NEW ASPECTS OF THE MODE OF ACTION OF NONSTEROID ANTI-INFLAMMATORY DRUGS

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This review surveys recent work on the possible mode of action and on the effects of that group of drugs variously known as non-narcotic analgesics, nonsteroidal anti-inflammatory drugs, aspirin-like drugs, antiphlogistic acids, and so on. These drugs show, to a greater or lesser degree, the therapeutic properties of reducing pain, inflammation, and fever and the side effects of causing gastrointestinal irritation and renal pathology. Some aspects of the pharmacology of these drugs have previously been reviewed in this series (1, 2).

MECHANISM OF ACTION

Several different hypotheses have been advanced to explain the actions of aspirin-like drugs. These include an interference with oxidative phosphorylation (3), the displacement of an endogenous anti-inflammatory peptide from plasma protein (4–6), interference with the migration of leucocytes (7, 8), inhibition of leucocytic-phagocytosis (9), stabilization of lysosomal membranes (10), inhibition of the generation of lipoperoxides (11), and hyperpolarization of neuronal membranes (12–14). Recently, interest in this field was stimulated enormously by the discovery (15–17) that aspirin-like drugs inhibit the synthesis of prostaglandins. This discovery disclosed a mechanism of action of aspirin-like drugs and coincidentally provided a valuable way of exploring the involvement of prostaglandins in other pathological and physiological processes. In the first of the three papers Vane (15) tested aspirin-like drugs as direct inhibitors of prostaglandin synthetase, using a cell-free preparation of prostaglandin synthetase that synthesized radioactive prostaglandin E_2 and $F_{2\alpha}$ from tritium-labelled arachidonic acid (18). Indomethacin, aspirin, and salicy-

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late strongly inhibited the synthesis of prostaglandins. Concurrently, Smith & Willis (16), using human platelets, tested their own hypothesis that aspirin-like drugs interfere with prostaglandin production, perhaps by inhibiting phospholipase. Aspirin had no effect on platelet phospholipase activity but reduced prostaglandin release, as did indomethacin. Since prostaglandins cannot be detected in platelets before thrombin treatment, they deduced that aspirin was interfering with prostaglandin production. Ferreira et al (17) confirmed the results in a more complex system, the dog perfused spleen, which released prostaglandins when contracted by either catecholamines or nerve stimulation. This release, also due to fresh synthesis of prostaglandins, was abolished by indomethacin.

POTENCY OF ASPIRIN-LIKE DRUGS AGAINST PROSTAGLANDIN PRODUCTION

Since those three pioneering papers, the inhibitory action of aspirin-like drugs on prostaglandin production has been amply confirmed and demonstrated in almost all laboratory species and many other biological preparations (Table 1), using a variety of assay techniques ranging from bioassay through radiometric, spectrophotometric, polarographic, chromatographic, to mass-spectrometric. Several interesting points have emerged, associated both with the absolute and relative potencies of the aspirin-like drugs.

Absolute Potencies

The absolute potency of the anti-inflammatory drugs against prostaglandin synthetase varies with the enzyme preparation used and the experimental conditions. On enzyme from bovine seminal vesicles (51, 52) ID_{50} concentrations for indomethacin were 10–100 times greater than those on synthetase from dog spleen or guinea pig lung. But the concentrations of arachidonic acid substrate used were also 15–30 times higher and the ID_{50} of indomethacin against sheep seminal vesicle enzyme varies directly with the substrate concentration (52). Since prostaglandin generation can be detected at much lower concentrations by bioassay than by chemical or physicochemical assay, absolute potency of aspirin-like drugs may also appear to vary with the type of assay used. Another factor that seems to influence activity is the way in which the enzyme is prepared. For instance, fluorindomethacin (38) and indomethacin (41) competitively inhibit microsomal enzyme preparations from sheep and bovine seminal vesicles; in contrast, on acetone-dried powder preparation of sheep vesicular glands, the inhibitory effect was irreversible and increased by pre-incubation (39)

Relative Potencies

The potencies of aspirin-like drugs as prostaglandin synthetase inhibitors, calculated on a molar basis relative to aspirin = 1, are presented in Table 2. In general, the overall rank order of potency is independent of the enzyme preparation, although there are some minor variations (even when the same preparation is used). However, there is a tremendous variation in the relative activities of aspirin and indomethacin

Table 1 Preparations in which aspirin or indomethacin have been shown to inhibit prostaglandin synthesis or release

