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THE EFFECT OF ANTI-INFLAMMATORY DRUGS ON VASCULAR SMOOTH MUSCLE

BY

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Histamine, 5-hydroxytryptamine (5-HT) and bradykinin have been implicated as mediators of inflammatory reactions in which fluid and plasma protein exude from the small blood vessels (Spector & Willoughby, 1963). Haddy (1960) has shown that the exudation produced by histamine is at least partly due to simultaneous dilatation of terminal arterioles and constriction of collecting venules. Several anti-inflammatory drugs antagonize venous constriction produced by histamine, 5-HT or bradykinin and it has been suggested that this might account for their anti-inflammatory effects (Northover, 1967a, b). The experiments to be described involve constant-rate perfusion of isolated arterial and venous segments, enabling numerous highly reproducible constrictor responses to be obtained. A wide range of substances which exert anti-inflammatory action *in vivo* has been tested as antagonists of vascular constriction. An account of this work was presented to the British Pharmacological Society Meeting in April, 1967.

METHODS

Isolated rat anterior mesenteric artery and vein

The dissection and cannulation of the artery and vein have previously been described in detail (Northover, 1967b). The cannulated blood vessel was connected *via* a heating coil (38° C) to a supply of aerated Tyrode solution. Perfusate which flowed from the cut ends of the branches of the blood vessel was collected in a small overflow bath (38° C) surrounding the tissue, as described by de la Lande & Harvey (1965). In this way both intimal and adventitial surfaces were bathed with perfusate. The rate of perfusion was controlled with a roller pump (Watson Marlow MHRE) and was adjusted at the beginning of the experiment to give a perfusion pressure of 10 cm water in the case of the vein and 30 cm water in the case of the artery. The perfusion pressure was recorded with a manometer, the float of which marked a smoked paper kymograph (Fig. 1). A change of perfusion pressure of 1 cm water caused the displacement of 0.48 ml. of fluid in the manometer. The flow rate for both artery and vein was usually in the range 4–10 ml./min and was held constant for the duration of a particular experiment. Constriction of the blood vessels causes a rise in perfusion pressure. Constrictor agents were dissolved in 0.05–0.1 ml. Tyrode solution and injected into the stream of perfusate close to the cannula.

Isolated guinea-pig anterior mesenteric vein

A technique similar to the one described for the rat anterior mesenteric vein was used, except that the rate of perfusion was usually in the range 8–14 ml./min.

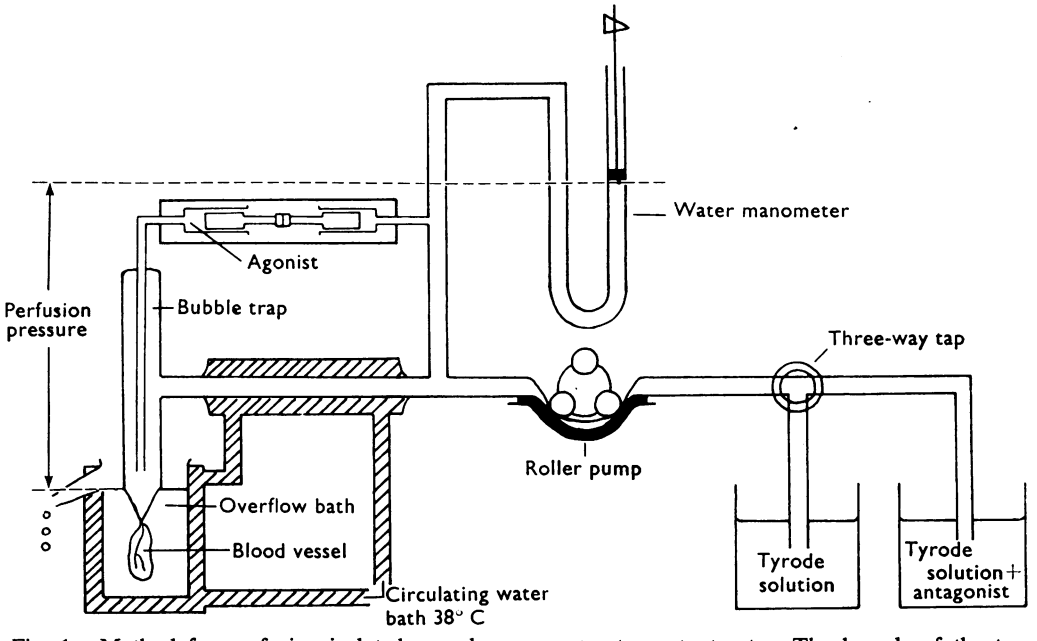

Fig. 1. Method for perfusing isolated vascular segments at constant rate. The barrels of the two syringes were clamped to a board. The two plungers were moved in the same direction so that the constrictor drug was injected into the stream of perfusate from one syringe and an equal volume of perfusate was withdrawn into the other. This avoids an injection artefact.

