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THE INFLUENCE OF ANALGESIC ANTIPYRETIC DRUGS ON THE RESPONSES OF GUINEA-PIG LUNGS TO BRADYKININ

BY

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Collier, Holgate, Schachter & Shorley (1959, 1960) stated that bradykinin causes bronchoconstriction in the guinea-pig anaesthetized with urethane. This bronchoconstriction is antagonized by acetylsalicylic acid, phenylbutazone and amidopyrine. Collier (1962) suggested that guinea-pig bronchial muscle contains receptors for bradykinin which are blocked by acetylsalicylate and related drugs. These were called A-receptors to distinguish them from bradykinin receptors elsewhere in the body not blocked by these drugs.

If these analgesics have a direct effect on the bronchial muscles they must also counteract the bronchoconstriction caused by bradykinin in the guinea-pig lung *in vitro*. Bhoola, Collier, Schachter & Shorley (1962) demonstrated that bradykinin contracted the isolated guinea-pig trachea and reduced the rate of tracheal perfusion through the isolated guinea-pig lung. With regard to the effects of analgesic antipyretic drugs on these responses of bradykinin they only mentioned that phenylbutazone and amidopyrine relaxed the tracheal strip and were more effective in depressing the response to bradykinin than that to either histamine or acetylcholine.

In the present investigation we examined the influence of some analgesic antipyretic drugs on the responses of isolated guinea-pig lung preparations to bradykinin. Since we could not demonstrate any antagonizing effect of these drugs in the experiments *in vitro*, we also investigated their action on the guinea-pig lung *in vivo*.

METHODS

Isolated guinea-pig lungs. Two different methods were used. In the first method, that of Sollman & von Oettingen (1928), the tracheo-bronchial tree was perfused with modified Krebs solution described by Bülbring & Hooton (1954). The flow of fluid escaping from superficial cuts in the surface of the lungs was recorded with a Palmer drop recording assembly. After administration of bradykinin and histamine, the increased resistance to flow may persist for some time. This was overcome by perfusing the lungs under a higher pressure till the rate of flow had approximately regained its original value.

In the second method, that of Bhattacharya & Delaunois (1955), both the trachea and the pulmonary artery were cannulated. The tracheal cannula was attached to a tube with three side-arms.

One arm was connected to a Marey-tambour to record bronchial calibre changes. A polythene cannula with a bore of 1.0 mm was pushed through a rubber cap on the second arm into the central tube; through this cannula 5% carbon dioxide in oxygen under a pressure of about 5 cm water was supplied to the lungs. The third arm served as a variable outlet. The cannulated pulmonary artery was perfused with the modified Krebs solution to which 3% dextran was added. The outflow from the opened left atrium was recorded by a Palmer drop recorder. Persisting high resistance in the airway and in the blood vessels after addition of bradykinin was overcome by temporarily increasing the pressures; when this remained without effect, 100 μg of isoproterenol was administered. In both preparations the dose of bradykinin (the reference dose) causing an obvious increase in airway resistance was established first. Thereafter the lungs were perfused either through the tracheo-bronchial or the vascular system with the fluid containing the analgesic under test. After perfusion for half an hour or more the test dose of bradykinin was administered. This dose was either identical to or double the reference dose.

Whole animal preparation. Guinea-pigs were prepared for recording the resistance of the lungs to inflation by the method of Konzett & Rössler (1940), as modified by Collier *et al.* (1960). The trachea was cannulated and the lungs were inflated with air by a miniature Starling pump (stroke volume 5 to 6 ml., usually at 72 strokes/min). The changes in pressure caused by the air escaping through the water valve (10 cm H_2O pressure) were recorded by a Statham transducer connected to a Honeywell Visicorder. Intravenous injections into the jugular vein were spaced at 10 min intervals.

Materials. Bradykinin was prepared by the action of crystalline trypsin on heated acidified ox plasma and was purified by chromatography on Amberlite CG-50. The two preparations used were standardized against synthetic bradykinin (Sandoz BRS 640), and were found to contain 3.0 and 5.3 μg bradykinin/mg respectively. The analgesic antipyretic drugs tested in the experiments were calcium acetylsalicylate (Ascal, Nedchem), sodium salicylate, phenazone, amidopyrine and sodium phenylbutazone (Butazolidin, Geigy).

