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Review

Vane's discovery of the mechanism of action of aspirin changed our understanding of its clinical pharmacology

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Abstract:
Aspirin exerts its analgesic, antipyretic and anti-inflammatory actions by inhibiting the enzyme cyclooxygenase and thus preventing the formation and release of prostaglandins. The elucidation by John Vane of the mechanism of action of aspirin in 1971 was followed twenty years later by the discovery of a second cyclooxygenase enzyme, COX-2 and the rapid development of selective inhibitors of this enzyme. The COX-2 inhibitors are potent anti-inflammatory drugs without the damaging side effects on the stomach mucosa of the non-selective aspirin-like inhibitors. More recently, two enzymes have been identified inhibition of which may explain the mechanism of action of paracetamol. These are a putative cyclooxygenase-3 which is a variant of cyclooxygenase-1 and derives from the same gene, and a COX-2 variant, induced with diclofenac, which may be involved in the resolution of inflammation.

Key words:
cyclooxygenase-1, cyclooxygenase-2, cyclooxygenase-3, paracetamol, diclofenac, analgesia, hypothermia

Abbreviations: BADGE – biphenol A diglycidyl ether, COX – 1-cyclooxygenase-1, COX-2 – cyclooxygenase-2, COX-3 – cyclooxygenase-3, HETE – 15R-hydroxyeicosatetraenoic acid, IL-6 – interleukin-6, IL-10 – interleukin-10, LPS – lipopolysaccharide, NFκBα – nuclear factor kappa Bα, PGES – prostaglandin endoperoxide synthase, PGI_2 – prostacyclin, PPARγ – peroxisome proliferator-activated receptor-γ, srIκBα – super repressor inhibitor kappa Bα, TGFβ – transforming growth factor β, TNFα – tumor necrosis factor α

Introduction

Although aspirin was synthesised in 1897 and marketed as an anti-inflammatory drug, its mechanism of action remained unknown for 74 years. However, in 1971, John Vane showed that aspirin and similar drugs such as indomethacin and salicylate inhibited the synthesis of prostaglandins by guinea pig lung homogenate in proportion to their anti-inflammatory potency in humans [42]. Felix Hoffman synthesised aspirin (acetylsalicylic acid) at Bayer Research Laboratories and Bayer's chief pharmacologist tested the new substance in comprehensive animal studies. Detailed pharmacological and toxicological tests confirmed that the actions of this newly developed drug were very satisfactory and Heinrich Dreser described the excellent analgesic and antipyretic effects of acetylsalicylic acid in a report printed in Pflüger's Archives in 1899 [12]. The clinical applications of ace-

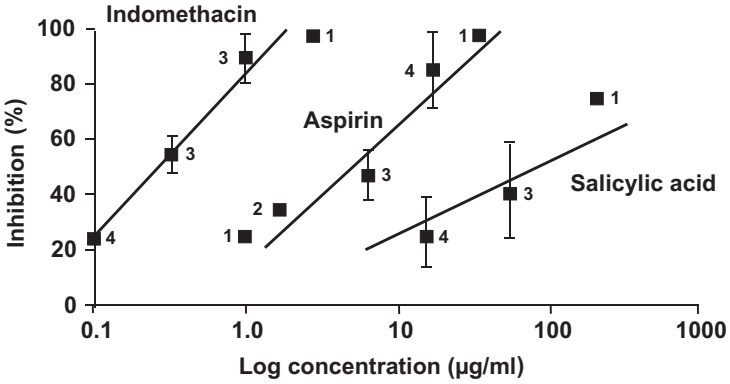

Fig. 1. Mechanism of action of aspirin. Aspirin, indomethacin and salicylic acid inhibited in a dose-related manner, production of prostanoids by a homogenate of guinea pig lung stimulated with arachidonic acid [42]

tylsalicylic acid were also tested in a year long study and the results demonstrated the improved pharmacological properties of the new compound compared to those of the archetypal sodium salicylate.

However, in time, some important side actions of aspirin became evident. Gastroscopic studies of the effect of aspirin on the human stomach showed the damaging effects of this drug on the gastric mucosa [11]. Many other clinically useful anti-inflammatory drugs, such as naproxen, indomethacin and phenylbutazone also caused visible gastric mucosal damage and erosion [22]. With the development of the platelet aggregometer by Born [5] it was also possible to detect the anti-platelet actions of aspirin both *in vitro* and *ex vivo* [32, 45].