Isolated guinea-pig ileum and vas deferens

A vas deferens or a 2–3 cm length of the terminal ileum was obtained from a freshly killed guinea-pig and suspended in a 20 ml. organ bath containing aerated Tyrode solution at 35° C. Longitudinal contractions were recorded on a smoked paper kymograph using an isotonic lever and frontal writing point giving approximately 30-fold magnification.

Tyrode solution

Unless otherwise stated the Tyrode solution had the following composition: NaCl 138 mM, KCl 2.74 mM, $NaHCO_3$ 10.1 mM, $MgCl_2$ 1.06 mM, $CaCl_2$ 0.582 mM, NaH_2PO_4 0.416 mM, glucose 5.68 mM.

For experiments in which the smooth muscle was "depolarized" the perfusate had the following composition: K_2SO_4 92 mM, $KHCO_3$ 10.0 mM, $MgCl_2$ 1.06 mM, NaH_2PO_4 0.416 mM, glucose 5.68 mM, with or without $CaCl_2$ 3.49 mM.

Anti-inflammatory drugs

The readily-soluble compounds were dissolved directly in Tyrode solution. The relatively water-insoluble substances of an acidic nature were first dissolved in 2% sodium carbonate solution to form the sodium salts. These solutions were then diluted to volume with Tyrode solution. The pH of the Tyrode solution both before and after adding the drug was adjusted to pH 7.6. Hydrocortisone was first dissolved in ethanol and the alcoholic solution then diluted 500-fold with Tyrode solution. The presence of 0.2% ethanol in the perfusate did not affect the responses of the blood vessels.

Other drugs

The doses of histamine hydrogen phosphate, 5-hydroxytryptamine creatine sulphate, (−)-adrenaline bitartrate, and (−)-noradrenaline bitartrate were calculated as their respective bases. Synthetic bradykinin was kindly supplied as a solid by Dr. H. O. J. Collier.

RESULTS

Rat anterior mesenteric vein

The vein is constricted by small doses of adrenaline or noradrenaline (0.1–1 μg), by rather larger doses of 5-HT or bradykinin (2–20 μg), but only by very large doses of histamine (20–200 μg). With the exception of histamine, responses to which were variable, the other drugs gave responses which could be reproduced at 15 min intervals for 2–6 hr. If the calcium chloride concentration exceeded 0.4–0.6 mM, spontaneous and progressive spasm occurred after several hours of perfusion, and if the concentration was less than 0.4 mM the constrictor responses of the blood vessel to drugs were reduced.

Addition of indomethacin to the perfusate inhibited the responses to all the constrictor agents tested to a similar degree (Fig. 2). The inhibitory effect of indomethacin depended upon its concentration in the perfusate, as shown in Fig. 3. In order to calculate the

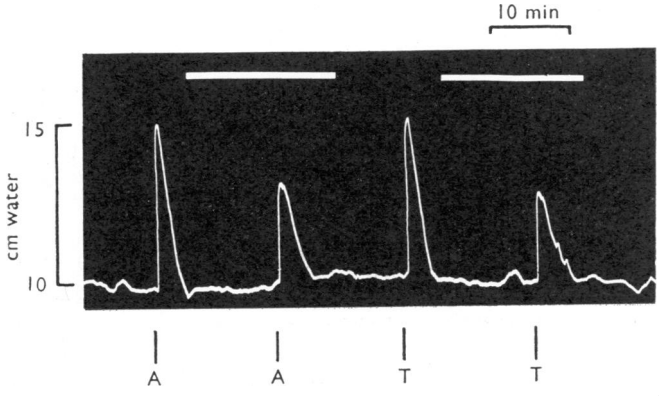

Fig. 2. Effect of indomethacin (1.5 mg/100 ml.) on constriction of the rat anterior mesenteric vein in response to 1 μg adrenaline (A) and to 8 μg 5-HT(T). Indomethacin was present in the perfusate during the horizontal bars.

