ANTAGONISTS OF BRADYKININ

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*Relationship of Drugs to the Kinin Activity of Plasma**

Figure 1 illustrates the probable mechanism by which kallidin is naturally released and inactivated.[1] The complexity of this mechanism means that, in a sample of blood, the amount of free, active kinin will depend on a balance of several processes. Consequently there are a good many ways in which foreign substances might increase or decrease the kinin activity of plasma. On the one hand, chemicals might increase the activity by (1) releasing kallikrein, through activating kallikreinogen or depressing inhibitors; (2) releasing kinin as histamine liberators release histamine; or (3) inhibiting kininase, the peptidase which destroys plasma kinin, as anticholinesterases inhibit enzymes destroying acetylcholine. On the other hand, chemicals might decrease the kinin activity of plasma by (1) depressing the synthesis in the body of precursors of kallikrein or kinin; (2) inhibiting the conversion of kallikreinogen into kallikrein; (3) reversibly or irreversibly inhibiting the action of kallikrein, as decarboxylase inhibitor depress conversion of amino acids into amines; (4) antagonizing kinin at its receptors, as antihistamines antagonize histamine; or (5) inactivating kinin, either by reinforcing plasma kininase or by direct action on kinin itself.

Table I gives some of the substances reported to change the kinin activity of plasma by some of the actions I have described. Of the inhibitors of plasma kallikrein given in the table, that in potatoes is the most active, the rest being more effective against kallikreins from other sources

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* The "kinin activity of plasma" refers to the extent to which plasma exerts the pharmacological effects of plasma kinin. The term "plasma kinin" is applied to any kinin liberated in plasma, of which two have so far been chemically identified.[28] One of these is bradykinin, which is liberated by trypsin or *Bothrops* venom. This has been characterized as H—L—Arg—L—Pro—L—Pro—Gly—L—Phe—L——Ser—L—Pro—L—Phe—L—Arg—OH. This nonapeptide, prepared by the action of trypsin on ox globulin, is trypsin bradykinin and, prepared by chemical combination of the appropriate amino acids, is synthetic bradykinin. Kallidin, the kinin liberated by kallikrein, has been distinguished by Pierce and Webster[28] into two peptides — Kallidins I and II. Kallidin I is chemically identical with bradykinin and Kallidin II is a decapeptide having the same chain as bradykinin with an added N-terminal lysine.

TABLE I

Some foreign substances affecting kinin activity of plasma

Effect on kinin activity	Mode of action	Substance
Increase	Release or activation of kallikrein	Trypsin;[2] acetone;[3] papain.[6]
	Release of kinin	Trypsin, *Bothrops* venom.[4]
	Inhibition of kininase	Cysteine;[5,6] Cd, Hg, Mn, ethylenediamine tetraacetic acid, 1,10-phenanthroline, ε-amino n-caproic acid, δ-amino n-valeric acid, arginine.[7]
Decrease	Inhibition of kallikrein	Potato extract;[8,9] soybean trypsin inhibitor, chloroethyl-dichlorovinyl phosphate.[10]
	Antagonism of kinin	Acetylsalicylate, phenylbutazone, amidopyrine, phenazone.[11,12,13]
	Activation of kininase	Co[7]
	Inactivation of kinin	Ninhydrin, hydroxylamine, diazobenzenesulphonic acid, fluorodinitrobenzene.[6]

than blood. The results which Dr Erdös[7] presented two days ago enable us to include in Table I a category for kininase activators and to extend the list of kininase inhibitors. Dr. Erdös has found that kallikrein inhibitors do not inhibit kininase, but we do not yet know whether inhibitors of kininase affect kallikrein.

Chemicals acting *in vivo* mainly in one of the ways shown in Table I are interesting, because they might help to elucidate the role of plasma kinin in the body and because they might be therapeutically useful. If known drugs were found active in one of these ways, this might explain their pharmacological effects. Of the several actions shown in Table I, I wish to consider now only direct antagonism of plasma kinin, much of the work I shall summarize being that on which Miss Shorley and I have been engaged for the past three years.

Antagonists of Bradykinin

While bradykinin is not the only kinin that can be liberated in plasma, it is the only one yet available in sufficient supply for antagonism studies. All our work has therefore been done with this peptide.

