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Antagonism of anti-inflammatory drugs on bradykinin-induced increase of capillary permeability

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Phenylbutazone, sodium salicylate, calcium acetylsalicylate and sodium mefenamate each antagonize the bradykinin-induced increase of capillary permeability in the rat paw. The method described was not affected to any extent by urethane anaesthesia nor by mecamylamine, papaverine, reserpine or chlorothiazide. Hexobarbitone partially inhibited the bradykinin effect, as did bretylium and acetic acid.

A possible method for evaluating the anti-inflammatory action of drugs is to test them as specific inhibitors of the humoral mediators of inflammation such as bradykinin. This plasma kinin provokes vasodilatation, increased capillary permeability, pain, and accumulation and migration of leucocytes (Lewis, 1963, 1964; Rocha e Silva, 1964). Bradykinin, when administered intravenously to guinea-pigs, also increases resistance of the lungs to inflation (Collier, 1963). The bronchospasm is inhibited by drugs like salicylate and phenylbutazone (Collier & Shorley, 1960, 1963). Increase of capillary permeability is an action of primary importance in the development of the inflammatory reaction; for this reason it seemed of interest to examine the effect of some anti-inflammatory drugs on the capillary permeability induced by bradykinin in the rat paw and to assess to what extent non-specific pharmacological effects interfered with this action of bradykinin.

Experimental

METHODS

Female Sprague Dawley rats weighing about 150 g were used.

The drugs (phenylbutazone, sodium salicylate, calcium acetylsalicylate, sodium mefenamate) were administered intraperitoneally dissolved in saline; 1 hr later bradykinin, histamine, or 5-hydroxytryptamine (5-HT) was injected dissolved in saline (pH 6·5–7·5) in a volume of 0·05 ml through a 26-gauge needle into the plantar tissue of the posterior right paw. Immediately after, azovan blue 1% solution (5 ml/kg) was administered intravenously. One hr later the animals were killed by exsanguination, and the paws were amputated at tarso-crural level and weighed. The dye content of the tissues was determined as described by Beach & Steinetz (1961).

Results

Phenylbutazone, sodium salicylate, calcium acetylsalicylate and sodium mefenamate antagonized the bradykinin-induced increase of capillary permeability. The effect of these drugs could be overcome by higher doses of bradykinin, the amount needed to produce an effect similar to that observed in control animals (i.e. the same content of dye in the tissues of the rat paw) increasing with the dose of antagonist administered.

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Phenylbutazone, sodium salicylate, and calcium acetylsalicylate also antagonized the effects of histamine and 5-HT, but to a lesser extent, whereas sodium mefenamate at higher dose enhanced these effects. A similar result was described by Collier & Shorley (1963) in experiments on guinea-pig lungs *in vivo*. Table 1 shows the results obtained.

TABLE 1. ANTAGONISM OF PHENYLBUTAZONE, SODIUM SALICYLATE, CALCIUM ACETYLSALICYLATE AND SODIUM MEFENAMATE TO THE BRADYKININ, HISTAMINE, OR 5-HT INDUCED INCREASE OF THE CAPILLARY PERMEABILITY IN RAT PAW

Antagonist	Dose mg/kg i.p.	Bradykinin (μg) needed to restore response (95% fiducial limits)*	Histamine	5-HT
Phenylbutazone	50	0·11 (0·07–0·15)	—	—
,,	100	0·24 (0·19–0·27)	1·23 (0·96–1·57)	0·13 (0·09–0·17)
,,	200	0·29 (0·24–0·32)	2·56 (1·97–3·12)	0·20 (0·15–0·24)
Sodium salicylate	100	0·07 (0·05–0·12)	—	—
,,	200	0·28 (0·20–0·36)	1·47 (1·05–2·12)	0·07 (0·04–0·90)
,,	400	0·41 (0·36–0·48)	4·56 (3·72–5·24)	0·23 (0·19–0·30)
Calcium acetylsalicylate	100	0·07 (0·04–0·10)	—	—
,,	200	0·24 (0·18–0·30)	1·36 (1·02–1·85)	0·09 (0·10–0·13)
,,	400	0·41 (0·35–0·48)	3·95 (3·15–4·71)	0·29 (0·22–0·37)
Sodium mefenamate	50	0·10 (0·07–0·16)	1·49 (1·18–1·97)	0·08 (0·04–0·10)
,,	100	0·20 (0·13–0·24)	—	—
,,	200	0·21 (0·16–0·26)	0·46 (0·32–0·68)	0·02 (0·01–0·05)

* After obtaining in untreated rats a standard response (a threefold increase of azovan blue dye content relative to controls) to 0·05 μg of bradykinin or to1·0 μg of histamine or to 0·05 μg of 5-HT, a dose of antagonist was administered and the dose of bradykinin or histamine, or 5-HT giving a response comparable to the standard was then determined. The results reported are the means of 20 determinations.