Discovery of the mechanism of action of aspirin

In 1971 the mechanism of the analgesic, antipyretic and anti-inflammatory actions of aspirin was understood. In his seminal experiment, using homogenate of guinea pig lung, John Vane concluded that the pharmacological actions of aspirin were due to the decreased production of prostaglandins by inhibition of the enzyme, cyclooxygenase (COX) [42]. Aliquots of the supernatant of a broken cell homogenate from guinea pig lung were incubated with arachidonic acid and with different concentrations of aspirin, indomethacin or sodium salicylate (Fig. 1). After 30 minutes incubation at 37°C, prostaglandin (PG)$F_{2\alpha}$ generation was estimated by bioassay on rat colon. There was a dose-dependent inhibition of prostaglandin formation by all three drugs, indomethacin being the most potent and sodium salicylate the least. Three control drugs, morphine, hydrocortisone and mepyramine had no effect on prostaglandin synthesis. Two other reports in the same issue of Nature lent support to this finding and extended it considerably. First, Smith and Willis [39] were investigating the effects of aspirin on platelet behavior. They obtained venous blood samples from three volunteers before and one hour after taking 600 mg aspirin orally. Platelets were isolated, washed and incubated with thrombin. Platelets from aspirin-treated blood did not aggregate and released lower levels of prostaglandins than those from volunteers not treated with aspirin. Thrombin added to a suspension of platelets *in vitro* to which various concentrations of aspirin had been added also released lower levels of prostaglandins and caused less aggregation. Second, Vane and his colleagues [14] demonstrated that aspirin and indomethacin blocked the release of prostaglandins from a perfused, isolated dog spleen subjected to sympathetic nerve stimulation.

These experiments explained why all the aspirin-like, or non-steroid anti-inflammatory drugs (NSAIDs) as they became known, shared the same pharmacological actions: anti-inflammatory, analgesic and antipyretic and the same side effects of damage to the stomach mucosa, toxicity to the kidney and inhibition of platelet aggregation. There was already evidence suggesting that PGE_1 was a pyretic agent in several species [29] and that PGE_2 mimicked the inflammatory response when injected intradermally [40]. Prostaglandins had also been detected in inflammatory exudates [10], so there were grounds for speculating that prostaglandins might be responsible, at least in part, for the genesis of fever and inflammation and that aspirin-like drugs might owe their therapeutic activity to their ability to prevent prostaglandin biosynthesis.

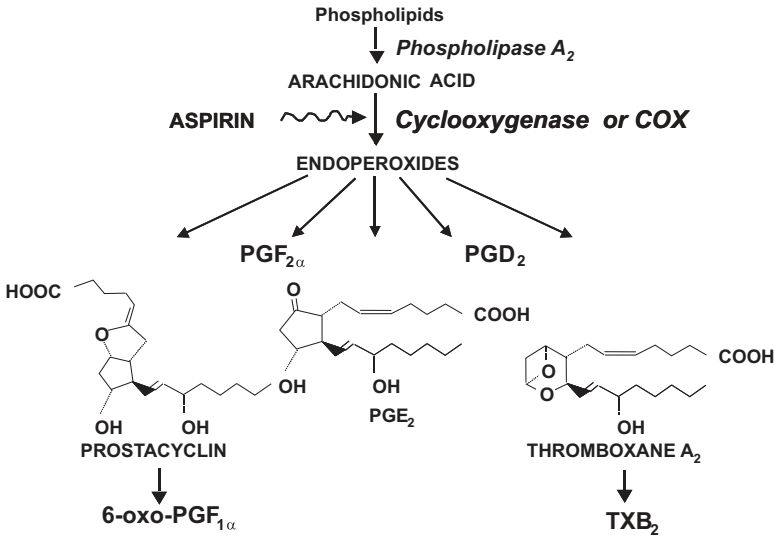

Fig. 2. The arachidonic acid cascade. The enzyme cyclooxygenase (COX) or prostaglandin endoperoxide synthase, converts arachidonic acid into unstable endoperoxide intermediates, PGG_2 and PGH_2. These undergo metabolism into the stable prostaglandins PGE_2, $PGF_{2\alpha}$ and PGD_2 as well as the unstable prostanoids, prostacyclin and thromboxane A_2. Aspirin prevents production of all these prostaglandins