RESULTS

Isolated guinea-pig lungs perfused via the tracheo-bronchial tree

The findings of Bhoola *et al.* (1962) that bradykinin and histamine cause an increase in resistance to flow in the tracheal perfusion channel were confirmed. In our hands the variation between lungs from different animals was great. The doses causing a definite increase in resistance ranged from 1.6 to 16.8 μg for bradykinin and from 80 to 320 μg for histamine. The responses to bradykinin and to histamine were fairly reproducible over a period of 2 hr, in spite of the fact that the lungs soon became oedematous. Since in most preparations the response to the first dose of bradykinin is higher than the response to subsequent doses, in all experiments a second (test) dose of this substance was given which was twice the reference dose. The effect of an analgesic was considered to be absent if the response to the test dose of bradykinin was higher than that to the reference dose; the effect was considered positive if the response to the test dose was either equal to or smaller than that to the reference dose and there was no reduction of the effect of histamine.

Figs. 1 and 2 show that calcium acetylsalicylate and sodium phenylbutazone in a concentration of 40 μg/ml. perfusion fluid had no inhibitory effect on the response to bradykinin. Assuming that the extracellular fluid of the guinea-pig is about 250 ml./kg body weight and the analgesics are distributed uniformly over this volume, the minimal intravenous effective doses of calcium acetylsalicylate and phenylbutazone according to Collier & Shorley (1960) will correspond to 8 and 32 μg/ml. extracellular fluid, respectively. Table 1 shows that calcium acetylsalicylate, sodium salicylate and sodium

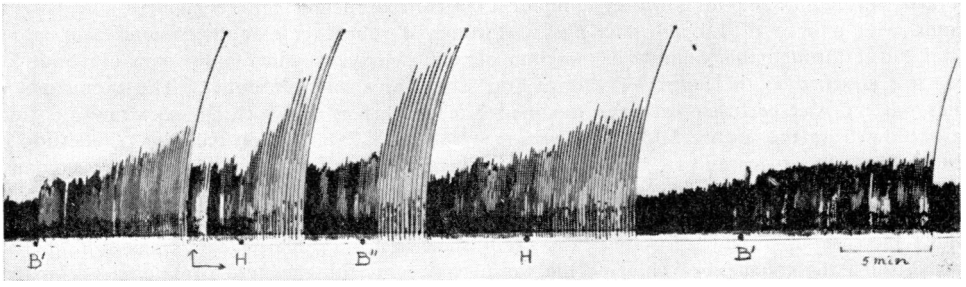

Fig. 1. Resistance to perfusion of tracheo-bronchial tree of guinea-pig lungs *in vitro* (Sollmann-von Oettingen preparation). Responses to bradykinin and histamine. Failure of calcium acetylsalicylate to reduce response to bradykinin. B′, 1.6 μg, and B″, 3.2 μg bradykinin; H, 20 μg histamine base. Starting at arrow calcium acetylsalicylate (40 μg/ml.) was present in the perfusion fluid.

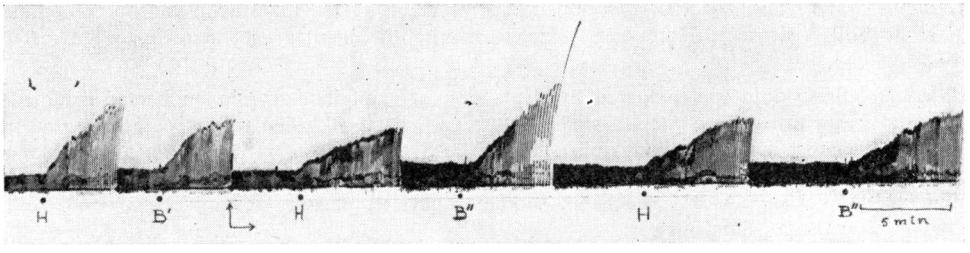

Fig. 2. Resistance to perfusion of tracheo-bronchial tree of guinea-pig lungs *in vitro* (Sollmann-von Oettingen preparation). Responses to bradykinin and histamine. Failure of sodium phenylbutazone to reduce response to bradykinin. B′, 6.4 μg, and B″, 12.8 μg bradykinin; H, 160 μg histamine base. Starting at arrow sodium phenylbutazone (40 μg/ml.) was present in the perfusion fluid.

