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REVIEW

The contribution of cyclooxygenase-2 to endocannabinoid metabolism and action

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The development of sensitive analytical methods for measurement of endocannabinoids, their metabolites, and related lipids, has underlined the complexity of the endocannabinoid system. A case can be made for an 'endocannabinoid soup' (akin to the inflammatory soup) whereby the net effect of a pathological state and/or a pharmacological intervention on this system is the result not only of changes in endocannabinoid levels but also of their metabolites and related compounds that affect their function. With respect to the metabolism of anandamide and 2-arachidonoylglycerol, the main hydrolytic enzymes involved are fatty acid amide hydrolase and monoacylglycerol lipase. However, other pathways can come into play when these are blocked. Cyclooxygenase-2 derived metabolites of anandamide and 2-arachidonoylglycerol have a number of properties, including effects upon cell viability, contraction of the cat iris sphincter (an effect mediated by a novel receptor), mobilization of calcium and modulation of synaptic transmission. Nonsteroidal anti-inflammatory agents, whose primary mode of action is the inhibition of cyclooxygenase, can also interact with the endocannabinoid system both *in vitro* and *in vivo*. Other enzymes, such as the lipoxygenase and cytochrome P450 oxidative enzymes, can also metabolize endocannabinoids and produce biologically active compounds. It is concluded that sensitive analytical methods, which allow for measurement of endocannabinoids and related lipids, should provide vital information as to the importance of these alternative metabolic pathways when the primary hydrolytic endocannabinoid metabolizing enzymes are inhibited.
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Abbreviations: AA, arachidonic acid; AEA, anandamide (arachidonoylethanolamide); 2-AG, 2-arachidonoylglycerol; AM251, *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide; CB, cannabinoid; COX, cyclooxygenase; FAAH, fatty acid amide hydrolase; ibu-am5, *N*-(3-methylpyridin-2-yl)-2-(4′-isobutylphenyl)propionamide; MGL, monoacylglycerol lipase; OA, oleic aid; OEA, oleoylethanolamide; PA, palmitic acid; PEA, palmitoylethanolamide; PG, prostaglandin; PG-EA, prostamide (prostaglandin ethanolamide); PG-GE, prostaglandin glycerol ester; PPAR, peroxisome proliferator-activated receptor

Introduction

The development of sensitive methods for the analysis of endocannabinoid levels in biological tissues has played a key role in our understanding of the effects of pathological conditions, genetic modifications and pharmacological intervention strategies upon endocannabinoid signalling processes in the body. Thus, for example, mice lacking the enzyme fatty acid amide hydrolase (FAAH) show large increases in brain anandamide (AEA) concentrations, as do animals treated with selective inhibitors of FAAH such as URB597 (3′-carbamoyl-biphenyl-3-yl-cyclohexylcarbamate) (Cravatt *et al*., 2001; Kathuria *et al*., 2003). The endocannabinoid system, however, is so much more than just AEA and 2-arachidonoylglycerol (2-AG), their synthetic and degradative enzymes and their receptors. In a wide view, it can be considered as comprising other potential endocannabinoids, other receptor systems capable of interacting with endocannabinoids, and endocannabinoid-related biologically active lipids (see the other articles in this themed issue of the *British Journal of Pharmacology*). Taking once again FAAH as an example, inhibition or genetic deletion of this enzyme will not only increase AEA (and in some tissues 2-AG) levels, but also affect other endogenous substrates for this enzyme. Examples are *N*-acylethanolamines such as palmitoylethanolamide (PEA) and oleoylethanolamide (OEA) (see Fegley *et al*., 2005 for an example of the effects of URB597 upon brain PEA and OEA levels). These compounds have important biological actions of their own. PEA, for example, is active in a variety of models of inflammation and inflammatory pain, and it has been argued that these effects are

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brought about by actions upon peroxisome proliferator-activated receptor-α (PPARα, Lo Verme *et al*., 2005). Other endogenous substrates, such as *N*-acyl amides and *N*-acyl taurines, also have biological actions of their own (Cravatt *et al*., 1995; Saghatelian *et al*., 2006).