Aspirin inhibits cyclooxygenase

A homogeneous, enzymatically active COX or prostaglandin endoperoxide synthase (PGES) was isolated in 1976 [18]. The enzyme was cloned and its structure elucidated by three different groups in 1988 [9, 28, 47]. This membrane-bound hemo- and glycoprotein with a molecular weight of 71 kDa is found in greatest amounts in the endoplasmic reticulum of prostanoid-forming cells [31]. It both cyclizes arachidonic acid and adds the 15-hydroperoxy group to form prostaglandin G_2 (PGG_2). The hydroperoxy group of PGG_2 is reduced to the hydroxy group of PGH_2 by a peroxidase (in the same enzyme protein) that utilizes a wide variety of compounds to provide the requisite pair of electrons (Fig. 2). The hydroperoxides also drive the cyclooxygenase reaction by maintaining a 'hydroperoxide tone' [20]. The endoperoxides become converted by tissue specific synthases to the stable prostaglandins, E_2, $F_{2\alpha}$ and D_2, as well as to the unstable prostanoids, prostacyclin (PGI_2) and thromboxane A_2. PGI_2 is degraded to the inactive, 6-oxo-$PGF_{1\alpha}$ and thromboxane A_2 to the inactive thromboxane B_2. Aspirin prevents the biosynthesis of these prostaglandins by inhibiting COX.

Picot, Loll and Garavito [35] determined the three-dimentional structure of COX. It is a homodimeric enzyme which integrates into only a single leaflet of the lipid bilayer of the cell membrane. This bifunctional enzyme comprises three independent folding units: an epidermal growth factor-like domain, a membrane-binding motif and an enzymatic domain. The

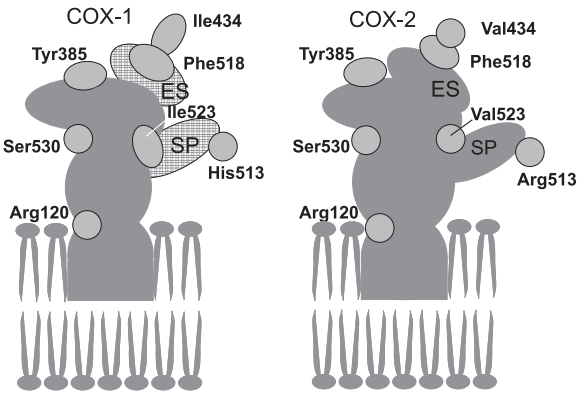

Fig. 3. The catalytic sites of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). Both catalytic channels have tyrosine (Tyr) 385 at the apex of the active site. Aspirin acetylates serine (Ser) 530 and prevents access of arachidonic acid to Tyr 385. The carboxylic acid groups of aspirin and salicylic acid bind weakly to arginine (Arg) 120 at the mouth of the channel. Substitution of isoleucine (Ile) 523 for valine (Val) 523 and histidine (His) 513 for arginine (Arg) 513 opens up a side pocket (SP) in COX-2. Substitution of Ile 434 by Val 434 creates an extra space (ES) at the apex of the active site. For simplicity, the numbering of the amino acids has been kept the same in COX-2 as in COX-1. (Reproduced with permission from "Principles of Immunopharmacology" second edition, Nijkamp F.P. and Parnham M.J. eds. 2005. Birkhauser Verlag. Basel, Switzerland. Page 505)

sites for peroxidase and cyclooxygenase activity are adjacent but spatially distinct. Three of the helices of the structure form an entrance channel to the active site and their insertion into the membrane allows arachidonic acid to gain access from the interior of the bilayer.

The COX active site is a long hydrophobic channel and Picot et al. [35] presented evidence that most of the aspirin-like drugs such as flurbiprofen inhibit COX by excluding arachidonate from the upper portion of the channel. Tyrosine (Tyr) 385 and serine (Ser) 530 are situated at the apex of the long active site. Tyrosine 385 forms a tyrosyl radical, abstracts hydrogen from the pro-S side of carbon 13 of arachidonic acid and creates an activated arachidonyl radical that undergoes the cyclization/oxygenation reaction which results in the formation of the unstable endoperoxides [37]. Aspirin inhibits COX by acetylation of the hydroxyl group of Ser 530, thereby excluding access for arachidonic acid to Tyr 385 by steric hindrance [36]. This covalent bond results in an irreversible inhibition of COX unlike the reversible inhibitory action of other NSAIDs. Loll and his colleagues [24] also postulated that the salicylate moiety of aspirin or salicylic acid binds with weak affinity to a second binding site below Ser 530 (probably to the carboxylate binding site, arginine (Arg)120). They suggested that the carboxylic acid of aspirin initially binds to Arg120, which is situated near the opening of the catalytic channel, followed by the covalent binding of the acetyl group to Ser 530 (Fig. 3).

In addition, when aspirin acetylates COX in platelets, this occurs in the pre-systemic circulation where there is a high concentration of aspirin in the portal vein before its metabolism by the liver. This inhibition of platelet function occurs with very low doses of aspirin which have no systemic effects and provides the basis for the use of daily 75 mg doses of aspirin in the prevention of heart attacks and strokes. It is irreversible and platelets lose their ability to aggregate until new platelets are formed, which is within 8 to 11 days in humans [43].