Species	Tissue	Preparation	Reference
Guinea pig	lungs	cell-free homogenates	15
		perfused	19
	whole body	in vivo	20
	uterus	in vitro	21
Dog	spleen	microsomal fraction	22
		chopped	23
		perfused	17
	kidney	in situ	24–26
	brain	cell-free homogenates	27
	platelets	in vivo and in vitro	28
Cat	spleen	perfused	29, 30
	kidney	in vivo	31
	CNS	in vivo	32
Rabbit	jejunum	isolated smooth muscle	33, 34
	brain	cell-free homogenates	27
	kidney	in vivo	35
	eye	in vivo	36
	spleen, kidney, iris-ciliary body, retina	cell-free homogenates	37
Ram	seminal vesicles	microsomal fraction	38
		acetone powder	39
Human	platelets	in vitro	16
		in vivo	16
	semen	in vivo	40
	whole body	in vivo	41
	skin	in vitro	42
	stomach	in vitro	43
Bull	seminal vesicles	microsomal fraction	44
Rat	pregnant uterus	in vitro	45–47
	skin inflammatory exudate	in vivo, in vitro	48
	kidney	in vivo	31
Mouse	tumors	homogenates	49
		tissue culture	50
	brain	freeze dried powder	47
Gerbil	brain	freeze dried powder	47

(the two substances most often studied) from preparation to preparation. On rabbit brain synthetase, for instance, the ratio is 19:1 whereas on bovine seminal vesicles it is 2140:1.

Table 2 Relative potencies of aspirin-like drugs against different preparations of prostaglandin synthetase[a]

Tissue	Guinea pig lung (15)	Dog Spleen (22)	Rabbit brain (27)	Bovine Semmal Vesicles (BSV) (44)	BSV (51)	BSV (52)	Sheep Seminal Vesicles (SSV) (38)	SSV (39)
Drug								
Aspirin[b]	1 [35]	1 [37]	1 [61]	1 [15,000]	1 [820]	1 [9000]	1 [83]	1 [9000]
Ibuprofen					0.7	4.5	55	
Phenylbutazone		5			1.9	6.4	6.6	
Naproxen				150	3.7	24	14	
Flufenamic acid					17		33	
Mefenamic acid		52			54		40	
Indomethacin	47	217	17	2140	410	236	185	900
Niflumic acid		336				76	68	
Meclofenamic acid		370				692		

[a] Reference numbers are given in parentheses.
[b] For aspirin, the ID_{50} concentrations (μM) are given in brackets.

Despite these variations in potency, inhibition of prostaglandin biosynthesis is clearly a general characteristic of aspirin-like drugs. It also seems to be a unique characteristic, for compounds representing many other types of pharmacological activity were inactive (<10% inhibition at 100 μg/ml); these included chloroquine, morphine, mepyramine, probenecid, azathioprine, *para*- and *meta*-hydroxybenzoic acid, promethazine, atropine, methysergide, phenoxybenzamine, propranolol, iproniazid, droperidol, chlorpromazine, and disodium cromoglycate. Tetrahydrocannabinol is said to be a prostaglandin synthetase inhibitor in concentrations as low as 10 μM, but the dose/response curve is very flat and the ID_{50} concentration (by extrapolation) is some 500 times higher (53).

ANTI-INFLAMMATORY ACTIVITY

Orally administered drugs have many hazards to face before circulating to a microsomal enzyme and it is therefore surprising to find any relationship between in vitro inhibition of prostaglandin synthetase and the anti-inflammatory activity. Yet for drugs where activities have been compared, the rank order was the same against carrageenin rat paw oedema as against spleen synthetase, except that indomethacin was out of order for the rat paw test (22). An even more striking correlation is shown by studying optical isomers of naproxen and indomethacin analogs. In each instance, the isomer with anti-inflammatory activity also strongly inhibited prostaglandin synthetase, whereas the one with weak anti-inflammatory activity was also weak against the synthetase (38, 44).

Sodium salicylate has only weak activity against prostaglandin synthetase in vitro (15) whereas it is as strong as aspirin in anti-inflammatory tests in vivo (54). A possible explanation of this anomaly was provided by Willis et al (48), who found the prostaglandin content of an inflammatory exudate in the rat was equally reduced by aspirin and sodium salicylate in vivo. However, when a broken cell preparation of the exudate was incubated in vitro, even though aspirin was still effective, salicylate had no action. They suggested (48) that salicylate may be converted in vivo to an active metabolite. Hamberg (41) also found salicylate to be as effective as aspirin against prostaglandin synthetase in man.