Analogous to the 'inflammatory soup', that is, the variety of mediators involved in the inflammatory response, we should consider the endocannabinoid system as an 'endocannabinoid soup' where the response to a given pathological system or intervention can be the sum of actions of a number of endogenous agents, not only *per se*, but also with respect to their ability to affect the actions of other agents (such as the ability of the 'entourage' compounds 2-palmitoylglycerol and 2-linoleoylglycerol to affect 2-AG function, Ben-Shabat *et al*., 1998). In the present review, the contribution of cyclooxygenase (COX) to this 'endocannabinoid soup' is considered.

Formation and function of prostamides (prostaglandin ethanolamides)

A simplified schematic showing the metabolism of AEA and 2-AG is shown in Figure 1. The primary route of AEA metabolism is via FAAH. 2-AG is a substrate for FAAH as well as monoacylglycerol lipase (MGL) (Goparaju *et al*., 1998; Dinh *et al*., 2002), and the effect of selective FAAH inhibition upon 2-AG levels will in consequence depend upon the relative affinities to, and availabilities of these enzymes in the tissue studied (Kathuria *et al*., 2003; Jhaveri *et al*., 2006; Maione *et al*., 2006).

Yu *et al*. (1997) reported that human recombinant COX-2 could oxygenate AEA in an analogous manner to that seen with arachidonic acid (AA). This has subsequently been confirmed by others, and a structure–activity relationship study has indicated that the hydroxyl-group of AEA is a key requirement for cyclooxygenation (Kozak *et al*., 2003). The incubation of cultured cells with AEA results in the formation of prostaglandin (PG)D_2-, PGE_2- and $PGF_{2\alpha}$-ethanolamides, and there is evidence that PGH_2-ethanolamide is formed as an intermediate in the production of $PGF_{2\alpha}$-EA (Yang *et al*., 2005). COX-1 is less efficient than COX-2 at metabolizing AEA (Yu *et al*., 1997), and it has been suggested that this may be related to the lack of a critical arginine residue in the active site (Kozak *et al*., 2003).

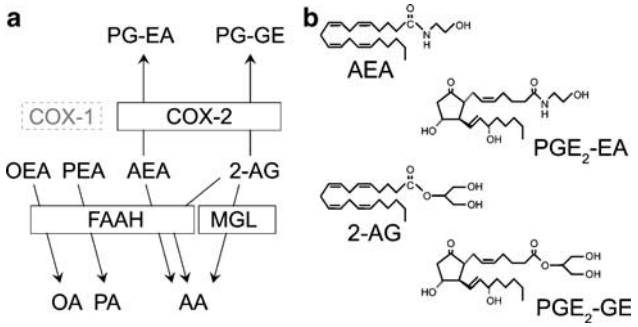

Figure 1 (**a**) Schematic representation of the roles of FAAH, MGL and COX-2 in the metabolism of AEA, 2-AG and the related *N*-acylethanolamines OEA and PEA. The endocannabinoids are poor substrates for COX-1 compared to COX-2, at least in cell-free systems. Further metabolism of the oleic acid (OA), palmitic acid (PA) and AA has not been shown in the figure, for reasons of simplicity. (**b**) Structures of AEA, 2-OG and representative COX-2 metabolites (PGE_2-EA and PGE_2-GE). 2-AG, 2-arachidonoylglycerol; AA, arachidonic acid; AEA, anandamide; COX, cyclooxygenase; FAAH, fatty acid amide hydrolase; MGL, monoacylglycerol lipase; OA, oleic acid; OEA, oleoylethanolamide; PA, palmitic acid; PEA, palmitoylethanolamide; PG-EA, prostaglandin ethanolamides ('prostamides'); PG-GE, prostaglandin glyceryl esters.