Discovery of cyclooxygenase-2

Thus it was established that low therapeutic doses of aspirin produced analgesia, antipyresis, ulceration of the stomach and inhibition of platelet aggregation, while high therapeutic doses were required for anti-inflammatory activity. In addition, the NSAIDs varied in the relative potency of their therapeutic actions compared to their activity in producing side effects. The reason for this remained unknown until the publication in 1991 of the discovery of a second COX encoded by a different gene from the first COX [46]. Cyclooxyenase-2 (COX-2), as it was named, is an inducible enzyme as opposed to the constitutive, cyclooxygenase-1 (COX-1). It is expressed in response to inflammatory stimuli released from bacteria such as lipopolysaccharide, cytokines released from macrophages, such as interleukin-1, and also mitogens and growth factors. The structure of COX-2 is 60% homologous with the amino acid structure of COX-1 and both isoforms have a molecular weight of 71 kDa. The human COX-2 gene at 8.3 kb is a small immediate early gene, whereas human COX-1 originates from a much larger 22 kb gene. The gene products also differ, with the mRNA for the inducible enzyme being approximately 4.5 kb and that of the constitutive enzyme being 2.8 kb [19, 33]. The active sites of the two enzymes are similar. However, the active site of COX-2 is slightly larger than that of COX-1 and provides access to a side pocket [21, 26]. It still retains serine at its active site, which is now in position 516 due to the change in structure. Aspirin acetylates serine 516 in COX-2, but due to the larger size of the catalytic channel, arachidonic acid is able to "squeeze past" the acetylated structure and form 15-R-hydroxyeicosatetraenoic acid (15-R-HETE) [27].

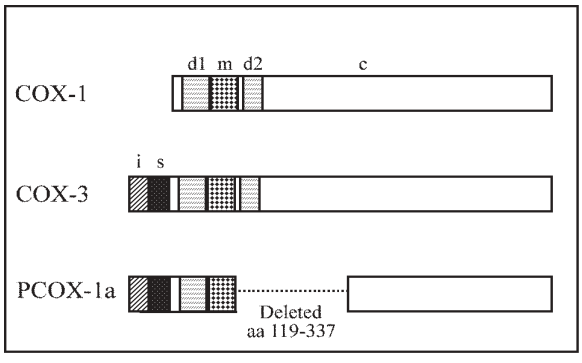

Fig. 4. Comparison of COX-3 and PCOX-1a with COX-1. COX-1 is represented by d1: dimerization domain 1, d2: dimerization domain 2, m: membrane binding domain, c: catalytic domain. COX-3 retains in addition, s: the signal peptide and i: a 30 amino acid sequence encoded by intron 1. PCOX-1a retains the intron 1 sequence but does not have amino acids 119-337. It does not synthesize prostaglandins [6]

The formation of the side pocket in the active site of COX-2 is due to the amino acid substitutions in the enzyme structure (Fig. 3). Thus, where COX-1 has isoleucine 523, this is replaced by the smaller valine 523 in COX-2 and allows access to the side pocket. In addition, isoleucine 434 near the apex of the active site is replaced by the smaller valine 434 thus increasing the size of the catalytic site [21, 26]. The important binding to Tyr 385 and Arg 120 remains the same in both enzymes. Binding to Arg 120 is essential for the activity of the carboxylic acid-containing inhibitors but not for non-carboxylic acid inhibitors. Based on these differences, it became possible to synthesize drugs which were selective inhibitors of COX-2 (such as celecoxib and rofecoxib), so that they possessed therapeutic anti-inflammatory actions but were without gastric side effects or anti-platelet activity [44].

Tab. 1. Selectivity of analgesic antipyretic drugs and NSAIDs for cyclooxygenase (COX) enzymes. Control drugs, caffeine and thalidomide did not inhibit the COX enzymes [6]

Drug	IC_{50} (μM)		
	COX-1	COX-2	COX-3
Acetaminophen	> 1000	> 1000	460
Aminopyrine*	> 1000	> 1000	688
Antipyrine	> 1000	> 1000	863
Dipyrone	350	> 1000	52
Phenacetin	> 1000	> 1000	102
Aspirin	10	> 1000	3.1
Diclofenac	0.035	0.041	0.008
Ibuprofen	2.4	5.7	0.24
Indomethacin	0.010	0.66	0.016
Caffeine	> 1000	> 1000	>1000
Thalidomide	> 1000	> 1000	>1000

All assays were carried out in the presence of 30 μM arachidonic acid. *4-dimethylaminoantipyrine