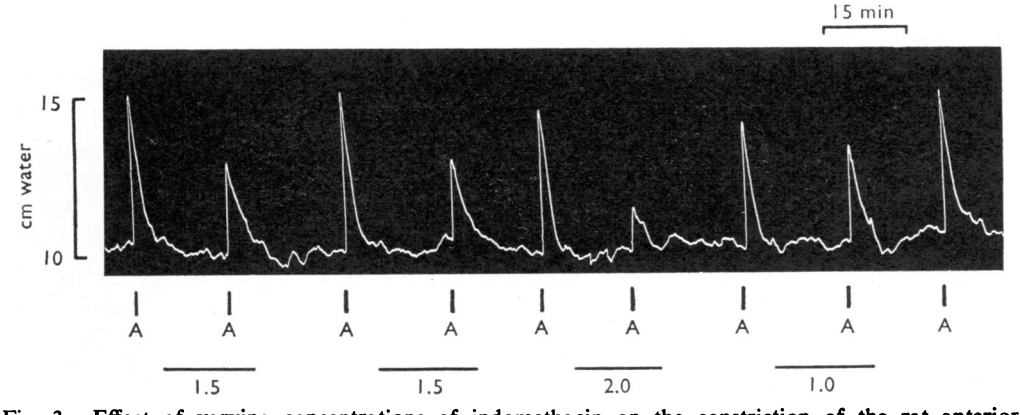

Fig. 3. Effect of varying concentrations of indomethacin on the constriction of the rat anterior mesenteric vein in response to 0.7 μg of adrenaline (A). Indomethacin (concentration expressed in mg/100 ml.) was present in the perfusate during the horizontal bars.

degree of inhibition the mean of the constrictor responses obtained immediately before and after indomethacin treatment was used as the control. The constrictor response obtained during indomethacin treatment was expressed as a percentage of the control, and is termed the percentage response. Figure 4 shows that the percentage response is

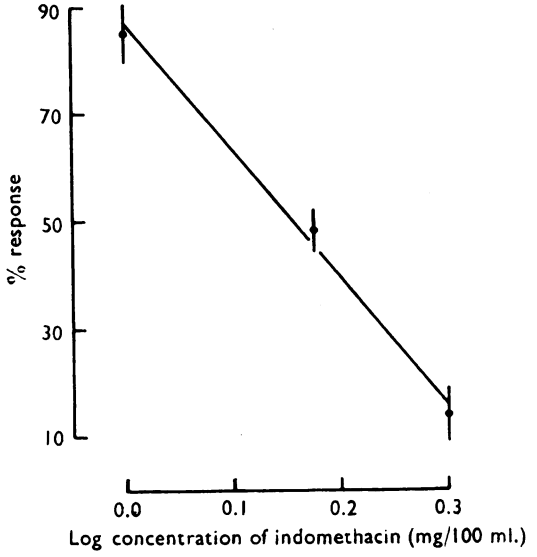

Fig. 4. Effect of varying concentrations of indomethacin on the constrictor response of rat anterior mesenteric vein to adrenaline. The vertical bars represent the standard errors of the means.

inversely proportional to the logarithm of the concentration of indomethacin in the perfusate. From such a graph the concentration of antagonist required to produce 50% response can be calculated, and is termed the ID_{50} concentration. Other anti-inflammatory drugs were tested in a similar way and the results are given in Table 1.

Rat anterior mesenteric artery

The artery is constricted by small doses of adrenaline, noradrenaline or 5-HT (0.1–1 μg), but histamine and bradykinin have rather weak and inconsistent effects. Similar findings were reported by McGregor (1965). Reproducible constrictor responses could be obtained once every 5 min, but, for the sake of comparison with the results obtained from the vein preparation, successive doses were applied every 15 min. In this way it was possible to obtain consistent responses for at least 8 hr.

Many anti-inflammatory drugs reduced the constrictor responses of the artery, the extent of the antagonism being independent of the nature of the constricting agent. This is in agreement with the findings of Starr & West (1966). Table 1 gives the ID_{50} concentrations of a number of anti-inflammatory agents against adrenaline-induced constriction of the artery. The more potent anti-inflammatory drugs showed inhibitory effects on the vein in lower concentrations than on the artery, but this difference was not seen with less potent antagonists.

TABLE 1

EFFECT OF ANTI-INFLAMMATORY DRUGS ON CONSTRICTOR RESPONSES TO ADRENALINE IN RAT ANTERIOR MESENTERIC VEIN AND ARTERY PERFUSED AT CONSTANT RATE

95% confidence limits in parentheses.