At first we used partially purified trypsin bradykinin; but, in order to make sure that the effects of bradykinin could not be due to impurities that activated the mechanism of kinin release, we later went over to synthetic bradykinin prepared by Nicolaides and De Wald.[14]

Most of our work was done *in vivo* in the guinea-pig, which was set up under urethane anaesthesia to record resistance of the lungs to artificial inflation, in the way first described by Drs. Konzett and Rössler.[15] Using this preparation, Dr. Holgate, Dr. Schachter, Miss Shorley and I[11, 12] found that small intravenous doses of bradykinin markedly raise the resistance of lungs to inflation. We concluded for several reasons that this effect was essentially due to bronchoconstriction. Later studies[16] have shown that bradykinin shortens isolated strips of guinea-pig trachea, albeit at doses larger than those of histamine or acetylcholine. We have also shown that bradykinin potently increases resistance to inflation, when dropped in solution onto the pleural surface of the lungs *in situ*. This effect resembles that which Dautrebande *et al.*[17] obtained in isolated guinea-pig lung with histamine, acetylcholine and 5-hydroxytryptamine.

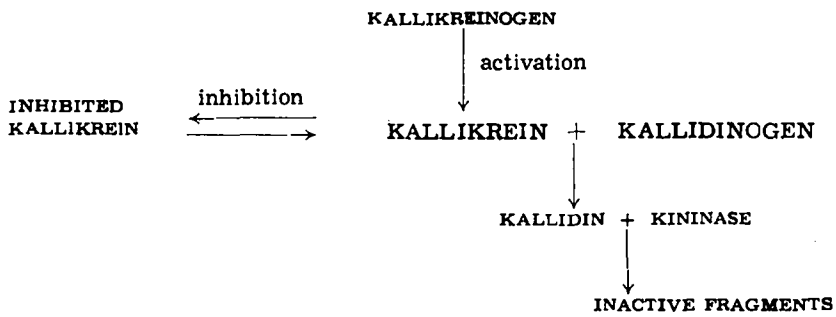

FIG. 1. Outline of probable mechanism of release and inactivation of kallidin in serum or plasma.[1]

Acetylsalicylate, phenylbutazone, amidopyrine and phenazone can each suppress the bronchoconstrictor response of guinea-pig lungs to bradykinin. Fig. 2 illustrates this effect, showing the following features: (1) one drug is effective by several routes; (2) responses to other bronchoconstrictor agents are unaffected; (3) doses of drug needed may be small; and (4) larger doses of bradykinin can overcome the suppression.

There are other features of this antagonism which Fig. 2 does not show. It is unaffected by cutting both vagi in the neck. The effect is seen when natural bradykinin is replaced by synthetic[18] or by wasp kinin. When an antagonist is administered before bradykinin, a fall in resistance to inflation is sometimes unmasked. This is well shown in Fig. 3, when 3·2 μg bradykinin was given after 100 mg/kg amidopyrine.

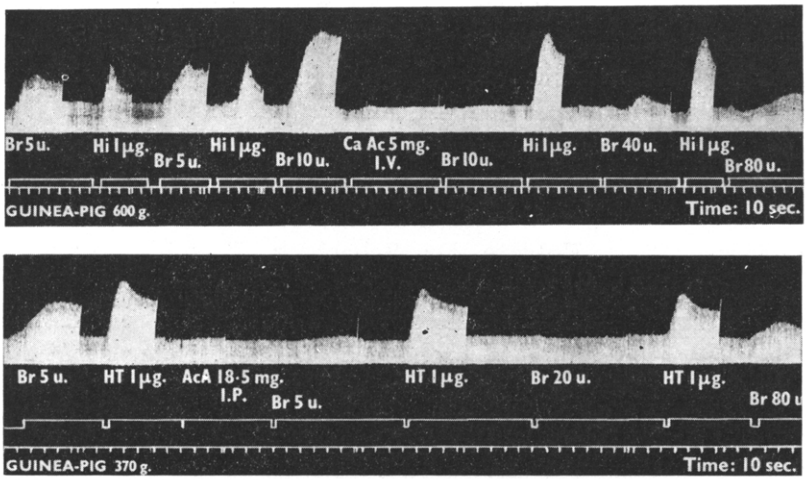

FIG. 2. Resistance to inflation of guinea-pig lungs *in vivo*. Suppression of response to bradykinin by acetylsalicylate[12]. Br, bradykinin (1 u = 0·08 µg); Hi, histamine; Ca Ac, calcium acetylsalicylate, dose as acid; HT, 5-hydroxytryptamine; Ac A, acetylsalicylic acid. All doses except Ac A given intranvenously at 5 min intervals. Dose of Ac A given intraperitoneally 4 min before bradykinin.