A COX-1 variant enzyme that retains intron-1

The discovery and characterization of COX-2 explained many anomalies connected with the actions of NSAIDs, but it failed to clarify the mechanism of action of paracetamol which had already been investigated by Flower and Vane in 1972 [15]. They concluded that brain COX was more sensitive to inhibition by paracetamol than COX in spleen, and suggested the possibility of tissue specific cyclooxygenases. Paracetamol has analgesic and antipyretic properties but no anti-inflammatory action. Its activity in cultured cell and human whole blood assays is that of a weak inhibitor of both COX-1 and COX-2. This led to speculation that variants of the currently established enzymes may exist with a different sensitivity to the NSAIDs. By serendipity, a COX-1 variant, named COX-3, present in high concentrations in dog brain was identified in 2002 [6]. COX-3 is a splice-variant of COX-1 that retains the intron-1 gene sequence at the mRNA level which encodes a 30 amino acid sequence inserted into the N-terminal hydrophobic signal peptide of the enzyme protein (Fig. 4). The variant was expressed in insect cells and its sensitivity to inhibition by paracetamol and other NSAIDs was measured. The variant enzyme was more sensitive to inhibition with paracetamol than either COX-1 or COX-2 and it was also more sensitive to the inhibitory action

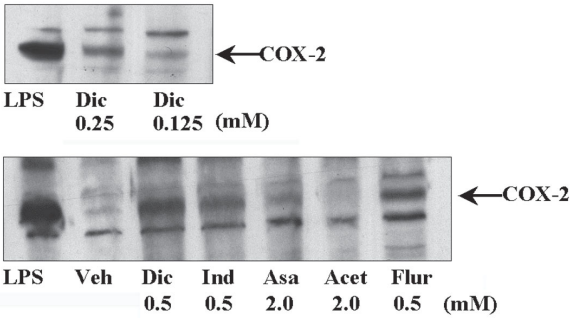

Fig. 5. Western blotting of COX-2 induced with NSAIDs. Induction with bacterial lipopolysaccharide (LPS) and vehicle (Veh) is recorded for comparison. Flubiprofen (Flur) and indomethacin (Ind) produced strong expression of enzyme protein. Aspirin caused moderate expression of COX-2, but acetaminophen (Acet) did not induce the protein. Diclofenac (Dic) (0.5 mM) induced strongly while lower doses (0.25 mM, 0.125 mM) stimulated expression in a dose related manner [38]

of non-selective NSAIDs including aspirin (Tab. 1). In the dog, COX-3 mRNA intron-1 is within frame, but in humans and rodents there is a frameshift mutation in intron-1 of the COX-3 transcript. However, COX-3 enzyme protein has been identified by Western blotting in human tissues [6] and in mouse brain (Fig. 6) [4], although the mechanism for conversion of COX-3 mRNA to active enzyme is not clear as yet.

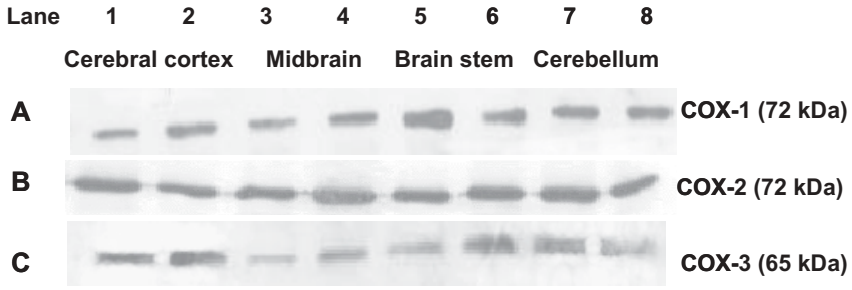

Fig. 6. Western blotting of COX enzymes in different regions of mouse brains. COX-1, COX-2 and COX-3 are distributed throughout the brains of mice, with the greatest concentration of COX-3 in the cerebral cortex [4]

However, this variant may be involved in the hypothermic and analgesic action of paracetamol in mice [2, 4], since paracetamol penetrates easily into the central nervous system [7].

Analgesia with paracetamol was produced in the writhing test in mice using intraperitoneal acetic acid as the nociceptive agent. Mice with the COX-1 gene deleted did not respond to paracetamol analgesia, whereas COX-2 gene deleted mice responded robustly to the analgesic action of paracetamol [4]. Thus, the analgesic action of paracetamol depends on a functioning COX-1 gene and involves inhibition of a COX-3 enzyme. The hypothermic action of paracetamol in mice was also partly dependent on the integrity of the COX-1 gene. The hypothermia was less marked in COX-1 gene deleted mice than in heterozygous animals [2].