The pharmacological properties of the prostamides are beginning to emerge. They have weak effects at prostanoid receptors (as compared to prostaglandins) and do not feedback inhibit FAAH or MGL (Ross *et al*., 2002; Matias *et al*., 2004; Fowler and Tiger, 2005). PGE_2-ethanolamide (PGE_2-EA) has no effect at concentrations of up to 10 μM on the binding of the cannabinoid (CB) receptor agonist [^{3}H]CP55,940 ([^{3}H](−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol) to mouse brain CB_1 receptors (Ross *et al*., 2002). However, the methanandamide analogue of $PGF_{2\alpha}$ showed a modest activity at CB_2 receptors (K_i value 0.7 μM), measured by the inhibition of [^{3}H]CP55,940 binding to human tonsil membranes (Berglund *et al*., 1999). The methanandamide analogue of PGE_2 was also found in this study to increase GTPγS (guanosine 5′-(3-O-thio) triphosphate) binding in rat brain membranes in a manner not affected by the CB_1 receptor antagonist/inverse agonist rimonabant (Berglund *et al*., 1999), suggesting an ability to activate a G-protein-coupled receptor other than CB_1 receptors. The receptor in question has not been identified.

Prostamides are potent contractors of the cat iris sphincter, with an order of potency $PGF_{2\alpha}$-EA (57, 11) > PGD_2-EA (499, 150) ≈ PGE_2-EA (564, 260) (Matias *et al*., 2004; values in parentheses are the EC_{50} values in nM for the prostamides followed by the corresponding prostaglandins). Given a high degree of metabolic stability of the prostamides and their modest effects at prostamide receptors (Kozak *et al*., 2001; Ross *et al*., 2002; Matias *et al*., 2004), it was argued by the latter authors that the effect upon the cat iris was produced by an action on a novel receptor by the prostamides themselves. Subsequent work has demonstrated that the compound AGN 204396 (3-(2-{(1*R*,2*R*,3*S*,4*R*)-3-[4-(4-cyclohexyl-butylcarbamoyl)-oxazol-2-yl]-7-oxa-bicyclo[2.2.1] hept-2-ylmethyl)-4-fluoro-phenyl)-propyl ethylamide) acts as an antagonist of the actions upon the iris of $PGF_{2\alpha}$-EA, PGD_2-EA and PGE_2-EA, but not of the corresponding prostaglandins or of PGE_2-GE (Woodward *et al*., 2007), further supporting the notion of a prostamide receptor in this tissue. Other actions of prostamides have also been reported. Thus, for example, treatment of HT29 colorectal carcinoma cells with 10 μM PGE_2-EA for 72 h results in a reduction of adherent cells with apoptotic changes (cleavage of poly (ADP-ribose) polymerase) being detected in the shed cells (Patsos *et al*., 2005). PGD_2-EA (30 μM), but not PGE_2-EA or $PGF_{2\alpha}$-EA, has been reported to increase the frequency of miniature inhibitory postsynaptic currents in hippocampal neurons in primary culture, a result in contrast to the decrease seen with PGD_2 (5 μM) (Sang *et al*., 2006). It would be interesting to determine whether or not the response to prostamides in these experiments is blocked by AGN 204396.

While there is no doubt that prostamides have interesting pharmacological properties when added exogenously, a key

question concerns their ability to be formed endogenously. When incubated with AEA (20 μM), activated RAW264.7 mouse macrophage cells produce PGD_2-EA in a manner blocked by the COX-2 inhibitor indomethacin phenethylamide (Kozak *et al*., 2002a). PGE_2-EA and $PGF_{2\alpha}$ were produced by human colon adenocarcinoma HCA-7 cells, following incubation with 20 μM AEA. The ability of cells to produce prostamides has raised a potentially important issue with respect to assay of prostaglandins. The measurements described in the study of Kozak *et al*. (2002a) utilized advanced liquid chromatography–mass spectrometry analytical methods. Commonly used commercially available assays measuring prostaglandin production rely on antibody recognition of the cyclooxygenated acyl side chain. However, this structure is the same for prostaglandins and prostamides, and several commercial antibodies have been shown not to discriminate between PGE_2 and PGE_2-EA (Glass *et al*., 2005). These authors showed further that amnion-derived WISH cells responded to interleukin-1β (0.2 ng ml^{-1}) plus AEA (10 μM) stimulation in a synergistic manner in terms of apparent PGE_2 production, but that the synergy was in fact primarily due to AEA-derived PGE_2-EA production.