TABLE 1

INFLUENCE OF A NUMBER OF ANALGESIC ANTIPYRETIC DRUGS ON BRONCHOCONSTRICTOR RESPONSE OF ISOLATED GUINEA-PIG LUNGS TO BRADYKININ INJECTED INTO THE TRACHEAL PERFUSION CHANNEL

After a reference dose of bradykinin and of histamine, perfusion with the fluid containing the drug under test was started. The test dose of bradykinin (twice the reference dose) was administered 40 to 60 min after the reference dose. The effect was considered positive if the analgesic reduced the response to the test dose of bradykinin to less than the response to the reference dose, without reducing that to histamine

Drug	Concentration of drug in μg/ml. perfusion fluid	Number of experiments	Effect on the response to bradykinin
Calcium acetylsalicylate	7	1	0
	15	1	0
	30	3	0
	40	1	0
Sodium salicylate	40	1	0
	80	2	0
Sodium phenylbutazone	40	1	0
	80	1	0
Phenazone	40	2	+
Amidopyrine	20	3	0 (1), + (2)
	40	1	0
	80	1	+

phenylbutazone were inactive in concentrations up to 40 to 80 μg/ml. In this isolated lung preparation, however, phenazone (40 μg/ml.) showed a positive effect, whereas the influence of amidopyrine on the response to bradykinin was variable. As in two out of five cases amidopyrine had no inhibitory effect and in many other experiments the lungs soon became oedematous accompanied by a loss of sensitivity, the observed inhibitory effects might be ascribed to non-specific effects. Therefore, the effects of all these analgesics were also investigated using the isolated guinea-pig lung preparation according to the method of Bhattacharya & Delaunois (1955).

Isolated guinea-pig lungs with vascular perfusion

The dose of bradykinin which in this isolated lung preparation caused a definite increase in airway resistance ranged from 2 to 6 μg ; doses in this range also caused a strong inhibition of the rate of flow in the lung vessels. The sensitivity of the bronchi to histamine on a weight basis was of the same order. When bradykinin and histamine was administered alternately the preparation soon became oedematous. This is to be expected since both agents increase the capillary permeability. Since it was shown in preliminary experiments that the analgesics investigated in the concentrations used did not inhibit the bronchoconstrictor effect of histamine, control determinations with histamine were omitted in most experiments.