Fig. 3 also shows the degree of specificity of amidopyrine against bradykinin. A dose of 100 mg/kg amidopyrine, which suppressed the bronchoconstrictor action of a dose of bradykinin 8 times the effective dose, briefly reduced the response to an effective dose of histamine. Acetylsalicylate was more specific than amidopyrine. Large doses of acetyl-

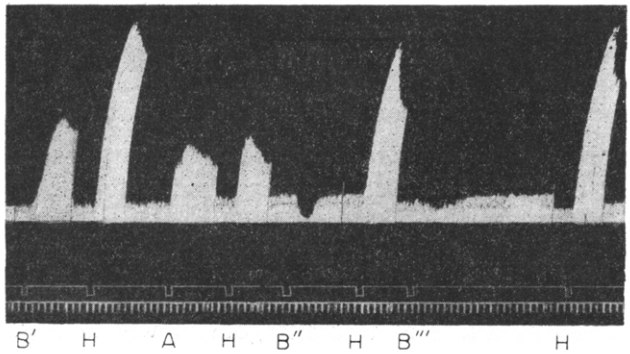

FIG. 3. Resistance to inflation of guinea-pig lungs *in vivo*. Degree of specificity of amidopyrine towards bradykinin and unmasking of probable dilator action of bradykinin on lung.[13] B′, 0·4 µg, B″, 3·2 µg and B‴ 6·4 µg of bradykinin; H, histamine, 2 µg; A, amidopyrine, 100 mg/kg. All doses were given intravenously, Weight of guinea-pig, 580 g. Time, 10 sec.

salicylate failed to suppress responses to histamine (Fig. 4), acetylcholine or 5-hydroxytryptamine. Fig. 4 also shows that a large dose of mepyramine did not suppress the response to bradykinin. Atropine and lysergic acid diethylamide were likewise ineffective against bradykinin, though they potently antagonized acetylcholine and 5-hydroxytryptamine respectively. Figs. 3 and 4 also show that large doses of amidopyrine, acetylsalicylate and mepyramine themselves increase resistance to inflation.

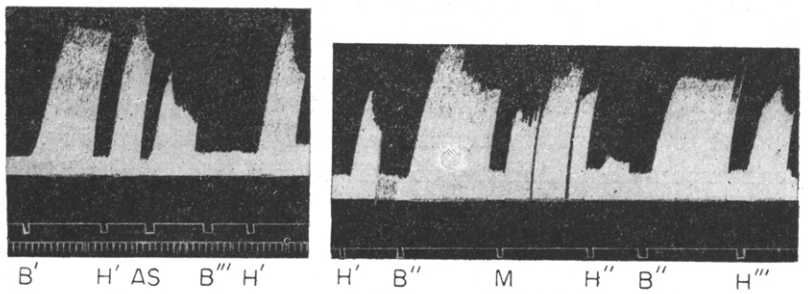

FIG. 4. Resistance to inflation of guinea-pig lungs *in vivo*. Failure of calcium acetylsalicylate to suppress response to histamine and of mepyramine to suppress response to bradykinin[13]. B′, 0·4 μg, B″, 0·8 μg, and B‴, 1·6 μg of bradykinin; H′, 1 μg H″, 1 mg, and H‴, 2 mg of histamine; AS, calcium acetylsalicylate, 100 mg/kg, dose as acid; M, mepyramine maleate, 10 mg/kg, dose as base. All doses were given intravenously. Guinea-pig weights: left, 460 g; right, 500 g. Time, 10 sec.

The reversal of the action of acetylsalicylate by high doses of bradykinin, illustrated in Fig. 2, may be measured by comparing the doses of bradykinin needed, before and after antagonist, to produce the same degree of bronchoconstriction. Table II gives the amounts of bradykinin

TABLE II

Restoration of bronchoconstrictor response of guinea-pigs to bradykinin after calcium acetylsalicylate[13]

A standard response was first obtained to 0·4 μg of bradykinin. After administering acetylsalicylate at three doses, the doses of bradykinin were determined which evoked a response similar to the standard. All doses were given intravenously.

Dose of acetylsalicylate (mg/kg)	Dose of bradykinin to restore response (μg)
1	1·6– 3·2
2	6·4–12·8
4	12·8–25·6

required to surmount the effects of various doses of acetylsalicylate. At three dose leveils, the ratio of this drug to bradykinin required to restore the response has roughly the value of 150, suggesting a competitive type of antagonism.