While the data described above clearly demonstrate that cells can produce prostamides when incubated with relatively high AEA concentrations, they do not prove one way or the other that such a process occurs *in vivo*. COX-2 is usually described in textbooks as an inducible enzyme, but it is constitutively active in the spinal cord (Ghilardi *et al*., 2004). Little information is available concerning the levels of prostamides in intact animals. However, Weber *et al*. (2004) reported that the treatment of $FAAH^{-/-}$ mice with AEA (50 mg kg^{-1} intravenous) produced detectable levels of $PGF_{2\alpha}$-EA and PGE_2-EA + PGD_2-EA in the liver, kidney, lung and small intestine. In contrast, levels of $PGF_{2\alpha}$-EA were below the limits of quantitation (defined as <50 pg ml^{-1} in the study) in all four tissues for normal (albeit not littermate) mice, regardless as to whether or not they had been treated with AEA. Quantitatable, but lower, levels of PGE_2-EA + PGD_2-EA were seen for the AEA-treated controls in the kidney and lung, but not in the other tissues (Weber *et al*., 2004). These data would suggest that while FAAH is the primary metabolic pathway for AEA, the alternative COX-2 metabolic route can come into play when FAAH is inhibited. This would be particularly apparent in damaged tissue, since the tissue damage *per se* causes an increased synthesis of AEA (see Berger *et al*., 2004 for an example demonstrating the dramatic increase in AEA and related *N*-acylethanolamines following ischaemic insult to the brain, a condition where COX-2 is induced, Collaço-Moraes *et al*., 1996). It remains to be seen whether the treatment of animals with a selective FAAH inhibitor results in measurable production of prostamides in damaged tissue, and whether this production impacts upon the damage.

Synthesis and biological actions of glyceryl prostaglandins

Just as AEA is a substrate for COX-2, 2-AG can be metabolized by this enzyme to produce prostaglandin glycerol esters (glyceryl prostaglandins, PG-GE) via PGH_2-GE as an intermediate (Kozak *et al*., 2000, 2002a). 2-AG is more avidly oxygenated by COX-2 than its regioisomer 1-AG or the analogue arachidonic acid 2-hydroxyethyl ester, while arachidonic acid 2-methoxyethyl ester is a poor substrate for COX-2 (Kozak *et al*., 2000). Incubation of HCA-7 cells with 20 μM 2-AG resulted in the production of both PGE_2-GE and $PGF_{2\alpha}$-GE in a manner inhibited by indomethacin phenylethylamide (Kozak *et al*., 2002a). Synthesis of PG-GE from endogenous 2-AG has also been demonstrated in lipopolysaccharide-treated murine resident peritoneal macrophages, in response to zymosan phagocytosis (Rouzer and Marnett, 2005), and in activated RAW264.7 macrophages in response to the calcium ionophore ionomycin (Kozak *et al*., 2000). In isolated enzyme assays, 2-AG was a poor substrate for COX-1 (Kozak *et al*., 2000). However, production of PG-GE was also seen in resident peritoneal macrophages in response to zymosan even when COX-2 was not induced by lipopolysaccharide treatment, and the additional zymosan-induced PG-GE production seen in the lipopolysaccharide-treated cells was reduced to the level seen in response to zymosan for the unstimulated cells following treatment with a COX-2-selective inhibitor (Rouzer and Marnett, 2005). In a follow-up study, this group showed that the production of PG-GE in response to zymosan was abolished when resident non-induced (that is, not treated with lipopolysaccharide) peritoneal macrophages were prepared from $COX\text{-}1^{-/-}$, but not $COX\text{-}2^{-/-}$ mice (Rouzer *et al*., 2006). Taken together, these studies indicate that in macrophages, both COX isoenzymes are involved in PG-GE production.

