28 – 99
Calcium acetylsalicylate	0·5	3·2	1·2– 8·4
	1	13	3·3– 51
	2	18	4·5– 69
	4	40	20 – 79
	8	115	52 –255
	16	98	25 –384
	32	50	19 –132
	64	85	32 –225
	128	216	55 –851

Using a limited amount of impure natural bradykinin, it was shown that the effect of acetylsalicylate could be surmounted by higher doses of bradykinin, the amount of agonist needed increasing with the dose of antagonist (Collier & Shorley, 1960). Subsequently, synthetic bradykinin became available, making it possible to extend this study and to raise the intravenous dose of antagonist, calcium acetylsalicylate, to 128 mg/kg, the maximum on the doubling scale used here that guinea-pigs would tolerate. This dose of acetylsalicylate and the highest tolerated intravenous doses (16 mg/kg, on this scale) of sodium mefenamate and flufenamate could each be

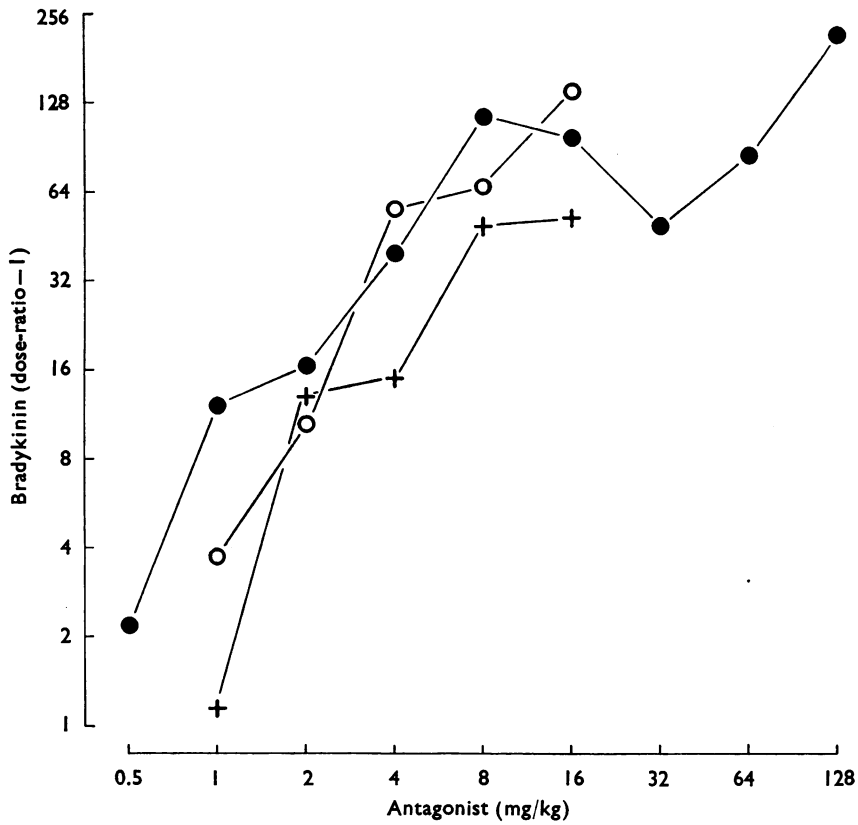

Fig. 3. Relationship of intravenous doses (abscissa) of calcium acetylsalicylate (● — ●), sodium mefenamate (○ — ○) and sodium flufenamate (+ — +) to dose of bradykinin (ordinate, as dose-ratio—1) required to surmount antagonism. For explanation of dose-ratios, see text.

surmounted by sufficiently high doses of bradykinin. The dose of bradykinin, given after antagonist, needed to produce a response of the same height as that to 1 μg/kg bradykinin, given before antagonist (the dose-ratio), was determined for a series of doses of each drug. The results are shown in Table 2. In Fig. 3, the dose-ratio is plotted against the dose of antagonist, both on logarithmic scales. Lines of approximately unit slope were obtained for mefenamate and flufenamate and for lower doses of acetylsalicylate. However, above 8 mg/kg the slope for calcium

acetylsalicylate flattened, the amount of bradykinin needed to overcome the antagonism not increasing significantly between 8 and 128 mg/kg.

Blood pressure

Intravenous doses of calcium acetylsalicylate (10 mg/kg), sodium mefenamate (8 mg/kg) and sodium flufenamate (8 mg/kg), though effective in suppressing the bronchoconstriction, failed to reduce the transient fall in blood pressure produced by intravenous injections of bradykinin (0.5 to 1 μg). On the contrary, the anti-inflammatory drugs slightly increased the fall in blood pressure, probably by preventing a secondary rise due to bronchoconstriction.

DISCUSSION

Mefenamic and flufenamic acids resemble acetylsalicylic acid and phenylbutazone in the main features of their antagonism of bradykinin-induced bronchoconstriction in the guinea-pig, except that acetylsalicylate is very long-acting. These drugs also delay erythema due to ultra-violet irradiation of guinea-pig skin, their order of potency being flufenamic acid>phenylbutazone>mefenamic acid>acetylsalicylic acid (Winder *et al.*, 1963). The order of antibradykinin potency by the duodenal route (flufenamic acid=phenylbutazone>mefenamic acid>acetylsalicylic acid) more closely resembles that against skin erythema than that against bradykinin by the intravenous route. Unpublished experiments have shown that certain other anthranilic acids, which are inactive in the skin erythema test, are also inactive against bradykinin. These facts strengthen the association already noted between antibradykinin action in lung and anti-erythema action in skin.

To pursue the implications of this correlation further would be premature, since Berry, Collier & Holgate (1963) recently reported that non-steroidal anti-inflammatory agents also antagonize bronchoconstriction due to slow reacting substance in anaphylaxis (SRS-A), which is chemically and pharmacologically distinct from kinins. Mefenamic and flufenamic acids also show this action, their potencies resembling those against bradykinin.

Winder *et al.* (1962, 1963) have found that mefenamic acid possesses, but flufenamic acid lacks, an analgesic action that is apparently central. Since the antibradykinin activities of these two drugs on guinea-pig bronchial muscle are about equal, there appears to be no relation between bradykinin antagonism and analgesia. This finding accords with the previously reported failure of centrally-acting analgesics, such as morphine and phencyclidine, to show antibradykinin action (Collier & Shorley, 1960).

We have previously suggested that in guinea-pig bronchial muscle there are receptors for bradykinin bronchoconstriction which are blocked by acetylsalicylate and other anti-inflammatory drugs. These were called A-receptors to distinguish them from receptors for bradykinin elsewhere in the body not blocked by anti-inflammatory drugs (Collier, 1962). The behaviour of mefenamic and flufenamic acids conforms so closely with that of known anti-inflammatory agents that they may be supposed to block the same bradykinin receptors in bronchial muscle.

The curves for mefenamate and flufenamate shown in Fig. 3 fulfil requirements for competitive antagonism. Over a similar dose range the curve for calcium acetylsalicylate also conforms with a competitive relationship to bradykinin, but at higher doses the antagonism by acetylsalicylate is relatively weaker. Possible explanations of the change in shape of this curve above 8 mg/kg of acetylsalicylate include: (1) that acetylsalicylate, itself a bronchoconstricting agent at higher doses, strengthens this action of bradykinin ; (2) that other receptors not blocked by acetylsalicylate are activated by higher doses of kinin ; and (3) that blood levels of acetylsalicylate do not increase proportionately with increasing dose. Too many unknown factors operate in this *in vivo* system for us to attempt to decide which explanation is correct.

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