The antipyretic drug 4-acetamidophenol (acetaminophen or paracetamol), which is 10 times less effective than aspirin on the dog spleen synthetase, has almost the same potency as aspirin on the brain enzymes (27, 48). Thus, the fact that paracetamol has antipyretic activity without anti-inflammatory activity can be explained by the differential sensitivity of the prostaglandin synthetases from different tissues. There are other examples of differential enzyme inhibition. Fenclozic acid inhibited guinea pig lung prostaglandin synthetase but appeared to stimulate prostaglandin synthesis by mouse ascites tumour homogenates (49). Bhattacherjee & Eakins (37) found a thousandfold variation in the ID_{50} of indomethacin against prostaglandin synthetases from different tissues of the rabbit. One enzyme from the spleen, ID_{50} was 0.05 μg/ml, from kidney 5.0 μg/ml, from the iris-ciliary body, 18.5 μg/ml, and from the retina, 50 μg/ml. Also of interest is the demonstration of species variation; a much higher dose (per kg) of indomethacin was needed to inhibit total prostaglandin production in guinea pigs (20) than in man (41).

For inhibition of prostaglandin biosynthesis to account for the anti-inflammatory action of aspirin-like drugs, normal therapeutic doses should lead to effective plasma concentrations. Certainly, free plasma concentrations during therapy with several aspirin-like drugs often exceed those needed to inhibit prostaglandin synthetase from dog spleen. Taking indomethacin as an example, the plasma concentration in man reaches 2 μg/ml. Because of protein binding (which is a property common to many of these drugs) the free plasma concentration would be 0.2 μg/ml. However, the ID_{50} for indomethacin in dog spleen synthetase is only 0.05 μg/ml (22).

That free plasma concentrations are more than sufficient to explain the anti-inflammatory activity by prostaglandin synthetase inhibition has also been shown in man. Therapeutic doses of indomethacin (200 mg daily), aspirin (3 g daily) or salicylate (3 g daily) reduced by 77–98% the output of prostaglandin metabolite in urine (41).

Comparison with Other Proposed Mechanisms of Action of Aspirin-Like Drugs

Inhibition of prostaglandin biosynthesis is clearly achieved by therapeutic doses of aspirin-like drugs. This contrasts with the much higher concentrations (15–60 μ*M*) of salicylate needed to uncouple oxidate phosphorylation (55). Similarly, indomethacin gave a 50% inhibition of prostaglandin synthetase at a concentration of 0.17 μ*M*, whereas as much as 250 μ*M* was required to produce 50% inhibition of oxidative phosphorylation in mitochondria (3). In fact, no convincing evidence relating uncoupling potency to anti-inflammatory activity has been obtained (55).

Inhibition of leucocyte phagocytosis (9) also occurs only with high concentrations of these drugs.

Another possible mechanism of action for aspirin-like drugs is the stabilization of lysosomal membranes. However, there is an inverse correlation between potency as anti-inflammatory agents and as stabilizers of lysosomal membranes (10). Furthermore, at doses that inhibit inflammation, some stabilization of the lysosomes might be expected, but in carrageenin exudates there was no consistent change in the content of free β-glucuronidase at a time when prostaglandin synthesis was inhibited by salicylates (48).

Smith and his colleagues (4–6) have shown that L-tryptophan is displaced from serum protein binding sites by anti-inflammatory drugs. They proposed that such drugs act by displacing peptides from their binding sites on serum proteins and that the free form of the peptide protects connective tissues from the effects of inflammatory insults. Confirmation of this hypothesis will depend upon isolating an anti-inflammatory peptide from serum. Thomas & West (56) have shown that crysteine (also an anti-oxidant) reduced an experimental inflammation but obtained no effect with serine or phenylalanyl-phenylalanine.

Interference with migration of leucocytes is no longer a tenable hypothesis, for aspirin and indomethacin affect only migration of monocytes (8, 57) and not of polymorphonuclear cells.

The remaining alternative hypotheses are interference with generation of lipoperoxides (11) and hyperpolarization of neuronal membranes (12, 13). Both of these could be explained by inhibition of prostaglandin synthetase.

Salicylates and other nonsteroid anti-inflammatory drugs inhibit protein biosynthesis in toxic amounts only and at these high plasma concentrations many cellular enzyme systems are blocked. Thus, the symptoms of salicylate intoxication may be the result of inhibition of many important enzymic activities (55).