Kim and Alger (2004) reported that depolarization-induced suppression of inhibition in rat hippocampal slices was not affected by URB597 (a result also seen by Makara *et al*., 2005), but was potentiated by the COX inhibitors meloxicam and nimesulide, and that the nimesulide potentiation was not seen in the presence of the CB_1 antagonist/inverse agonist AM251 (*N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide). These authors explained their findings in a model whereby 2-AG was released by the pyramidal cell to act retrogradely upon CB_1 receptors located on the axon terminal before reuptake and degradation by COX-2 (Kim and Alger, 2004). A further study utilizing hippocampal cultures indicated a similar effect of the COX-2-selective inhibitor NS398 (*N*-[2-cyclohexyloxy-4-nitrophenyl]methanesulfonamide, 20 μM) (Sang *et al*., 2006). Furthermore, induction of COX-2 by interleukin-1β treatment reduced the observed depolarization-induced suppression of inhibition in the cultures (Sang *et al*., 2006). The lack of MGL-selective inhibitors has precluded investigation as to the relative importance of MGL and COX-2. However, Makara *et al*. (2007) reported that the non-selective inhibitor methyl arachidonoyl fluorophosphonate potentiated depolarization-induced suppression of inhibition in hippocampal slices, which is consistent with a significant role of MGL in regulating the intensity of this process given that URB 597 was without effect.

PG-GEs may have important biological actions, particularly in the brain, although the synthetic compounds used have been based upon the 1-AG regioisomer rather than 2-AG, presumably for reasons of stability. Most work has been undertaken with the PGE_2-GE compound, which has

weak effects upon prostanoid receptors and does not feedback inhibit MGL or FAAH, but increases the levels of inositol-(1,4,5)-trisphosphate and mobilizes calcium in RAW264.7 cells, and increases the frequency of miniature inhibitory postsynaptic currents in hippocampal neurons in primary culture in a manner not blocked by rimonabant (Nirodi *et al*., 2004; Fowler and Tiger, 2005; Sang *et al*., 2006). The effect of PGE_2-GE upon the frequency of miniature inhibitory postsynaptic currents in hippocampal neurons was mimicked by PGD_2-GE and $PGF_{2\alpha}$-GE and was reduced by the mitogen-activated protein kinase inhibitor PD98059 (2-(2-amino-3-methoxyphenyl)-4H-1-benzopyran-4-one) and by the non-selective inositol-(1,4,5)-trisphosphate receptor antagonist 2-aminoethoxydiphenyl borane (Sang *et al*., 2006). Further, induction of COX-2 in the cultures by treatment with interleukin-1β also resulted in an increased frequency of the miniature inhibitory postsynaptic currents in a manner resistant to rimonabant, but attenuated by PD98059 and 2-aminoethoxydiphenyl borane (Sang *et al*., 2006). These data suggest that there is an endogenous production of PGE-GEs in the cultures. Their conclusion that the 'enhanced COX-2 activity resulting from inflammation, traumatic injury, epilepsy or degenerative disorders will have significant impact on … eCB [endocannabinoid, my note]-derived prostanoid signalling in synaptic activity' (Sang *et al*., 2006) motivates further investigation.

Interaction of non-steroidal anti-inflammatory drugs with the endocannabinoid system

Given the ability of endocannabinoids to interact with COX-2, and the structural similarities between both arachidonic acid, AEA and 2-AG, as well as between COX-2 and FAAH (Bracey *et al*., 2002), it is perhaps not surprising that compounds with primary actions upon COX enzymes also interact with the endocannabinoid system, including an ability directly to inhibit FAAH (Table 1). Under basal conditions, blockade of COX-2 by the non-steroidal anti-inflammatory drugs (NSAIDs) would be unlikely to raise levels of AEA (even in the presence of a partial FAAH inhibitory action of the compounds). Indeed, local administration of ibuprofen does not significantly increase levels of the *N*-acylethanolamines AEA, PEA or OEA in the paw, a result also seen with rofecoxib (Guindon *et al*., 2006b). However, ibuprofen (and rofecoxib) can potentiate *N*-acylethanolamine levels when administered with AEA (Guindon *et al*., 2006b). What is not clear from this study is whether the FAAH inhibitory actions of the NSAIDs contribute to the findings. This should, however, be possible to investigate experimentally by comparing compounds with different relative potencies towards FAAH and COX. In this respect, ibu-am5 (*N*-(3-methylpyridin-2-yl)-2-(4′-isobutylphenyl)propionamide), the 6-methyl-pyridin-2-yl analogue of ibuprofen (which has a greater potency towards FAAH relative to COX-1 and -2 than ibuprofen, Holt *et al*., 2007) and the COX-2 selective compounds nimesulide and SC-58125 (5-(4-fluorophenyl)-1-[4-(methylsulfonyl)phenyl]-3-(trifluoromethyl)-1H-pyrazole) (which do not inhibit FAAH, Fowler *et al*., 2003) may be useful in teasing out the contribution of FAAH inhibition to the pharmacological actions of NSAIDs.