Anti-inflammatory drug	ID_{50} concentrations (mg/100 ml.) Vein	Artery
Indomethacin	1·5 (1·3–1·7)	3·5 (3·2–3·8)
Ibufenac	7·6 (6·7–8·5)	9·0 (7·9–10·1)
2-Methoxyphenylacetic acid	>200	>200
α-(4-*tert*-Butylphenoxy) propionic acid	22 (19–25)	24 (15–33)
α-(4-*sec*-Butylphenoxy) propionic acid	28 (22–34)	25 (18–32)
α-(4-Phenylphenoxy) propionic acid	23 (17–29)	27 (20–34)
Flufenamic acid	0·51 (0·42–0·60)	1·8 (1·6–2·0)
Mefenamic acid	1·6 (1·2–2·0)	2·4 (2·3–2·5)
Anthranilic acid	>200	>200
Cinchophen	4·3 (3·6–5·0)	4·6 (4·1–5·1)
Oxycinchophen	1·8 (1·4–2·2)	8·0 (7·4–8·6)
Aminopyrine	5·0 (4·6–5·4)	29 (26–32)
Phenylbutazone	9·7 (8·4–11·0)	32 (27–37)
Oxyphenbutazone	8·6 (7·6–9·6)	35 (29–41)
p-Nitrophenylbutazone	12 (10–14)	19 (16–22)
Sulphinpyrazone	23 (18–28)	13 (10–16)
Phenazone	96 (86–106)	92 (80–114)
Chloroquine diphosphate	0·54 (0·47–0·61)	0·61 (0·54–0·67)
Hydroxychloroquine diphosphate	0·76 (0·65–0·87)	1·1 (1·0–1·2)
Desethylchloroquine dihydrochloride	0·23 (0·17–0·29)	0·45 (0·40–0·50)
Salicylic acid	81 (73–89)	83 (73–93)
5-Chlorosalicylic acid	48 (40–56)	12 (9–15)
3-Methylsalicylic acid	18 (15–21)	27 (22–32)
3,5-Dibromo-2,6-dihydroxybenzoic acid	2·1 (1·9–2·3)	2·6 (2·3–2·9)
3-Hydroxybenzoic acid 4-Hydroxybenzoic acid 2,4-Dihydroxybenzoic acid 2,5-Dihydroxybenzoic acid 2,6-Dihydroxybenzoic acid 3,5-Dihydroxybenzoic acid	>200	>200
2-(p-Methylsulphonylphenyl)imidazo-[1,2-α]-pyridine	0·97 (0·92–1·1)	0·80 (0·66–0·94)
Benzindamine hydrochloride	0·18 (0·14–0·22)	0·14 (0·10–0·18)
Oxolamine citrate	4·2 (3·6–4·8)	5·1 (4·3–5·9)
Sodium aurothiomalate	>5	>5
Hydrocortisone sodium succinate	>10	>10
Hydrocortisone	>10	>10

"Depolarized" smooth muscle

The rat anterior mesenteric vein was constricted by adrenaline almost as effectively when "depolarized" with K_2SO_4 as when perfused with ordinary Tyrode solution. The presence of calcium chloride in the perfusate (3.49 mM) was as necessary for obtaining responses of the "depolarized" muscle as it was when ordinary Tyrode solution was used. Cuthbert & Sutter (1965) have also reported that drug-induced contraction of venous smooth muscle is dependent upon calcium ions but independent of membrane

potential. Anti-inflammatory drugs which antagonized adrenaline-induced venous constriction in ordinary Tyrode were approximately equally effective when tested on the " depolarized " vein (Table 2). This suggests that anti-inflammatory drugs do not exert their action by preventing depolarization of the smooth muscle membrane.

TABLE 2

A COMPARISON OF THE EFFECTS OF ANTI-INFLAMMATORY DRUGS ON CONSTRICTOR RESPONSES TO ADRENALINE IN RAT ANTERIOR MESENTERIC VEIN PERFUSED AT CONSTANT RATE WITH TYRODE SOLUTION AND WITH " DEPOLARIZING " SOLUTION

95% confidence limits in parentheses.

Anti-inflammatory drug	ID_{50} concentrations (mg/100 ml.)	
	Tyrode solution	" Depolarizing " solution
Flufenamic acid	0·51 (0·42–0·60)	0·63 (0·50–0·76)
Indomethacin	1·5 (1·3–1·7)	1·4 (1·2–1·6)
Phenylbutazone	9·7 (8·4–11·0)	9·3 (8·5–10·1)

Cuthbert & Sutter (1965) have suggested that adrenaline and certain other smooth muscle contracting agents cause calcium ions to be released into the interior of the muscle cell, and that it is the intracellular calcium ions which are responsible for the shortening of the contractile proteins. In view of this suggestion, experiments were performed to test whether anti-inflammatory drugs interfere with calcium-induced vascular constriction. The rat anterior mesenteric vein was perfused with calcium-free " depolarizing " solution. Without interrupting the experiment the perfusate was changed to a " depolarizing " solution containing calcium chloride (3.49 mM), resulting in constriction. Partial relaxation of the constriction occurred spontaneously during continued perfusion with the calcium-containing solution, but complete relaxation occurred only after return to the calcium-free " depolarizing " solution. The experiment was repeated with flufenamic acid (1 mg/100 ml.) in the perfusate throughout, but this time the change from calcium-free to calcium-containing solution failed to constrict the vein. This suggests that flufenamate may prevent the entry of calcium into the cell.