A COX-2 variant enzyme induced with high doses of diclofenac

In 1995, Dan Simmons discovered a COX-2 variant enzyme in chicken embryo fibroblasts, that was induced with high concentrations of NSAIDs, which at the same time produced apoptosis of these cells [25]. The most effective NSAID was 0.5 mM diclofenac, which induced high levels of a COX-2-like protein in cultured J774.2 mouse macrophages (Fig. 5). The diclofenac-induced COX-2 was sensitive to inhibition with paracetamol while COX-2 induced with lipopolysaccharide in the cultured J774.2 cells could not be inhibited [38]. Moreover, the non-selective NSAIDs, such as flurbiprofen and tolfenamic acid were less potent in inhibiting the diclofenac-induced enzyme than the lipopolysaccharide-induced COX-2. Aspirin induced the NSAID-inducible COX-2 to a measurable degree but did not inhibit it in doses up to 2 mM. To induce this COX-2 variant, diclofenac stimulates a PPAR-γ receptor [23], the endogenous ligand for which is 15-deoxy$^{\Delta 12\text{-}14}$PGJ$_2$ [8].

The COX-2 variant induced with 0.5 mM diclofenac differs from COX-2 induced with bacterial lipopolysaccharide (LPS) in a number of ways summarized in Table 2. For example, induction of COX-2 with LPS results in the release of inflammatory cytokines such as TNFα and IL-6, while induction of diclofenac-induced COX-2 releases cytokines such as TGFβ and IL-10 which are anti-inflammatory and involved in the resolution of inflammation [3]. Thus, an important function performed by the diclofenac-induced COX-2 may be in the resolution of inflammation. Furthermore, it would be tempting to speculate that the diclofenac-induced COX-2 is similar to the COX-2 enzyme involved in the resolution of rat experimental pleurisy described by Gilroy et al. [16, 17].

Tab. 2. LPS-induced COX-2 is different from diclofenac-induced COX-2. LPS induces COX-2 by activating NFκBα and diclofenac induces COX-2 by stimulating PPARγ. Induction is blocked by different inhibitors and antagonists. LPS releases inflammatory cytokines TNFα and IL-6. Diclofenac releases anti-inflammatory cytokines TGFβ and IL-10 [3]

LPS-induced	Diclofenac-induced
NFκBα - mediator	PPARγ - mediator
Inhibitor - srIκBα	Also induced by – 15 deoxy $\Delta^{12,14}$ PGJ$_2$
Cytokines released: TNFα} inflammatory IL-6 } "	Antagonists: GW9662 BADGE
	Cytokines released: TGFβ} anti-inflammatory IL-10} "

Conclusions

Thus, the discovery made by John Vane that aspirin inhibits COX and prevents synthesis of prostaglandins has led to the later discovery of COX-2 and the development of the selective COX-2 inhibitors. These useful drugs retain the anti-inflammatory properties of NSAIDs but without the damaging effects on the gastric mucosa of the non-selective inhibitors of COX-1 and COX-2. Further studies uncovered a COX-1 variant enzyme, named COX-3, the inhibition of which may explain the mechanism of action of paracetamol. Interestingly, a COX-2 variant enzyme induced by stimulation of PPARγ may be instrumental in the resolution of the inflammatory response.

John Vane's legacy has also given us aspirin as an anti-thrombotic drug for the prevention of heart attacks and strokes [1] while it remains a popular over-the-counter remedy for headache, fever and inflammation. The anti-thrombotic properties of low doses of aspirin have also been applied to the treatment of recurrent fetal growth retardation, where blood clots form in the umbilical vessels resulting in habitual abortion [13]. Epidemiological studies have demonstrated a beneficial effect of aspirin in protecting against several forms of cancer, especially that of the colon [41]. Its reputation as a universal remedy for many common diseases still remains strong.

References:

1. Antiplatelet Trialists Collaboration. Secondary prevention of vascular disease by prolonged antiplatelet treatment. Br Med J, 1988, 296, 320–331.
2. Ayoub SS, Botting RM, Goorha S, Colville-Nash PR, Willoughby DA, Ballou LR: Acetaminophen-induced hypothermia in mice is mediated by a prostaglandin endoperoxide synthase 1 gene-derived protein. Proc Natl Acad Sci USA, 2004, 101, 11165–11169.
3. Ayoub SS, Botting RM, Joshi AN, Seed MP, Colville-Nash PR: Activation of macrophage peroxisome proliferators-activated receptor-γ by diclofenac results in the induction of cyclooxygenase-2 protein and the synthesis of anti-inflammatory cytokines. Mol Cell Biochem, 2009, 327, 101–110.
4. Ayoub SS, Colville-Nash PR, Willoughby DA, Botting RM: The involvement of a cyclooxygenase 1 gene-derived protein in the antinociceptive action of paracetamol in mice. Eur J Pharmacol, 2006, 538, 57–65.
5. Born GVR: Aggregation of blood platelets by adenosine diphosphate and its reversal. Nature, 1962, 194, 927.
6. Chandrasekharan NV, Dai H, Roos KLT, Evanson NK, Tomsik J, Elton TS, Simmons D: COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: Cloning, structure and expression. Proc Natl Acad Sci USA, 2002, 99, 13926–13931.
7. Courade J-P, Besse D, Delchambre C, Hanoun N, Hamon M, Eschalier A, Caussade F, Cloarec A: Acetaminophen distribution in the rat central nervous system. Life Sci, 2001, 69, 1455–1464.
8. Cuzzocrea S, Wayman NS, Mazzon E, Dugo L, Paolo RD, Serraino I, Britti D et al.: The cyclopentone prostaglandin 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 attenuates the development of acute and chronic inflammation. Mol Pharmacol, 2002, 61, 997–1007.
9. DeWitt DL, Smith WL: Primary structure of prostaglandin G/H synthase from sheep vesicular gland determined from the complementary DNA sequence. Proc Natl Acad Sci, USA, 1988, 8, 1412–1416.
10. Di Rosa M, Giroud JP, Willoughby DA: Studies of the mediators of the acute inflammatory response induced in rats in different sites by carrageenin and turpentine. J Pathol, 1971, 104, 15–29.
11. Douthwaite AH, Lintott GM: Gastroscopic observation of the effect of aspirin and certain other substances on the stomach. Lancet, 1935, ii, 1222–1225.
12. Dreser H: Pharmacologisches über Aspirin (Acetylsalicyl-saüre). Pflügers Arch Gesamte Physiol Menschen Tiere, 1899, 76, 306–318.
13. Elder MG, de Sweit M, Robertson A, Elder MA, Flloyd E, Hawkins DF: Low dose aspirin in pregnancy. Lancet, 1988, i, 410.
14. Ferreira SH, Moncada S, Vane JR: Indomethacin and aspirin abolish prostaglandin release from spleen. Nature, 1971, 231, 237–239.
15. Flower RJ, Vane JR: Inhibition of prostaglandin synthetase in brain explains the antipyretic activity of paracetamol (4-acetamidophenol). Nature, 1972, 240, 410–411.
16. Gilroy DW, Colville-Nash PR, Willis D, Chivers J, Paul-Clark MJ, Willoughby DA: Inducible cyclooxygenase may have anti-inflammatory properties. Nat Med, 1999, 5, 698–701.
17. Gilroy DW, Colville-Nash PR, McMaster S, Sawatzky DA, Willoughby DA, Lawrence T: Inducible cyclooxygenase-derived 15-deoxy$\Delta^{12\text{-}14}$$PGJ_2$ brings about acute inflammatory resolution in rat pleurisy by inducing neutrophil and macrophage apoptosis. FASEB J, 2003, 17, 2269–2271.
18. Hemler M, Lands WEM, Smith WL: Purification of the cyclooxygenase that forms prostaglandins: Demonstration of two forms of iron in the holoenzyme. J Biol Chem, 1976, 251, 5575–5579.
19. Herschman HR: Prostaglandin synthase 2. Biochim Biophys Acta, 1996, 1299, 125–140.
20. Kulmacz RJ, Pendleton RB, Lands WEM: Interaction between peroxidase and cyclooxygenase activities in prostaglandin-endoperoxide synthase. Interpretation of reaction kinetics. J Biol Chem, 1994, 269, 5527–5536.
21. Kurumbail RG, Stevens AM, Gierse JK, McDonald JJ, Stegeman RA, Pak JY, Gildehaus D et al.: Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. Nature, 1996, 384, 644–648.