Section of the spinal cord at various levels and general anaesthesia are known to reduce the development of local oedemas (Domenjoz, 1954). Hexobarbitone (70 mg/kg i.p.) produced a threefold increase in the dose of bradykinin needed to produce an extravasation of azovan blue dye comparable to that observed in non-anaesthetized rats, but the barbiturate did not affect the antagonistic potency of the anti-inflammatory drugs examined. In fact the ratios between the threshold dose of bradykinin before and after administration of test compounds in normal and anaesthetized rats were similar Urethane did not interfere with the action of bradykinin. The results are in Table 2.

TABLE 2. INFLUENCE OF URETHANE OR HEXOBARBITONE ANAESTHESIA ON THE "BLUEING" OF THE RAT PAW CAUSED BY BRADYKININ AND ON THE ANTAGONISTIC EFFECT OF ANTI-INFLAMMATORY DRUGS

Treatment	Threshold dose of bradykinin μg*		
	Normal	Urethane 700 mg/kg i.p.	Hexobarbitone 70 mg/kg i.p.
Saline 3 ml/kg i.p.	0·05 (0·02–0·09)	0·06 (0·02–0·09)	0·13 (0·07–0·02)
Phenylbutazone 200 mg/kg i.p.	0·28 (0·21–0·37)	0·31 (0·02–0·42)	0·59 (0·32–0·78)
Sodium salicylate 200 mg/kg i.p.	0·28 (0·19–0·37)	0·30 (0·21–0·43)	0·73 (0·06–0·95)
Calcium acetylsalicylate 200 mg/kg i.p.	0·23 (0·16–0·32)	0·25 (0·18–0·35)	0·69 (0·49–0·85)
Sodium mefenamate 200 mg/kg i.p.	0·20 (0·13–0·36)	0·23 (0·14–0·39)	0·59 (0·29–0·74)

* The threshold dose is the dose of bradykinin which provokes a threefold increase in azovan blue dye content of the rat paw relative to control animals. In brackets the range of dose in 10 determinations.

As a number of non-specific pharmacological effects are known to interfere with development of local oedemas (Garattini, Jori & others,

1964) the effects of an antihypertensive drug (bretylium), a ganglion-blocking drug (mecamylamine), a vasodilator (papaverine), a catecholamine-depleting agent (reserpine), a diuretic drug (chlorothiazide), and a general irritant (acetic acid) have been evaluated on the bradykinin-induced blueing of the rat paw. The results in Table 3 show that, of the

TABLE 3. EFFECT OF VARIOUS AGENTS ON THE INCREASED CAPILLARY PERMEABILITY INDUCED BY BRADYKININ

Compounds	% Inhibition of azovan blue dye extravasation in rat paw induced by bradykinin (0·05 μg)	No. of experiments
Bretylium 20 mg/kg i.p.	47 (32–59)*	15
Mecamylamine 5 mg/kg s.c.	7 (2–13)	15
Papaverine 10 mg/kg i.p.	8 (3–16)	15
Reserpine 5 mg/kg i.p. (for 3 consecutive days)	12 (7–19)	20
Chlorothiazide 20 mg/kg s.c.	11 (5–19)	20
Acetic acid 0·6–10 ml/kg i.p.	65 (31–83)	15

* Figures in parentheses give range.

compounds examined, bretylium and acetic acid inhibit significantly the extravasation of dye induced by bradykinin whereas the other drugs were without effect.

Discussion and conclusion

The anti-inflammatory drugs examined inhibited the bradykinin-induced increase of capillary permeability.

Over the dose range used, the order of antibradykinin potency was: phenylbutazone > mefenamate > salicylate > acetyl salicylate. This order agrees with the findings of Collier & Shorley (1963) in the guinea-pig bronchospasm test and is similar to that obtained in the skin erythema test (Winder, Wax & others, 1963).

The results reported here also demonstrate differential effects on different agonists when the dose of antagonist is raised.

The capillary permeability induced by bradykinin appears to be less sensitive to systemic pharmacological effects than do local oedemas induced by various agents; changes in cardiovascular function, depletion of tissue catecholamines and induced diuresis do not affect the test. The inhibitory action of bretylium could be ascribed to its enhancing effect on free or circulating adrenaline and noradrenaline which are likely to be acting as local anti-inflammatory hormones (Spector & Willoughby, 1964). The effect of acetic acid is a particular aspect of a known "inhibitory" effect of the general irritation on the inflammatory processes (Garattini & others, 1964).

Urethane anaesthesia does not influence the capillary permeability induced by bradykinin, whereas hexobarbitone anaesthesia partially inhibits this effect of bradykinin, possibly through depression of the central

nervous system, the role of which in the inflammatory response has been pointed out by Chapman & Goodell (1966). However the barbiturate does not modify the antagonistic action of anti-inflammatory drugs expressed in terms of dose-ratios.

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