Guinea-pig anterior mesenteric vein, terminal ileum and vas deferens

Collier (1963) has shown that anti-inflammatory drugs antagonize bradykinin-induced bronchoconstriction in the guinea-pig but not in other species tested. A similar species limitation does not, however, apply to the antagonism of constrictor responses in the perfused mesenteric vein. The guinea-pig mesenteric vein constricted in response to small doses of adrenaline, noradrenaline, histamine, or bradykinin (0.1–1 μg), and the responses were antagonized by several anti-inflammatory drugs in concentrations similar to those required on the rat mesenteric vein (Table 3). The inhibitory effect of anti-inflammatory drugs is exerted on several different types of smooth muscle. The isolated guinea-pig ileum contracted in response to small doses of histamine (0.05–0.2 μg) and the isolated guinea-pig vas deferens contracted in response to noradrenaline (1–5 μg). These contractions were reduced by the addition of indomethacin or phenylbutazone to the organ bath 2–5 min before the agonist. This inhibition was produced, however, only by concentrations of indomethacin or phenylbutazone considerably greater than were needed with guinea-pig mesenteric vein. No inhibition was obtained with indomethacin at less

than 3.5 mg/100 ml. or with phenylbutazone at less than 38 mg/100 ml. In order to reduce the size of the isotonic contractions of the ileum in response to histamine by 50% the concentrations of indomethacin and phenylbutazone required were 11 and 109 mg/100 ml. respectively. The concentrations of indomethacin and phenylbutazone required to reduce the size of the isotonic contractions of the vas deferens in response to noradrenaline were 12 and 101 mg/100 ml. respectively.

TABLE 3

A COMPARISON OF THE EFFECTS OF ANTI-INFLAMMATORY DRUGS ON CONSTRICTOR RESPONSES TO ADRENALINE IN RAT, AND HISTAMINE IN GUINEA-PIG ANTERIOR MESENTERIC VEINS

95% confidence limits in parentheses

Anti-inflammatory drug	ID_{50} concentrations (mg/100 ml.)	
	Rat	Guinea-pig
Indomethacin	1·5 (1·3–1·7)	0·97 (0·86–1·11)
Flufenamic acid	0·51 (0·42–0·60)	3·5 (3·1–3·9)
Aminopyrine	5·0 (4·6–5·4)	12 (10–14)
Phenylbutazone	9·7 (8·5–11·0)	14 (11–17)
Phenazone	96 (86–106)	21 (16–26)
5-Chlorosalicylic acid	48 (40–56)	38 (32–44)
Chloroquine diphosphate	0·54 (0·47–0·61)	0·49–0·44–0·54)

DISCUSSION

Several workers have reported that anti-inflammatory drugs of the analgesic-antipyretic type reduce constrictor responses of blood vessels. Probably the first were Jaques & Domenjoz (1950) who demonstrated that histamine reduced the rate of outflow of perfusate from an isolated rabbit ear perfused with an electrolyte solution. This response was greatly reduced by a number of anti-inflammatory drugs. Glover, Marshall & Whelan (1957) showed that 5-HT administered intra-arterially caused vasoconstriction in the human forearm, which was reduced in the presence of a plasma concentration of salicylate of 20 mg/100 ml. They ascribed the antagonism, however, to an indirect effect of salicylate. Strom & Coffman (1963) found that intravenously administered adrenaline or noradrenaline reduced blood flow in the intact human forelimb. These responses were smaller if the subject had ingested 3.6 g aspirin daily for the 2 days before the test. Greeff & Moog (1964) reported that histamine, 5-HT and several kinins produced both vasoconstriction and bronchoconstriction in the isolated perfused guinea-pig lung. Phenylbutazone and aspirin antagonized both vasoconstrictive and bronchoconstrictive effects of bradykinin, and antagonized the vasoconstrictive (but not the bronchoconstrictive) effect of histamine, but had no effect upon the responses to 5HT. Klupp & Konzett (1965) found that bradykinin constricted the pulmonary artery of the guinea-pig and that this was prevented if the animal was pretreated with phenylbutazone. Dhawan & Ahmad (1966) found that sodium salicylate treatment reduced the pressor effect of ephedrine in the anaesthetized dog, and Hauge, Lunde & Waaler (1966) found that phenylbutazone and sodium salicylate reduced vasoconstriction in blood-perfused rabbit lung caused by a variety of agents.

The reports mentioned so far do not enable responses of pre- and post-capillary vessels

to be examined separately. Starr & West (1966) have recently studied the responses of isolated perfused arterial segments. They showed that anti-inflammatory drugs of the analgesic-antipyretic type reduced the constrictor responses of arterial smooth muscle, and that the degree of antagonism was independent of the nature of the constrictor agent. The present findings with the rat anterior mesenteric artery confirm those of Starr & West, and, in addition, they demonstrate that other types of smooth muscle are inhibited non-specifically by anti-inflammatory drugs. Venous muscle tends to be more sensitive than arterial muscle to the more potent anti-inflammatory drugs, but venous and arterial muscles are almost equally sensitive to the less potent anti-inflammatory drugs. Smooth muscle from the ileum and vas deferens, however, is much less sensitive than vascular smooth muscle to inhibition with these drugs.