A clinically more pressing correlate of the above discussion is whether increasing the FAAH inhibitory component of NSAIDs may improve upon their therapeutic properties and/or modify their adverse effect profile. Little is known in this respect, although the potential exists for increased efficacy, given that the NSAID ketorolac produces additive effects with the CB agonist WIN55212-2 ((*R*)-(+)-[2,3-dihydro-5-

Table 1 Selection of *in vitro* and *in vivo* effects of NSAIDs upon the endocannabinoid system

NSAID	In vitro *actions*	In vivo *actions*
Ibuprofen	pH-dependent inhibition of rat brain and recombinant FAAH (IC_{50} value 130 μM at pH 6) (Holt *et al*., 2001; Holt *et al*., 2007); no inhibition of MGL (Holt *et al*., 2007); partial inhibition of [^{3}H]CP55,940 binding to CB_1 and CB_2 receptors only at 300 μM but not at 100 μM (Holt *et al*., 2007)	Local administration to paw and formalin test of inflammatory pain in rats: no AM251-sensitive action *per se*, but AM251-sensitive synergy with AEA (Guindon *et al*., 2006a). Synergistic effect with AEA on paw AEA, PEA and OEA levels (Guindon *et al*., 2006b)
Indomethacin	pH-dependent inhibition of rat brain FAAH (IC_{50} value 17 μM at pH 6) (Fowler *et al*., 2003); no inhibition of MGL (Holt *et al*., 2007). Inhibits [^{3}H]CP55,940 binding to CB_1 and CB_2 at concentrations $\geqslant$100 μM (Holt *et al*., 2007)	Intrathecally administered indomethacin is active in formalin test in mice in a manner blocked by AM251 (Gühring *et al*., 2002). This effect of indomethacin is not seen in $CB^{-/-}$ mice (Gühring *et al*., 2002). In contrast, the ability of oral indomethacin to attenuate visceral pain (*p*-phenylquinone stretch model) is not affected by either rimonabant or SR144528 (Anikwue *et al*., 2002)
Flurbiprofen	pH-dependent inhibition of rat brain FAAH (IC_{50} values 31 and 13 μM for (*R*)- and (*S*)-enantiomers at pH 6) (Fowler *et al*., 2003); 3 μM flurbiprofen decreases calcitonin gene-related peptide release from perfused rat spinal cord in a manner blocked by AM251 (Seidel *et al*., 2003)	Intrathecally administered flurbiprofen active in formalin test in rats in a manner blocked by AM251 (Ates *et al*., 2003)
Rofecoxib	No data, but the related compound celecoxib is a weak FAAH inhibitor (IC_{50} value $\sim$300 μM, not pH-dependent) (Fowler *et al*., 2003)	Local administration to paw and formalin test in rats: synergistic effect with AEA on paw AEA, PEA and OEA levels (Guindon *et al*., 2006b)