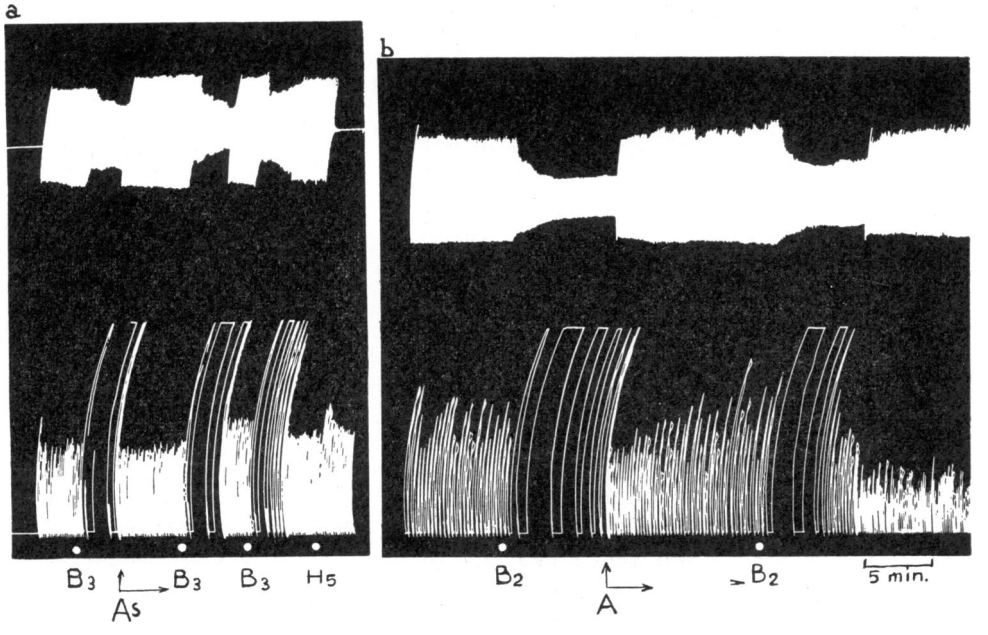

Fig. 3. Resistance in airway (upper curve) and in the blood vessels (lower curve) of guinea-pig lungs *in vitro* (Bhattacharya and Delaunois preparation). Responses to bradykinin and histamine. Failure of calcium acetylsalicylate (*a*) and amidopyrine (*b*) to reduce response to bradykinin. B, 2 and 3 μg bradykinin ; H, 5 μg histamine. Starting at arrow either calcium acetylsalicylate (As, Fig. 3,*a*) or amidopyrine (A, Fig. 3,*b*) was present in the perfusion fluid in a concentration of 40 μg/ml.

In these experiments the test dose of bradykinin was identical to the reference dose. As in six control experiments the bronchoconstrictor responses to a second dose of bradykinin were 38, 11, 44, 34, 36 and 44% smaller than to a first dose, the effect of an analgesic was considered positive if the bronchoconstrictor response to the test dose of bradykinin was less than half the response to the reference dose. Fig. 3,*a* shows a typical experiment in which the influence of calcium acetylsalicylate (40 μg/ml.) was investigated. A dose of 3 μg bradykinin caused a definite increase in resistance in the airway and in the blood vessels. Both effects were not affected by calcium acetylsalicylate. Fig. 3,*b* shows that amidopyrine (40 μg/ml.) also failed to reduce the increased resistance both in the airway and in the pulmonary vessels due to bradykinin. The results of these experiments are summarized in Table 2. Calcium acetylsalicylate, sodium salicylate, sodium phenylbutazone and phenazone in concentrations of 40 to 80 μg/ml. did not block the bronchoconstrictor response to bradykinin.

Amidopyrine in a concentration of 20 μg/ml. was ineffective in two experiments; the effect of amidopyrine 40 μg/ml. was positive in one out of three experiments.

TABLE 2

INFLUENCE OF A NUMBER OF ANALGESIC ANTIPYRETIC DRUGS ON THE INCREASE IN RESISTANCE OF THE AIRWAY OF ISOLATED GUINEA-PIG LUNGS DUE TO BRADYKININ INJECTED INTO THE PULMONARY ARTERY

After a reference dose of bradykinin, perfusion with the fluid containing the analgesic under test was started. The test dose of bradykinin, equal to the reference dose, was administered 30 min later. The effect was considered positive if the analgesic reduced the response to the test dose of bradykinin to less than half that to the reference dose

Drug	Concentration of drug in μg/ml. perfusion fluid	Number of experiments	Effect on the response to bradykinin
Calcium acetylsalicylate	40 μg	4	0
	80 μg	1	0
Sodium salicylate	40 μg	4	0
Sodium phenylbutazone	40 μg	4	0
Phenazone	40 μg	9	0
Amidopyrine	20 μg	2	0
	40 μg	3	0 (2), + (1)

Whole animal preparations

The experiments with the isolated lung preparations have shown that the analgesics used do not block the bronchoconstrictor response to bradykinin *in vitro*. This finding is in contrast to the effects *in vivo* described by Collier & Shorley (1960, 1963). Therefore, the *in vivo* system was reinvestigated. In nine guinea-pigs anaesthetized with urethane (625 mg/kg intraperitoneally and 625 mg/kg intramuscularly) the intravenous threshold dose of bradykinin, causing an increased resistance to inflation of the lungs, was determined. After this threshold was established calcium acetylsalicylate was injected intravenously and the dose of bradykinin needed to produce a response of about the same magnitude as that obtained by the threshold dose was determined. This bradykinin dose was expressed as a multiple of the threshold dose: the dose-ratio (Gaddum, Hameed, Hathway & Stephens, 1955). The results are presented in Table 3.