The release of rabbit aorta contracting substance (RCS) (58) from guinea pig lungs during anaphylaxis is blocked by aspirin and its congeners at concentrations as low as those required to inhibit prostaglandin generation. Several indications, including RCS formation from arachidonic acid (59, 60), its appearance always with prostaglandins (61), its instability (59), and the inhibition of its release by aspirin-like drugs suggested to Gryglewski & Vane (23, 62) that RCS is the cyclic endoperoxide postulated as an unstable intermediate in the biosynthesis of prostaglandins. Recently Hamberg & Samuelsson have isolated the cyclic endoperoxide intermediate and shown that it contracts rabbit aorta (63). These results indicate an interference by aspirin-like drugs at an early stage in the synthesis of prostaglandins. The work of Takeguchi & Sih (51) points in the same direction. Oxidation of the co-factor epinephrine occurs during the transformation of the hydroperoxide to the endoperoxide. This oxidation was inhibited by several aspirin-like drugs. Recently, Flower et al (52) studied the effect of several nonsteroid anti-inflammatory agents on the generation of prostaglandin E_2, $F_{2\alpha}$, D_2, and malondialdehyde by prostaglandin synthetase from bovine seminal vesicles. The ID_{50} concentrations of aspirin-like drugs were mostly similar for all the products, suggesting that they acted prior to formation of the endoperoxide intermediate. An

exception was phenylbutazone; at the ID_{50} concentration for prostaglandins E_2 and $F_{2\alpha}$ it had no effect on the formation of either prostaglandin D_2 or malondialdehyde, suggesting that phenylbutazone interferes with the enzymes that convert the cyclic endoperoxide to prostaglandins E_2 and $F_{2\alpha}$.

The Contribution of Prostaglandins to Inflammation

How can we fit the release of prostaglandins into the overall inflammatory process? Certainly we cannot ignore the roles of other known and established mediators, such as histamine, 5-hydroxytryptamine, bradykinin, and slow-reacting substances in anaphylaxis (SRS-A); nor can we adequately review their roles in the space available. For such a review, the reader is referred to Spector & Willoughby (64) and Rocha e Silva & Garcia-Leme (65). Perhaps the most practical way is to outline in general terms the sequence of events, but to concentrate on evidence for the involvement of the prostaglandins. In doing so, we shall develop the idea, propounded by Ferreira (66), that low concentrations of prostaglandins sensitize pain receptors to stimulation by other inflammatory mediators. Such a sensitization may also hold for the other facets of the inflammatory response, such as oedema (67). If it does, the inhibition of prostaglandin biosynthesis by aspirin-like drugs will also seemingly decrease the actions of histamine, bradykinin, and the other mediators.

Release of Chemical Mediators

In different types of inflammation, some mediators may have more prominent roles than others because of the relative sensitivities of the tissues in which they are released. The sequence of mediator release may also be important. For instance, in anaphylactic shock in the lung there is an explosive and simultaneous release of histamine, SRS-A, RCS, and prostaglandins E_2 and $F_{2\alpha}$ (see 19). However, in the inflammatory response to subcutaneous injection of carrageenin in the rat, there is a sequential release (67a). At first there is an output of histamine, which then declines, perhaps because the preformed stores in mast cells have been exhausted. This is followed by bradykinin formation. There is little prostaglandin activity ($<$ 5 ng/ml) until 3 hr after the carrageenin injection but then the concentration gradually rises to an average plateau of 80 ng/ml between 18–24 hr. As the concentration of prostaglandins rose, so did that of histamine, once more reaching more than 1 μg/ml at 24 hr. This secondary release of histamine may be associated with fresh synthesis, for in many situations (68) "nascent histamine" formation has been described, due to increased activity of histidine decarboxylase.

Di Rosa et al (7) used depleting agents or antagonists to study the role of different mediators in rat foot-paw oedema induced by carrageenin. To abolish the first phase of the response, they had to use a combination of antagonists of histamine and 5-hydroxytryptamine or deplete both agents with compound 48/80. A kininogen-depleting agent (cellulose sulfate), presumably preventing formation of bradykinin, depressed the 1.5–2.5 hr oedema. They agreed that prostaglandins were released thereafter and also noted (8) that the "prostaglandin phase" of the oedema (which coincided with the arrival of PMN leucocytes in large numbers) was most susceptible to aspirin-like drugs. Other results (7) suggested that the early phase of turpen-

tine-induced pleurisy in the rat was mainly histamine-mediated and that 5-HT and kinins were much less important in this type of inflammation.

Prostaglandin generation occurs in many forms of damage to the skin, including contact dermatitis (69), inflammation due to ultraviolet light (70), and scalding (71).