The concentrations of anti-inflammatory drugs required to antagonize venous constriction *in vitro* (Table 1) are within the range of plasma concentrations attained *in vivo* after the administration of doses which are sufficient to produce an anti-inflammatory effect. It is unlikely, however, that a concentration sufficient to antagonize contractions of the gastrointestinal tract or the vas deferens would be reached *in vivo*. Plasma concentrations of indomethacin of up to 6 mg/100 ml. have been reported by Hucker, Zacchei, Cox, Brodie & Cantwell (1966) after the intravenous administration of 10 mg/kg to the rat. Plasma concentrations in man, however, after oral administration of 200 mg indomethacin rose only to 0.74 mg/100 ml. Burns, Yu, Dayton, Gutman & Brodie (1960) administered 600 mg of either phenylbutazone or oxyphenbutazone intravenously to man, and found plasma concentrations of 7 mg/100 ml. 1 hr later. Kampmann & Frey (1966) report similar plasma concentrations in other species and with various routes of administration. The plasma concentration of salicylate required for an anti-inflammatory action in man is generally agreed to be approximately 35 mg/100 ml. (Ansell, 1963). Continuous oral administration of chloroquine or hydroxychloroquine for 3 months produced plasma concentrations of these drugs which gradually rose to 0.3 mg/100 ml. (McChesney & McAuliff, 1961). This slow rise in plasma concentration would account for the fact that these drugs usually need to be administered to man for several weeks before an anti-inflammatory effect is seen.

The extent to which venous constriction accounts for the exudation of plasma in acute inflammation is still not clear. Post-capillary vessel (venous) constriction will cause a rise in the pressure of blood in the small blood vessels unless pre-capillary (arterial) channels are constricted at least to the same extent. It is now established that veins constrict, under suitable circumstances, to many of the suggested mediators of inflammation (Sutter, 1965; Hughes & Vane, 1967) but such information is insufficient to establish that venous constriction occurs when the vessel is *in situ*. Reports of the effects of mediators of inflammation on venous channels *in situ* are conflicting, as discussed by Northover (1967b). Venous constriction is produced *in vitro* by smaller amounts of the mediator substances than are required to produce arterial constriction (Inchley, 1926; Moog & Fischer, 1964). When studied *in situ*, however, most of the possible mediators of inflammation dilate arterial channels (Haddy, 1960). By a combination of pre-capillary dilation and post-capillary constriction the pressure of blood in the small blood vessels will be greatly increased.

A rise in small vessel pressure tends to cause oedema, but the oedema fluid or

inflammation is characterized by a high plasma protein concentration. Rowley (1964) claims that venous constriction raises the pressure in the small blood vessels to such an extent that, in addition to causing oedema, the vessel walls lose the ability to retain plasma protein. Some support for this concept is provided by the present findings. If venous constriction is responsible for the exudative phase of inflammation, there should be a correlation between anti-inflammatory activity and the ability to prevent venous constriction. One way to study this correlation is in a series of closely related chemical compounds. Table 1 contains a number of hydroxybenzoic acid derivatives. Unlike salicylate (2-hydroxybenzoate), 3-hydroxy-, 4-hydroxy-, 2,4-dihydroxy-, 2,5-dihydroxy-, 2,6-dihydroxy- and 3,5-dihydroxybenzoic acids have been found inactive as anti-inflammatory agents (Smith, 1960; Northover, 1963, 1964; Whitehouse, 1965; Lim, 1966). Likewise only salicylate was active in the present test. Lightowler & Rylance (1963) and Northover (1963, 1964) have shown that certain halogen- and alkyl-substituted hydroxybenzoates are more potent anti-inflammatory agents than salicylate, and Table 1 shows that they are also more potent than salicylate in preventing vascular constriction. Another way to study the correlation between anti-inflammatory activity and the ability to prevent venous constriction is to examine a series of anti-inflammatory agents with widely differing chemical structure. Oxolamine (Silvestrini & Pozzatti, 1961), benzindamine (Silvestrini, 1965; de Gregorio, 1965) and 2-(p-methylsulphonylphenyl) imidazo-[1,2-α]-pyridine (Almirante, Polo, Mugaini, Provincialli, Rugarli, Biancotti, Gamba & Murman, 1965) are heterocyclic compounds of widely differing structure which inhibit inflammatory reactions. Table 1 shows that they also prevent vascular constriction.