In order to assess the potencies of drugs as antagonists of bradykinin bronchoconstriction, we obtained from the Konzett and Rössler preparation reference responses to small doses of bradykinin and histamine. A dose of drug was then given on the scale 512, 256... 2,1 etc. mg/kg. This was followed by the same dose of histamine and by double the dose of bradykinin given previously. The minimal effective dose (MED) of a drug was taken as the lowest dose which reduced the response to the *second* dose of bradykinin to less than half that to the *first*, without affecting the response to histamine. This procedure is illustrated in Fig. 5, from an experiment in which 2 mg/kg acetylsalicylate was effective but 1 mg/kg was not.

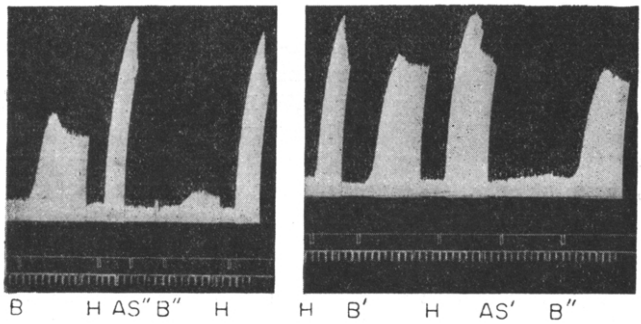

FIG. 5. Resistance to inflation of guinea-pig lungs *in vivo*. Estimation of intravenous minimal effective dose of calcium acetylsalicylate[13]. B′, 0·4 μg and B″, 0·8 μg of bradykinin; H, histamine, 2 μg; AS′, 1 mg/kg and AS″, 2 mg/kg, calcium acetylsalicylate, doses as acid. All doses given intravenously. Guinea-pig weights: left, 450 g; right, 380 g. Time, 10 sec.

We subjected a number of compounds to this procedure, with results summarized in Table III which shows that those of appreciable activity belong to the group of anti-inflammatory, analgesic, antipyretic drugs. Griseofulvin was tested because it has anti-inflammatory activity in the cotton-pellet test in rats and the tuberculin-sensitivity test in guinea-pigs,[19] but no unequivocal antibradykinin action was shown at the largest intravenous dose at which the solution in dimethyl formamide was well tolerated. Hydrocortisone and γ-resorcylic acid, though effective at higher doses in depressing the response to bradykinin, also depressed that to histamine. Substances such as adrenaline and isoprenaline, not shown in the table, had a non-specific dilator action.[12]

Significance of the Antagonism to Bradykinin

Many of the compounds that specifically suppress bradykinin bronchoconstriction, shown in Table III, also delay the appearance of erythema in the skin of guinea-pigs exposed to ultra-violet irradiation.[20, 21] Correlation of rank order of potencies in the two types of test is high. In both, corticoids show little or no activity.

TABLE III

Potencies of various agents in suppressing bronchoconstriction due to bradykinin in guinea-pigs[13]

The minimal effective dose (MED) is the least dose of an antagonist that reduces the response to an intravenous dose of bradykinin, which is twice the preceding dose, to less than half the preceding response, without reducing that to histamine.

Agent	MED (mg acid or base/kg)			
	Intravenous	Oral	Intraduodenal	Other routes
Acetylsalicylic acid	2*	32	64	—
Sodium salicylate	64	>512	256	—
Salicylamide	—	>256	>512	—
γ-Resorcylic acid	>64**	—	—	—
Gentisic acid	>64**	—	—	—
4-Hydroxyisophthalic acid	>64**	—	>512	I.P.>512
Cinchophen	32**	>512	256	—
Sodium phenylbutazone	4	16	16	—
Amidopyrine	8	16	16	—
Phenazone	8	64	128	—
Acetanilide	—	>512	256	—
Phenacetin	—	>512	512	—
Paracetamol	16	512	128	—
Amodiaquine phosphate	—	>512	>512	—
Cortisone	—	—	—	I.M.>16
Hydrocortisone sodium succinate	>32	—	—	—
D, L-Aldosterone	> 2	—	—	—
Morphine sulphate	>32	—	—	—
Methadone hydrochloride	> 4	—	—	—
Phencyclidine hydrochloride	> 8	—	—	—
Griseofulvin	>32	—	—	—

* administered as calcium salt; ** administered as sodium salt; — not tested; I.P. intraperitoneal; I.M. intramuscular.

These facts might lead us to suppose that ultraviolet irradiation releases bradykinin in skin and that analgesic-antipyretic drugs depress the resulting erythema by antagonizing the bradykinin released. Unfortunately, there are objections to this hypothesis. Bradykinin, injected intra-

dermally in the guinea-pig, produces wealing but not obvious erythema. This failure to produce erythema might be due to a failure to reproduce by intradermal injection of bradykinin the natural conditions of its release as regards site, concentration or time-course; but potent antagonists of bradykinin bronchoconstriction do not seem to suppress the skin wealing that we can readily produce by intradermal injection of bradykinin.