The invasion of the inflamed area by PMN cells may also be important for the maintenance of prostaglandin generation (and thereby of the inflammation). Higgs & Youlten (72) showed that phagocytosis was accompanied by prostaglandin release and suggested that this could constitute a control mechanism for further influx of phagocytes, since prostaglandin E_1 is leucotactic (73, 74). Phagocytosis (and therefore leucotaxis) would continue as long as the injurious agent or tissue debris was present.

As in peripheral inflammatory responses, there is a generation of prostaglandin E-like substance in the central nervous system during fever (75).

The Inflammatory Effects

ERYTHEMA Prostaglandins of the E and F series (the ones likely to be generated in inflammation) cause erythema and prostaglandin E_1 is effective at doses as low as 1 ng; for $F_{1\alpha}$ 1 μg is needed (76, 77). There are, however, two features of the vascular effects of prostaglandins not shared by other putative mediators of inflammation. The first is a sustained action and the second is the ability to counteract the vasoconstriction caused by substances such as norepinephrine and angiotensin. The erythema induced by intradermal injection or subdermal infusions (66) illustrates well the long-lasting action of prostaglandins (sometimes up to 10 hr). This long-lasting action confers an important property upon the prostaglandins, in that the appearance and the magnitude of their effects not only depend on the actual concentration but also upon the duration of their release or infusion (66).

In contrast to the long-lasting effects upon cutaneous vessels and superficial veins, the vasodilator actions of prostaglandins on other vascualr beds vanish within a few minutes. However, sometimes, there then remains a long-lasting reduction in response to vasoconstrictor substances (78). It could be that the long duration of prostaglandin erythema in man is partially due to a reduced local reactivity to the sympathetic mediator. In addition to this direct effect on the skin vessels, prostaglandins may also be causing vasodilatation by blockade of the sympathetic control mechanism since prostaglandins are known to inhibit the release of the adrenergic mediator (79). Such a mechanism, however, has not yet been proved to operate in vivo.

OEDEMA Prostaglandins, like bradykinin, histamine, and 5-hydroxytryptamine, cause increased vascular permeability by inducing vascular leakage at the postcapillary and collecting venules (74). Although most active substances exhibit a general relationship between ability to increase vascular permeability and erythema formation, these effects result from actions on different components of the vessel. Erythema represents a local pooling of blood due to a relaxation of the smooth muscles of the walls of the arterioles and venules, whereas increased vascular permeability is thought to result from the contraction of the venular endothelial cells (80). In fact,

prostaglandins, produce vasodilatation more effectively than oedema. Prostaglandin E_1, when compared with histamine in the guinea pig skin, produces a similar degree of erythema (but much longer-lasting) and a smaller wheal (76). Similarly, in man histamine, bradykinin, and prostaglandin E_1 each cause erythema and oedema when injected intradermally. However, prostaglandin E_1 induces long-lasting erythema and a much less pronounced oedema (66). There is no difference in the duration of the increased vascular permeability induced by histamine or prostaglandin (81).

Prostaglandins E_1, E_2, and A_2, but not $F_{2\alpha}$ caused oedema when injected into the hind paws of rats (82). Prostaglandin E_1 (on a weight basis) was as effective as bradykinin, though higher doses (40–80 μg), instead of causing increased effects like bradykinin, produces erythema without oedema. When prostaglandin E_1 was given together with histamine or 5-hydroxytryptamine, it elicited an additive effect rather than a synergistic one. We reinvestigated this problem and found that the rat paw oedema caused by mixtures of prostaglandin E_1 with histamine or bradykinin was substantially greater than that expected by simple addition. Moreover, histamine-bradykinin mixtures produced effects no greater than histamine alone (67).

If a prostaglandin is sensitizing blood vessels to the permeability effects of other mediators (as happens with pain receptors: see later), then the actions of anti-inflammatory drugs on oedema can be explained by removal of this sensitization. Thus, the contribution that prostaglandins make to the oedema of inflammation is by increasing the effects of the other known mediators, such as histamine and bradykinin. To test this idea, carrageenin-induced paw swelling was measured in rats treated with indomethacin. Low concentrations of prostaglandin E_1 or E_2 added to the carrageenin injection strikingly increased oedema formation. Clearly, prostaglandin E can sensitize the blood vessels to the permeability-increasing effects of the other mediators locally released by carrageenin, and removal of endogenous prostaglandin generation (and therefore of the sensitization) explains the anti-oedema effects of aspirin-like drugs (67).