Most of the drugs which are at present used for the treatment of inflammatory diseases in man inhibit vascular constriction. The adrenal corticosteroids and organic gold compounds, however, are exceptions, which suggests that their anti-inflammatory action is through a different mechanism.

SUMMARY

1. The isolated anterior mesenteric artery or vein of the rat and guinea-pig was perfused with Tyrode solution at constant rate. Constriction of the blood vessels causes a rise in perfusion pressure.

2. Small amounts of adrenaline, noradrenaline or 5-HT constricted both the vein and artery of the rat. Small amounts of bradykinin constricted the vein but larger amounts were necessary to constrict the artery.

3. The guinea-pig vein was constricted by small amounts of adrenaline, noradrenaline, histamine and bradykinin.

4. The constrictions of both rat and guinea-pig vessels were inhibited by several types of anti-inflammatory drugs, and any one of these drugs inhibited the responses to all the constrictor agents to about the same extent.

5. The concentrations of anti-inflammatory drugs required to antagonize vascular constriction are within the range of plasma concentrations attained in animals given doses sufficient to produce an anti-inflammatory effect.

6. Adrenaline still constricted the rat vein after "depolarization" with potassium sulphate, and the response to adrenaline was still inhibited by anti-inflammatory drugs.

7. Contractions of the isolated guinea-pig ileum in response to histamine and of the isolated guinea-pig vas deferens in response to noradrenaline were antagonized by anti-inflammatory drugs, but in higher concentrations than were required with vascular smooth muscle.

8. Hydrocortisone and sodium aurothiomalate failed to inhibit vascular constriction.

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REFERENCES

ALMIRANTE, L., POLO, L., MUGAINI, A., PROVINCIALLI, E., RUGARLI, P., BIANCOTTI, A., GAMBA, A. & MURMAN, W. (1965). Derivatives of imidazole. I. Synthesis and reactions of imidazo-[1,2-α]-pyridines with analgesic, antipyretic, and anticonvulsant activity. *J. med. Chem.*, **8**, 305–312.

ANSELL, B. M. (1963). Relationship of dosage and type of salicylate therapy to plasma levels in patients with rheumatoid arthritis. In *Salicylates*, ed. DIXON, A. St. J., MARTIN, B. K., SMITH, M. J. H. & WOOD, P. H. N., pp. 35–37. J. & A. Churchill, London.

BURNS, J. J., YU, T. F., DAYTON, P. G., GUTMAN, A. B. & BRODIE, B. B. (1960). Biochemical pharmacological considerations of phenylbutazone and its analogues. *Ann. N.Y. Acad. Sci.*, **86**, 253–262.

COLLIER, H. O. J. (1963). Antagonism by aspirin and like-acting drugs of kinins and SRS-A in guinea pig lung. In *Salicylates*, ed. DIXON, A. St. J., MARTIN, B. K., SMITH, M. J. H. & WOOD, P. H. N., pp. 120–126. J. & A. Churchill, London.

CUTHBERT, A. M. & SUTTER, M. C. (1965). The effect of drugs on the relation between the action potential discharge and tension in a mammalian vein. *Br. J. Pharmac. Chemother.*, **25**, 592–601.

DE GREGORIO, M. (1965). Some chemical data on benzindamine. In *Non-steroidal Anti-inflammatory Drugs*, ed. GARATTINI, S. & DUKES, M. N. G., pp. 422–429. Excerpta Medica Foundation, Amsterdam.

DE LA LANDE, I. S. & HARVEY, J. A. (1965). A new and sensitive bioassay for catecholamines. *J. Pharm. Pharmac.*, **17**, 589–593.

DHAWAN, B. N. & AHMAD, A. (1966). Effect of salicylates on vascular responses. *Jap. J. Pharmac.*, **16**, 338–341.

GLOVER, W. E., MARSHALL, R. J. & WHELAN, R. F. (1957). The antagonism of the vascular effects of 5-hydroxytryptamine by BOL 148 and sodium salicylate in the human subject. *Br. J. Pharmac. Chemother.*, **12**, 498–503.

GREEFF, K. & MOOG, E. (1964). Vergleichende Untersuchungen über die bronchoconstrictorische und gefabconstrictorische Wirkung des Bradykinins, Histamins und Serotonins an isolierten Lungenpräparaten. *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **248**, 204–215.

HADDY, F. J. (1960). Effect of histamine on small and large vessel pressures in the dog foreleg. *Am. J. Physiol.*, **198**, 161–168.

HAUGE, A., LUNDE, P. K. M. & WAALER, B. A. (1966). The effect of bradykinin, kallidin and eledoisin upon the pulmonary vascular bed of an isolated blood-perfused rabbit lung preparation. *Acta. physiol. scand.*, **66**, 269–277.