TABLE 3

INFLUENCE OF CALCIUM ACETYLSALICYLATE ON THE INCREASED RESISTANCE TO INFLATION OF LUNGS CAUSED BY BRADYKININ IN GUINEA-PIGS ANAESTHETIZED WITH URETHANE

Equi-effective doses of bradykinin were determined before (threshold dose) and after (test dose) intravenous injection of 2 mg/kg and 8 mg/kg calcium acetylsalicylate. The test dose was expressed as a multiple of the threshold dose (dose-ratio)

Guinea-pig no.	Threshold dose of bradykinin (μg/kg)	Dose-ratios after calcium acetylsalicylate	
		2 mg/kg	8 mg/kg
1	1·6	2	2
2	0·4	2	16
3	1·6	1	4
4	3·2	1	—
5	4·0	2	4
6	0·4	4	8
7	0·8	4	4
8	2·0	4	4
9	8·0	1	1
Average	2·4 μg/kg	2·3	4·7

This table shows that there is a great variation in the sensitivity for bradykinin, threshold doses ranging from 0.4 to 8.0 μg/kg. The dose-ratios after 2 mg of calcium acetylsalicylate per kg range from one to four and therefore are about four to eight times lower than those found by Collier & Shorley (1960, 1963). Furthermore, Table 3 shows that in four out of eight experiments identical dose-ratios were found after 2 mg/kg and 8 mg/kg calcium acetylsalicylate.

In guinea-pigs anaesthetized with a combination of sodium pentobarbitone (50 mg/kg, intraperitoneally) and sodium barbitone (160 mg/kg, intravenously) instead of urethane, the potency of calcium acetylsalicylate (2 mg/kg) was much higher. Table 4 shows that in seven out of nine experiments the dose-ratio for bradykinin was 16 or more. In five of these experiments a second dose of 8 mg/kg of calcium acetylsalicylate was not followed by a further increase in dose-ratio.

TABLE 4

INFLUENCE OF CALCIUM ACETYLSALICYLATE ON THE INCREASED RESISTANCE TO INFLATION OF LUNGS CAUSED BY BRADYKININ IN GUINEA-PIGS ANAESTHETIZED WITH BARBITURATES

Equi-effective doses of bradykinin were determined before (threshold) dose and after (test dose) intravenous injection of 2 mg/kg and 8 mg/kg calcium acetylsalicylate. The test dose was expressed as a multiple of the threshold dose (dose-ratio)

Guinea-pig no.	Threshold dose of bradykinin (μg/kg)	Dose-ratios after calcium acetylsalicylate	
		2 mg/kg	8 mg/kg
1	0·8	16	—
2	0·8	2	8
3	1·0	16	16
4	1·0	16	32
5	1·0	16	16
6	2·4	16	—
7	2·4	16	16
8	4·8	4	4
9	0·3	64	64
Average	1·6 μg/kg	18·4	22·3

TABLE 5

INFLUENCE OF VAGOTOMY ON THE RESPONSE OF GUINEA-PIG LUNGS *IN VIVO* TO BRADYKININ AND ON THE POTENCY OF CALCIUM ACETYLSALICYLATE IN SUPPRESSING THIS RESPONSE

The guinea-pigs were anaesthetized with barbiturates. The doses of bradykinin given after 2 and 8 mg calcium acetylsalicylate/kg respectively, needed to produce a response of about the same height as that to the threshold dose before calcium acetylsalicylate, were determined and expressed as dose-ratios (multiples of threshold dose after vagotomy)

Guinea-pig no.	Threshold dose of bradykinin (μg/kg)		Dose-ratios after calcium acetylsalicylate in vagotomized animals	
	before vagotomy	after vagotomy	2 mg/kg	8 mg/kg
1	0·4	1·6	8	8
2	2·6	5·2	4	—
3	1·2	1·2	4	4
4	1·6	1·6	8	8
5	2·4	4·8	2	2
6	1·6	6·4	2	2
7	2·1	4·2	2	2
8	0·8	1·6	8	8
9	1·2	4·8	2	2
Average	1·5 μg/kg	3·5 μg/kg	4·4	4·5

The effect of intravenous bradykinin was also studied before and after vagotomy in nine guinea-pigs anaesthetized with the barbiturate mixture. Table 5 shows that in two experiments the response to bradykinin was not modified, whereas in seven experiments two to four times more bradykinin was needed after vagotomy to obtain a response of the same magnitude as before vagotomy. In these vagotomized animals the antibradykinin effect of an intravenous dose of 2 mg/kg calcium acetylsalicylate was on the average one-fourth of that in non-vagotomized guinea-pigs (compare Tables 4 and 5), while a dose of 8 mg/kg calcium acetylsalicylate was not followed by a further increase in bradykinin suppressing activity.