PAIN In man, prostaglandins cause headache and pain along the veins into which they are infused (83, 84). When given intradermally (66) or intramuscularly (85) in concentrations higher than those expected to occur in inflammation (67a, 70), prostaglandin E_1 causes long-lasting pain. However, induction of hyperalgesia (i.e. a state in which pain can be elicited by normally painless mechanical or chemical stimulation) seems to be a typical effect of prostaglandins. Prostaglandins injected into dog knee joints produce incapacitation (86). Prostaglandin E_1 was 10 times more potent than prostaglandin E_2; the reactions to both began within 15 min and lasted for several hours. With prostaglandin $F_{2\alpha}$ there was an initial brief effect followed by a delayed gradual increase over 4 hr.

A long-lasting hyperalgesia occurred when minute amounts of prostaglandin E_1 were given intradermally (77) or infused subdermally (66). Ferreira's (66) subdermal infusion experiments, which were carried out in order to mimic the continuous release of mediators at the site of an injury, showed that the hyperalgesic effects of prostaglandins were cumulative, since they depended not only on concentration, but also on duration of the infusions. This cumulative sensitizing activity of the pain

receptors was later also observed in dog spleen (87, 88) and rat paw (89). In Ferreira's experiments, during separate subdermal infusions of prostaglandin E_1, bradykinin, or histamine (or a mixture of bradykinin and histamine) there was no overt pain; but when prostaglandin E_1 was added to bradykinin or histamine (or a mixture of both), strong pain occurred.

Another important observation concerned pruritus (66). Neither histamine, bradykinin, nor prostaglandin E_1 infusions by themselves caused itch. However, when prostaglandin E_1 was infused along with histamine, itching was always recorded. This role of prostaglandins in potentiating the effects of histamine has recently been confirmed (89a). When prostaglandin E_1 was infused with bradykinin, there was pain rather than itch.

When applied to a blister base, prostaglandins do not cause pain (90). This may be because a blister base is already an inflamed site, made hyperalgesic by the local release of prostaglandins. Thus, in an area already saturated with prostaglandins, a further application will not elicit a further response. Presumably, endogenous prostaglandin production in the blister base enhances the pain-producing properties of added substances. This sensitizing action of prostaglandins to pain induced by bradykinin has recently been shown to occur in dog spleen also, a preparation used by Lim and his colleagues (91–93) to show that aspirin-like drugs act peripherally as analgesics. Injection of bradykinin or epinephrine released prostaglandins from dog spleen in similar amounts, both in vitro and in vivo (87, 88, 94). As epinephrine is a much weaker pain-producing substance than bradykinin in this system, a prostaglandin could not be the *mediator* of the pain-producing activity of bradykinin.

The reflex rise in blood pressure induced by intra-arterial bradykinin injections into the spleen of lightly anaesthetized dogs was also used as an indication of sensory stimulation (93). Doses of bradykinin that released prostaglandin from the spleen caused reflex increases in blood pressure in proportion to the dose used and these were reduced by indomethacin. When prostaglandin E_1 was given with the bradykinin in the indomethacin-treated dogs, the reflex increase in blood pressure was restored, sometimes to greater than control values (87, 89).

An explanation of the analgesic action of aspirin-like drugs is shown diagramatically in Figure 1. It is generally agreed that the analgesic action of morphine occurs centrally, whereas the work of Lim et al (91–93) clearly showed that aspirin had a peripheral effect. By preventing prostaglandin release in inflammation, aspirin prevents the sensitization of the pain receptors to mechanical stimulation or to the other chemicals. This hypothesis also explains why aspirin is ineffective as an analgesic in uninflamed tissues, as shown by the Randall-Selito test (95). Presumably, aspirin is only effective as an analgesic in tissues in which prostaglandin formation is taking place.

Fatty acid hydroperoxides can also cause pain in man (66). Intensity of the pain produced by intradermal injections of hydroperoxides of arachidonic, linoleic, and linolenic acids was greater than that induced by either the parent fatty acids or acetylcholine, bradykinin, histamine, or prostaglandin E_1. Thus lipoperoxides formed during prostaglandin biosynthesis may also be important as pain-producing

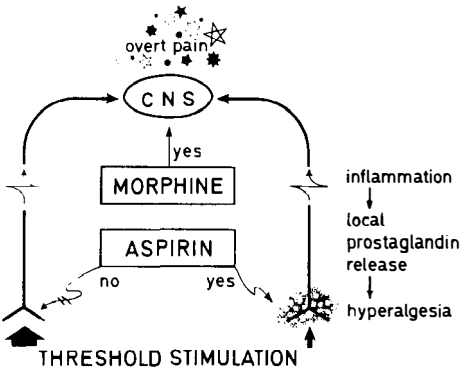

Figure 1 The role of prostaglandins in pain production.

substances. Certainly RCS, which may be the cyclic endoperoxide precursor of prostaglandins (63), has strong pharmacological activity in that it contracts rabbit aorta and many other arterial muscle strips (58, 96), as do the lipoperoxides generated by lipoxidase acting on unsaturated fatty acids such as arachidonic, linoleic, and linolenic acid (23, 28).