HUCKER, H. B., ZACCHEI, A. G., COX, S. V., BRODIE, D. A. & CANTWELL, N. H. R. (1966). Studies on the absorption, distribution and excretion of indomethacin in various species. *J. Pharmac. exp. Ther.*, **153**, 237–249.

HUGHES, J. & VANE, J. R. (1967). An analysis of the responses of the isolated portal vein of the rabbit to electrical stimulation and to drugs. *Br. J. Pharmac. Chemother.*, **30**, 46–66.

INCHLEY, O. (1926). Histamine shock. *J. Physiol., Lond.*, **61**, 282–293.

JAQUES, R. & DOMENJOZ, R. (1950). Histaminantagonistische Wirkung bei Pyrazolen und Antihistaminen. *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **212**, 124–134.

KAMPMANN, E. & FREY, H. H. (1966). Serum concentration of phenylbutazone in tests for antiphlogistic activity and under clinical treatment. *Nature, Lond.*, **209**, 519.

KLUPP, H. & KONZETT, H. (1965). Der Einfluss von Bradykinin und Kallidin auf den Druck in der Arteria pulmonalis. *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **249**, 479–485.

LIGHTOWLER, J. E. & RYLANCE, H. J. (1963). Substituted dihydroxybenzoic acids as possible anti-inflammatory agents. *J. Pharm. Pharmac.*, **15**, 633–638.

LIM, R. K. S. (1966). Salicylate analgesia. In *The Salicylates*, ed. SMITH, M. J. H. & SMITH, P. K., pp. 155–202. Wiley, New York.

MCCHESNEY, E. W. & MCAULIFF, J. P. (1961). Laboratory studies of the 4-aminoquinoline antimalarials: I. Some biochemical characteristics of chloroquine, hydroxychloroquine and SN-7718. *Antibiotics & Chemother.*, **11**, 800–810.

MCGREGOR, D. D. (1965). The effect of sympathetic nerve stimulation on vasoconstrictor responses in perfused mesenteric blood vessels of the rat. *J. Physiol., Lond.*, **177**, 21–30.

MOOG, E. & FISCHER, J. (1964). Vergleich der Wirkung verschiedener biogener Amine und Polypeptide auf den arteriellen und venösen Lungenkreislauf. *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **247**, 325.

NORTHOVER, B. J. (1963). The permeability to plasma proteins of the peritoneal blood vessels of the mouse, and the effect of substances that alter permeability. *J. Path. Bact.*, **85**, 361–370.

NORTHOVER, B. J. (1964). The action of anti-inflammatory compounds in mice with peritonitis. *J. Path. Bact.*, **87**, 395–404.

NORTHOVER, B. J. (1967a). The effect of anti-inflammatory drugs on the deposition of colloidal carbon in the walls of venules. *J. Path. Bact.*, in press.

NORTHOVER, B. J. (1967b). The antagonism between anti-inflammatory drugs and substances that constrict veins. *J. Path. Bact.*, in press.

ROWLEY, D. A. (1964). Venous constriction as the cause of increased vascular permeability produced by 5-hydroxytryptamine, histamine, bradykinin and 48/80 in the rat. *Br. J. exp. Path.*, **45**, 56–67.

SILVESTRINI, B. (1965). General implications from a study on the anti-inflammatory activityof benzindamine. In *Non-steroidal Anti-inflammatory Drugs*, ed. GARATTINI, S. & DUKES, M. N. G., pp. 180–189. Excerpta Medica Foundation, Amsterdam.

SILVESTRINI, B. & POZZATTI, C. (1961). Pharmacological properties of 3-phenyl-5-β-diethylaminoethyl-1,2,4-oxadiazole. *Br. J. Pharmac. Chemother.*, **16**, 209–217.

SMITH, P. K. (1960). The pharmacology of salicylates and related compounds. *Ann. N.Y. Acad. Sci.*, **86**, 38–63.

SPECTOR, W. G. & WILLOUGHBY, D. A. (1963). The inflammatory response. *Bact. Rev.*, **27**, 117–154.

STARR, M. S. & WEST, G. B. (1966). The effect of bradykinin and anti-inflammatory agents on isolated arteries. *J. Pharm. Pharmac.*, **18**, 838–840.

STROM, E. A. & COFFMAN, J. D. (1963). Effect of aspirin on circulatory responses to catecholamines. *Arthritis Rheumat.*, **6**, 689–697.

SUTTER, M. C. (1965). The pharmacology of isolated veins. *Br. J. Pharmac. Chemother.*, **24**, 742–751.

WHITEHOUSE, M. W. (1965). Some biochemical and pharmacological properties of anti-inflammatory drugs. *Progr. Drug Res.*, **8**, 321–429.