22. Lanza FL, Royer GL Jr, Nelson RS, Chenn TT, Seckman CE, Rack MF: The effects of ibuprofen, indomethacin, aspirin, naproxen and placebo on the gastric mucosa of normal volunteers: a gastroscopic and photographic study. Dig Dis Sci, 1979, 24, 823–828.
23. Ledwith BJ, Pauley CJ, Wanger LK, Rokos CL, Alberts DW, Manam S: Induction of cyclooxygenase-2 expression by peroxisome proliferators and non-tetradecanoylphorbol 12,13-myristate-type tumor promoters in immortalized mouse liver cells. J Biol Chem, 1997, 272, 3707–3714.
24. Loll PJ, Picot D, Garavito RM: The structural basis of aspirin activity inferred from the crystal structure of inactivated prostaglandin H_2 synthase. Nature Struct Biol, 1995, 2, 637–643.
25. Lu X, Xie W, Reed D, Bradshaw WS, Simmons DL: Nonsteroidal anti-inflammatory drugs cause apoptosis and induce cyclooxygenases in chicken embryo fibroblasts. Proc Natl Acad Sci USA, 1995, 92, 7961–7965.
26. Luong C, Miller A, Barnett J, Chow J, Ramesha C, Browner MF: Flexibility of the NSAID binding site in the structure of human cyclooxygenase-2. Nature Struct Biol, 1996, 3, 927–933.
27. Mancini JA, O'Neill GP, Bayly C, Vickers PJ: Mutation of serine-516 in human prostaglandin G/H synthase-2 to methionine or aspirin acetylation of this residue stimulates 15-R-HETE synthesis. FEBS Lett, 1994, 342, 33–37.
28. Merlie JP, Fagan D, Mudd J, Needleman P: Isolation and characterisation of the complementary DNA for sheep seminal vesicle prostaglandin endoperoxide synthase (cyclooxygenase). J Biol Chem, 1988, 263, 3550–3553.
29. Milton AS, Wendlandt S: A possible role for prostaglandin E_1 as a modulator of temperature regulation in the central nervous system of the cat. J Physiol, 1970, 207, 76–77.
30. Mitchell JA, Akarasereenont P, Thiemermann C, Flower RJ, Vane JR: Selectivity of nonstroidal anti-inflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. Proc Natl Acad Sci USA, 1993, 90, 11693–11697.
31. Miyamoto T, Ogino N, Yamamoto S, Hayaishi O: Purification of prostaglandin endoperoxide synthase from bovine vesicular gland microsomes. J Biol Chem, 1976, 251, 2629–2636.
32. O'Brien JR: Effects of salicylates on human platelets. Lancet, 1968, i, 779.
33. Otto JC, Smith WL: Prostaglandin endoperoxide synthases-1 and -2. J Lipid Mediat Cell Signal, 1995, 12, 139–156.
34. Patrignani P, Panara MR, Greco A, Fusco O, Natoli C, Iacobelli S, Cipollone F et al.: Biochemical and pharmacological characterisation of the cyclooxygenase activity of human blood prostaglandin endoperoxide synthases. J Pharmacol Exp Therap, 1994, 271, 1705–1712.
35. Picot D, Loll PJ, Garavito RM: The X-ray crystal structure of the membrane protein prostaglandin H_2 synthase-1. Nature, 1994, 367, 243–249.
36. Roth GJ, Stanford N, Majerus PW: Acetylation of prostaglandin synthetase by aspirin. Proc Natl Acad Sci USA, 1975, 2, 3073–3076.
37. Simmons DL, Botting RM, Hla T: Cyclooxygenase isozymes: the biology of prostaglandin synthesis and inhibition. Pharmacol Rev, 2004, 56, 387–437.
38. Simmons DL, Botting RM, Robertson PM, Madsen ML, Vane JR: Induction of an acetaminophen-sensitive cyclooxygenase with reduced sensitivity to nonsteroid anti-inflammatory drugs. Proc Natl Acad Sci, USA, 1999, 96, 3275–3280.
39. Smith JB, Willis AL: Aspirin selectively inhibits prostaglandin production in human platelets. Nature, 1971, 231, 235–237.
40. Solomon LM, Juhlin L, Kirschbaum MB: Prostaglandins in cutaneous vasculature. J Invest Derm, 1968, 51, 280–282.
41. Thun MJ, Namboodiri MM, Heath Jr CW: Aspirin use and reduced risk of colon cancer. N Engl J Med, 1991, 325, 1593–1596.
42. Vane JR: Inhibition of prostaglandin synthesis as a mechanism of action for the aspirin-like drugs. Nature, 1971, 231, 232–235.
43. Vesterqvist O, Green K: Urinary excretion of 2,3-dinorthromboxane B_2 in man under normal conditions, following drugs and during some pathological conditions. Prostaglandins, 1984, 27, 677–644.
44. Warner TD, Giuliano F, Vojnovic I, Bukasa A, Mitchell JA, Vane JR: Nonsteroidal drug selectivities for cyclooxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: A full in vitro analysis. Proc Natl Acad Sci USA, 1999, 96, 7563–7568.
45. Weiss HJ, Aledort LM: Impaired platelet connective tissue reasction in man after aspirin ingestion. Lancet, 1967, ii, 495.
46. Xie W, Chipman JG, Robertson DL, Erikson RL, Simmons DL: Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. Proc Natl Acad Sci USA, 1991, 88, 2692–2696.
47. Yokoyama C, Takai T, Tanabe T: Primary structure of sheep prostaglandin endoperoxide synthase deduced from cDNA sequence. FEBS Lett, 1988, 231, 347–351.

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