Except for isolated guinea-pig trachea[16] and possibly also rabbit blood pressure,[22] no other preparations, either *in vitro* or *in vivo*, have shown a specific antagonism of bradykinin by analgesic-antipyretic drugs. *In vivo* preparations failing to show this effect include guinea-pig skin, guinea-pig and rat blood pressure and rat uterus *in situ*. *In vitro* preparations giving similar negative results include guinea-pig ileum, rat uterus and rat duodenum. Their failure shows that these drugs do not chemically inactivate bradykinin.

Although we cannot therefore establish a definite connection between antagonism to bradykinin and antiinflammatory action, a number of interesting implications arise from the effects observed in lung. In Edinburgh, Lahiri and Brocklehurst[23] recently demonstrated that administration of antigen to sensitized guinea-pigs, rats and rabbits sharply raises the kinin concentrations of their blood. In the guinea-pig these workers reported a peak value of about 40 ng/ml., which should be sufficient to cause bronchoconstriction. Although release of plasma kinin therefore probably contributes to anaphylactic bronchospasm in the guinea-pig, its share is less than that of histamine, as two observations show Herxheimer[24] could not, with phenylbutazone or salicylate, protect sensitized guinea-pigs against antigen given by aerosol; and we[13] failed, with large doses of acetylsalicylate, to overcome the bronchospasm that intravenous antigen evoked after a very brief latency. In both experiments, however, mepyramine was partially effective.

Histamine does not seem to contribute so overwhelmingly to human asthma as to anaphylactic bronchospasm in the guinea-pig lung. The questions whether bradykinin plays a significant part in asthma and whether analgesic, antipyretic drugs relieve this condition are therefore interesting. In a preliminary communication, Herxheimer and Stresemann[25] have reported that inhalation of bradykinin solution as an aerosol reduces the vital capacity of asthmatic subjects. They commented that phenazone is a common constituent of proprietary asthma powders and that this drug increases the vital capacity in many cases of asthma.

Further studies of the action of bradykinin and its antagonism in the lungs of various species have produced some unexpected results. In the rabbit, bradykinin increases resistance of the lungs to inflation, but larger doses are needed and acetylsalicylate, phenylbutazone and amidopyrine do not antagonize the effect.[16] In the cat, larger doses of bradykinin also decrease lung distensibility;[26] but in the dog it appears to have a

slight bronchodilator action.[27] In the guinea-pig, as Fig. 3 showed, anti-bradykinins unmask a dilator action of bradykinin in the lung. In this species too, while intravenous or intracardiac injection of bradykinin causes obvious respiratory distress, its intraperitoneal injection or the inhalation of bradykinin aerosol do not.[25] In man, although Herxheimer and Stresemann found that asthmatic subjects respond to bradykinin aerosol, we[16] failed to evoke with bradykinin a contraction from strips of human bronchus, obtained from pieces of lung excized in bronchial carcinoma. Yet histamine and acetylcholine were active in this preparation.

CONCLUSION

The results I have outlined show that there are certain pieces missing from this jig-saw puzzle. We can make some guesses as to what these may be. Since drugs that, in the guinea-pig, antagonize bradykinin bronchoconstriction, do not antagonize its apparent bronchodilator action nor its effects on many other tissues, we may suppose that there are at least two types of receptors for bradykinin. The receptors for bronchoconstriction in the guinea-pig, blocked by analgesic-antipyretic drugs, might provisionally be called A (Antipyretic) receptors. This type of receptor is also sensitive to at least one other kinin — that of wasp venom.

There is also the correlation between rank orders of potency of drugs acting in the guinea-pig against bradykinin bronchoconstriction and against skin erythema due to ultra-violet irradiation. Assuming this correlation is not coincidental or due to some trivial or superficial cause, the simplest hypothesis I can suggest is that irradiation releases a substance in skin that acts on receptors for erythema. These receptors are supposed not to respond to bradykinin, but to be blocked by analgesic, antipyretic drugs. This hypothetical substance is also supposed to resemble bradykinin and wasp kinin enough to act on A receptors in lung.

Whatever the explanation of the complexities that have been met, and I know very well that other explanations are possible and even likely, a striking fact has emerged. Acetylsalicylate, phenylbutazone, amidopyrine and like-acting drugs antagonize the bronchoconstrictor action of bradykinin in the guinea-pig, potently, specifically and surmountably.

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