Abbreviations: AEA, anandamide; CB, cannabinoid; FAAH, fatty acid amide hydrolase; IC_{50}, half-maximal inhibitory concentration; MGL, monoacylglycerol lipase; NSAID, non-steroidal anti-inflammatory drug; OEA, oleoylethanolamide; PEA, palmitoylethanolamide.
Indomethacin has also been reported to reduce carrageenan-induced oedema in pentobarbital-treated mice in a manner prevented by SR144528 (*N*-[(1*S*)-endo-1,3,3-trimethyl bicyclo [2.2.1] heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylben-zyl)-pyrazole-3-carboxamide) (Holt *et al*., 2005). It is not, however, clear whether this reflects the ability of SR144528 to block CB_2 receptors (Rinaldi-Carmona *et al*., 1998) or to prevent effects mediated by PPARα (Lo Verme *et al*., 2006).

methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone) in the mouse in the acetic acid-induced writhing model of inflammatory visceral pain (Ulugöl *et al*., 2006), and that ibu-am5 is more efficacious than ibuprofen in the corresponding model for the rat (Cocco *et al*., 2003). With respect to unwanted effects, ibu-am5 showed a lower acute ulcerogenic propensity than ibuprofen in the rat (Cocco *et al*., 2003), although this is probably related to the physicochemical properties of the compounds rather than their relative activities towards FAAH and COX (for discussion, see Holt *et al*., 2007). Very little information is available concerning the potential importance of modulation of the endocannabinoid system over a long period of time upon NSAID pharmacology: the only study to my knowledge investigating repeated administration protocols reported that $10\,mg\,kg^{-1}$ intraperitoneal (i.p.) (b.i.d. for 6.5 days) Δ^9-tetrahydrocannabinol treatment of mice reduced the sensitivity of the animals to the analgesic effects of several NSAIDs in the *p*-phenylquinone test for visceral nociception (Anikwue *et al*., 2002). In contrast, repeated methanandamide ($10\,mg\,kg^{-1}$ i.p.) treatment did not affect the sensitivity of the animals to NSAID treatment (Anikwue *et al*., 2002), which would suggest at least to this author that pharmacokinetic mechanisms may contribute to the interaction between repeated Δ^9-tetrahydrocannabinol treatment and NSAID sensitivity.

Other players in the 'endocannabinoid soup'

The focus of this review has been on AEA and 2-AG and their FAAH/MGL- and COX-2-derived metabolites. However, as intimated in the introduction, this represents only part of the picture. Firstly, AEA and 2-AG are by no means the only endogenous compounds with effects upon CB receptors, and other arachidonoyl compounds including noladin ether (2-arachidonoyl glyceryl ether, Hanuš *et al*., 2001) and virodhamine (*O*-arachidonoylethanolamine, Porter *et al*., 2002) have been proposed to act as endocannabinoids. With respect to the *N*-acylethanolamine family of compounds, Hanuš *et al*. (1993) reported the isolation of homo-γ-linolenylethanolamide and docosatetraenylethanolamide from porcine brain. The compounds had very similar affinities to AEA to the CB_1 receptor (Hanuš *et al*., 1993). Furthermore, astrocytes in culture produce these compounds in about the same concentrations as AEA, and in a manner stimulated by ionomycin (Walter *et al*., 2002). Perhaps the major difference between these compounds and AEA is the degree to which they have been studied—a simple PubMed search for 'docosatetraenylethanolamide' conducted in April 2007 gave six hits. The corresponding search for AEA gave 1659 hits. This issue was discussed at the 'hot topic' session 'Detecting endocannabinoids and their metabolites' chaired by this author at the Focussed meeting of the *British Pharmacological Society* and *3rd European Workshop in Cannabinoid Research*, held in Nottingham, 20–21 April 2007. It was argued that the availability (or, to be more precise, the lack thereof) of deuterated isotopes of the homo-γ-linolenylethanolamide and docosatetraenylethanolamide was a major limiting factor precluding analytical studies of these potentially important endogenous lipids. It is to be hoped that such compounds will become available in the near future.