It is worth noting that aspirin-like drugs do not affect the hyperalgesia or the pain caused by direct action of prostaglandins. Aspirin, phenylbutazone, or indomethacin were ineffective against the incapacitation induced by prostaglandins in the dog knee joint (86). Indomethacin diminished the nociceptive effect of many agents injected intraperitoneally in mice (97) or intra-arterially in dog spleen (93), but it did not abolish either the writhing response in mice or the sensitization of dog splenic sensory nerves induced by prostaglandins (87, 97).

Thus, the anti-inflammatory acids do not reduce the effects of prostaglandins but reduce those effects caused by substances that induce generation of prostaglandins. A possible exception to this is the action of *fenamates,* which have some antagonist action at receptors for prostaglandins (98) as well as potent antisynthetase activity.

FEVER Fever is often associated with an inflammatory process. Prostaglandin E_1 is the most powerful pyretic agent known, when injected either into cerebral ventricles, or directly into the anterior hypothalmus (99, 100). The hyperthermic effect is dose-dependent, almost immediate, and lasts for about 3 hr. Prostaglandin E_1 or E_2 causes fever by an action on the same region as that on which monoamines and pyrogens act to affect temperature.

Fever always occurs during induction of human abortion with prostaglandin $F_{2\alpha}$ (101); however, prostaglandin $F_{2\alpha}$ is a rather ineffective pyretic agent in cats and rabbits (99). The pyrogenic action of prostaglandin E_2 is greater than that of $F_{2\alpha}$ in animals, but when used for induction of abortion, it is infused at one tenth the rate used for prostaglandin $F_{2\alpha}$ and only 15% of the patients showed an increase in temperature (102). In the human studies, elevation of temperature showed better correlation with the infusion rate than with the time course of the abortion. Thus,

the generation of prostaglandins in some areas of the central nervous system or its presence in the general circulation may induce fever in animals, including man. In man, the remission of pain and fever is often the first indication of the effectiveness of therapeutic doses of aspirin-like drugs.

Generation of a prostaglandin E-like substance in the central nervous system has been measured during fever (75) and the concentrations in the CSF rise after intravenous pyrogen by 2.5–4 times, sometimes to as much as 35 ng/ml.

Aspirin-like drugs do not abolish either the formation of endogenous pyrogen by leucocytes (103) or the pyretic action of prostaglandins injected into the third ventricle of cats (99). However, they inhibit both the generation of prostaglandins in the central nervous system and the fever caused by pyrogens or 5-hydroxytryptamine given into the cerebral ventricles (32).

MIGRATION OF LEUCOCYTES AND GRANULOMA FORMATION There is no conclusive evidence that prostaglandins are leucotactic in an inflammatory process. In vitro only prostanglandin E_1 in a concentration at least 10 times higher than that found in inflammatory exudates produces a modest migration of polymorphonuclear leucocytes when compared with activated plasma (73, 74). In man, with a skin window technique, prostaglandin E_1 and prostaglandin $F_{1\alpha}$ did not alter the cellular sequence and number of cells of the exudate of a cutaneous inflammation (104). Arora et al (105) found no increased leucocytic emigration into a skin area previously treated (1–4 hr) by local injection of prostaglandin E_1.

One important aspect of an inflammatory reaction is the granuloma formation associated with an increased production of collagen. Local prostaglandin E_1 enhances the granuloma formation by cotton pellets (105) and increases collagen synthesis in chick embryo tibiae (106). However, in rats when prostaglandin E_1 was inoculated locally into air pouches at high dosages (50–100 μg), it did not elicit a granulomatous reaction (82). Aspirin-like drugs diminished granuloma formation by cotton pellets (107). However, emigration of PMN cells in an acute inflammatory reaction was not modified by indomethacin or phenylbutazone although monocyte emigration was greatly reduced (8, 57). This observation indicates that prostaglandins may be the leucotactic factor responsible for the accumulation of monocytes, one of the aspects of the conversion of an acute inflammatory reaction into a chronic one.