DISCUSSION

Collier & Shorley (1960) found that the analgesic antipyretic drugs specifically antagonized the increase in resistance to inflation of guinea-pig lungs *in vivo* caused by bradykinin. They stated that the dose of bradykinin required to overcome antagonism by calcium acetylsalicylate increased with the dose of acetylsalicylate given, the ratio being approximately constant. Collier (1962) suggested that guinea-pig bronchial muscle contains receptors for bradykinin bronchoconstriction which are blocked by acetylsalicylate and other analgesic antipyretic drugs.

In the present investigation, the finding of Collier & Shorley (1960, 1963) that a dose of 2 mg/kg calcium acetylsalicylate blocked the increase in resistance to inflation of the lungs caused by a small dose of bradykinin was confirmed. In our experiments, however, this antibradykinin effect of calcium acetylsalicylate was influenced by the anaesthetic used. In guinea-pigs anaesthetized with urethane, we found a low antibradykinin effect of 2 mg/kg calcium acetylsalicylate, whereas in most animals anaesthetized with a combination of sodium pentobarbitone and sodium barbitone the effect was four to eight times greater. Moreover, in most of these experiments a dose of 8 mg/kg calcium acetyl-

salicylate was not more effective than the dose of 2 mg/kg. From these findings it is evident that the antibradykinin effect of calcium acetylsalicylate is in most cases already maximal at a dose of 2 mg/kg. This notion is consistent with that of Gjuriš & Westermann (1965) who showed that acetylsalicylic acid abolishes the action of small doses only of bradykinin.

In guinea-pigs anaesthetized with barbiturates vagotomy had little effect on the threshold dose of bradykinin. In these vagotomized animals the antibradykinin effect of 2 mg/kg calcium acetylsalicylate was about a quarter of that found in non-vagotomized animals under the same anaesthesia. This observation is at variance with the statement of Collier & Shorley (1960) that vagotomy did not affect the potency of calcium acetylsalicylate.

In isolated guinea-pig lung preparations, calcium acetylsalicylate, sodium salicylate, sodium phenylbutazone, phenazone and amidopyrine in concentrations of 40 μg/ml. perfusion fluid did not suppress the bronchoconstriction due to bradykinin.

Although in the isolated lung preparation with vascular perfusion plasmakininase is absent, the dose of bradykinin needed to produce bronchoconstriction was higher than the intravenous dose causing increased resistance *in vivo*. In these isolated lungs the bronchoconstrictor response to bradykinin was accompanied by a marked constriction of the lung vessels. This effect was also not suppressed by the analgesics used.

The findings that the inhibitory influence of calcium acetylsalicylate on the response to bradykinin of guinea-pig lungs *in vivo* is dependent on the anaesthetic used and is abolished for the greater part by vagotomy suggest that the effect of calcium acetylsalicylate is indirect, and probably mediated by the central nervous system. The fact that calcium acetylsalicylate and the other analgesics tested do not suppress either the bronchoconstrictor or the vasoconstrictor effect of bradykinin in isolated guinea-pig lung preparations, is in accordance with this assumption.

SUMMARY

1. The effect of a number of analgesic antipyretic drugs on the response of the guinea-pig lung to bradykinin has been investigated *in vitro* and *in vivo*.

2. Relatively high doses of bradykinin were needed to cause bronchoconstriction *in vitro*. This effect was accompanied by a notable constriction of the perfused lung vessels. Calcium acetylsalicylate, sodium salicylate, phenylbutazone, amidopyrine and phenazone had no effect on either of these responses.

3. The inhibitory effect of calcium acetylsalicylate on the increased resistance to inflation of the lungs *in vivo* after small doses of bradykinin varied with the anaesthetic used and was reduced by vagotomy. This inhibitory effect was marked at a dose of 2 mg/kg. On increasing the dose to 8 mg/kg the effect remained practically unchanged.

4. It is concluded that the analgesics used inhibit the effect of bradykinin on the guinea-pig lungs *in vivo* by an effect on the central nervous system.

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