A second factor in the complexity of the system is the presence of additional enzyme pathways that are capable of the metabolism of AEA and 2-AG. Some of these, like other esterases such as *N*-acylethanolamine-hydrolysing acid amidase (Ueda *et al*., 2001), human neuropathy target esterase (van Tienhoven *et al*., 2002) and a hitherto unidentified MGL-like activity distinct from MGL (Muccioli *et al*., 2007), as well as the newly discovered FAAH-2 present in primates but not rodents (Wei *et al*., 2006) will result in the hydrolysis of these endocannabinoids to give arachidonic acid. The net effect of an FAAH or an MGL inhibitor upon the levels of AEA or 2-AG within a given tissue will thus represent a combination of factors including inhibitor selectivity for FAAH/MGL vs these other enzymes, the relative roles played by the enzymes towards the metabolism of AEA/2-AG in the tissue in question, pharmacokinetic parameters, and in the case of inflamed tissue, the influence of the extracellular pH upon the potency of the inhibitor (which can be considerable, see Paylor *et al*., 2006). Other enzymes, such as lipoxygenases (Hampson *et al*., 1995; Ueda *et al*., 1995), *P*450 oxidizing enzymes (Snider *et al*., 2007 and references therein), monoacylglycerol kinases (Simpson *et al*., 1991) and an as yet unidentified enzyme converting *N*-acylethanolamines to their phosphorylcholine derivatives (Mulder and Cravatt, 2006) will produce other derivatives that in some cases have been shown to possess biological activity. Thus, for example, 15-hydroxyeicosatetraenoic acid glyceryl ester, produced by the action of 15-lipoxygenase upon 2-AG, has agonist actions upon PPARα (Kozak *et al*., 2002b). Incubation of human polymorphonuclear leukocytes with AEA results in the production of 12- and 15-(*S*)-hydroxyarachidonoylethanolamide, of which the former retains affinity for CB receptors (see also Hampson *et al*., 1995; Edgemond *et al*., 1998).

As if the system was not complex enough, an additional factor to be considered is the fact that AEA and 2-AG can produce biological effects by acting as precursors for arachidonic acid. The ability, for example, of AEA to increase pulmonary artery pressure in isolated rabbit lungs is not mimicked by the stable analogue methanandamide, is blocked by the nonspecific FAAH inhibitor methyl arachidonoyl fluorophosphonate, and by both aspirin and the COX-2-selective inhibitor nimesulide, suggesting that the effect of AEA in this model is mediated by its FAAH-catalysed conversion to AA and thereafter to an active COX-2-derived metabolite (Wahn *et al*., 2005). Similarly, ethanolamine, produced by FAAH-catalysed cleavage of *N*-acylethanolamines, should be considered, at least *in vitro*, in the light of the finding that it mediates the protective effect of AEA against low serum-induced apoptosis of N18TG2 neuroblastoma cells (Matas *et al*., 2007).

Conclusions

The present review has primarily highlighted the connection between the endocannabinoid and COX systems, and suggested that the latter may play a role under conditions of heightened endocannabinoid synthesis. Certainly, a case

can be made for the study of PG-EA and PG-GE levels following treatment with FAAH inhibitors under such conditions. Throughout this article, I have suggested that these alternative pathways come into play primarily when FAAH and/or MGL are inhibited and when endocannabinoid synthesis is activated following tissue damage. This conclusion is in some respects based upon the sensitivity of the analytical procedures used in the work so far published in peer-reviewed journals. The finding that COX-2 inhibitors inhibit, in a manner reversed by AM251, basal excitatory transmission in hippocampal slices (Slanina and Schweitzer, 2005) suggests that in some cases, the pathways may contribute to the removal of endocannabinoids even under basal conditions. These authors pointed out that since COX-2 activity is rapidly and transiently induced by an increase in glutamatergic synaptic activity (Yamagata *et al*., 1993), this enzyme may have a role in the regulation of endocannabinoid levels in a manner dependent upon neuronal activity (Slanina and Schweitzer, 2005). The first sentence of this review concerned the development of assays capable of the measurement of endocannabinoid levels in biological tissue. It is fitting that the final sentence should also recognize the important role that such methods will continue to have, and to suggest that future studies should widen the net, not only to other endocannabinoids and related lipids, but also to delineate the importance of these alternative endocannabinoid metabolic pathways.

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Conflict of interest

The author states no conflict of interest.

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