SIDE EFFECTS

Some of the toxic effects of large doses of aspirin-like drugs may be due to inhibition of other enzyme systems, as discussed earlier. However, inhibition of prostaglandin biosynthesis may also lead to unwanted side effects in organs that depend upon prostaglandins for normal physiological function.

The aspirin-like drugs all induce gastrointestinal irritation, which may lead to ulceration. Prostaglandin synthesis and release can be provoked by many different forms of mechanical stimulation, including gentle massage (19, 108). Thus, mechanical stimulation of the mucosa associated with gastrointestinal contractions, may

lead to synthesis intramurally of a prostaglandin that in some way protects the mucosa from damage.

There are several possible protective mechanisms. Prostaglandin E_1 inhibits gastric acid secretion (109) so a locally released prostaglandin may be a braking mechanism to prevent hyperacidity, which can lead to mucosal damage. Such a mechanism is supported by the fact that indomethacin increased a submaximal secretion of acid induced by pentagastrin in rats (110). However, Bennett et al (111) found that submaximal gastric secretion in man, also induced by pentagastrin, was generally decreased slightly by indomethacin. They suggested that the function of locally released prostaglandins in the stomach may be to increase bloodflow to the mucosa and that the vasoconstriction consequent upon removal of this effect by aspirin-like drugs may lead to ischaemia, tissue death, and bleeding.

Another possibility might be that inhibition of prostaglandin biosynthesis in the stomach leads to a local accumulation of a prostaglandin precursor, such as arachidonic acid, and that this causes the irritation.

The anti-inflammatory acids also cause varying degrees of nephrotoxicity, with some incidence of papillary necrosis. Some, like phenylbutazone, lead to retention of sodium chloride and water. The prostaglandins are natruiretic (112, 113) and are found in renal medulla (114–116), together with prostaglandin synthetase (117), which is located in cells forming the collecting tubules (118). Thus, some of the renal side effects of anti-inflammatory drugs may depend upon their interaction with prostaglandin synthetase in the kidney.

Recently, indomethacin has been shown to be slightly more potent than theophylline as a phosphodiesterase inhibitor (119). If other aspirin-like drugs have a similar effect, this may also account for some of their side effects.

The major concern in the current research for new drugs is to minimize their side effects. To minimize the side effects commonly associated with broad-spectrum, nonspecific, anti-inflammatory drugs, Shen (120) suggested a cocktail mixture consisting of several narrow spectrum agents, each acting specifically at one of the many facets of complex inflammatory reactions, such as lysosomal enzymes, inflammatory mediators, etc. Considering the differential sensitivity of the prostaglandin synthetases, it may also be possible to develop specific inhibitors for the synthetase of each tissue, or group of tissues. If such a search could include the requirement that the synthetase of stomach and kidney were *not* inhibited, the common side effects may be eliminated.

CONCLUSIONS

Nonsteroid anti-inflammatory drugs inhibit prostaglandin biosynthesis in concentrations likely to be found in body fluids during therapy. The evidence that we have assembled, together with the actions of prostaglandins (erythema, pyresis, sensitization of vascular tissue to increased permeability, and sensitization of pain receptors), overwhelmingly supports our theory that this antienzyme effect is the mechanism of action of aspirin-like drugs. Intermediates in prostaglandin biosynthesis may also play a part in the inflammatory process. Prostaglandin synthetases prepared from

different tissues show different sensitivities to aspirin-like drugs. This property, which may reflect a series of isoenzymes, can explain the variations in activity within the group of compounds.

Inhibition of prostaglandin biosynthesis by aspirin-like drugs, especially indomethacin (as one of the most potent) can also be used to explore the involvement of prostaglandins in pathological and physiological processes. Already, it has demonstrated the involvement of prostaglandins in the maintenance of tone of isolated smooth muscle preparations (33, 34, 121), in the control of uterine activity in vivo and in vitro (45, 46, 122–125), in normal ovary function (126–129), in modulation of lipolysis in isolated fat cells (130), in modulation of sympathetic nervous activity in vitro (30, 79), and in regulation of blood flow in the kidney (25, 26, 130a), adipose tissue (131), and during haemodynamic shock (132).

The fact that aspirin is a relatively nontoxic drug, consumed in enormous quantities throughout the world, suggests that prostaglandin synthetase is not an enzyme vital for the existence of the organism. This fits with the concept that prostaglandins are modulators of the activity of the body, perhaps mainly involved in local communication between cells, especially in defensive reactions induced by damage or stress.

This review covers literature available to us in England before June 1973. Other reviews that discuss the general relationship between prostaglandins and aspirin-like drugs are references 48 and 133–139.

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