Review

Regulation of cyclooxygenase-2 expression by cyclic AMP

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Abstract

Prostaglandins (PG) regulate many biological processes, among others inflammatory reactions. Cyclooxygenases-1 and -2 (COX-1 and COX-2) catalyse PG synthesis. Since this step is rate limiting, the regulation of COX expression is of critical importance to PG biology. Contrary to COX-1, which is constitutively expressed, COX-2 expression is subject to regulation. For example, COX-2 levels are increased in inflammatory reactions. Many signalling pathways can regulate COX-2 expression, not least those involving receptors for COX products themselves. Analysis of the intracellular signal transducers involved reveals a crucial role for cAMP, albeit as a modulator rather than direct inducer. Indeed, the influence of cAMP on COX-2 expression is complex and dependent on the cell type and cellular environment. This review aims to summarise various topics related to cAMP-dependent COX-2 expression. Firstly, the main aspects of COX-2 regulation are briefly considered. Secondly, the molecular basis for *COX-2* gene (post)-transcriptional regulation is reviewed. Lastly, a detailed overview of the effects of cAMP-dependent signalling on COX-2 mRNA and protein expression in various human and rodent cells is provided. There is a large number of marketed, clinical and preclinical concepts promoting the elevation of intracellular cAMP levels for therapeutic purposes (e.g., β_2-agoinsts, PG receptor agonists, phosphodiesterase inhibitors). In this respect, the role of cAMP in the regulation of COX-2 expression, especially the human enzyme, is of significant clinical importance.
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1. Introduction

1.1. Prostanoid signalling

Prostanoids ($PGF_{2\alpha}$, PGE_2, PGD_2, PGI_2, TxA_2) are derived from arachidonic acid (AA) via a sequence of enzyme-catalysed steps, involving in order phospholipase A_2, Cyclooxygenases-1 and -2 (COX-1 and -2) and one of several terminal prostaglandin synthases. The COX catalysed step results in the formation of PGH_2 and is rate limiting. Hence, levels of COX expression are a critical determinant of prostanoid levels.

Of the two COX isozymes, one is constitutively expressed, COX-1, while the other, COX-2, is regulated. Many cytokines and growth factors govern COX-2 regulation (for a review, see

[1]). These signalling mediators use several different intracellular pathways, e.g., NFkB, fos/jun. While those pathways can influence COX-2 expression directly by acting on their respective sites on the COX-2 promoter, they also do so indirectly via the modulation of cAMP signalling. For instance PKA function might be modulated (see Fig. 1). Furthermore, other signalling pathways involved in COX-2 levels regulation act by directly affecting cAMP levels. For instance ligands for Gi- or Gs-coupled GPCR receptors influence cAMP levels directly by modulating adenylate cyclase activity: Gi-coupled receptors inhibit adenylate cyclase activity while Gs-coupled receptors stimulate it. Indeed, this is illustrated by COX-2 autoregulatory mechanisms involving the whole PG pathway: prostaglandin E_2 signals through four different G-protein-coupled receptors (e.g.,
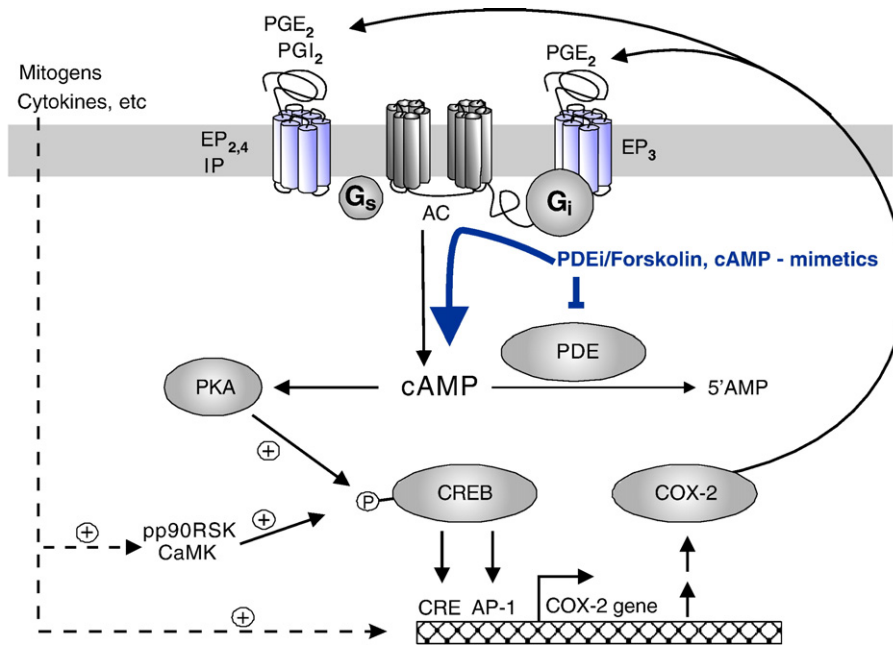
Fig. 1. Illustration of PG mediated induction of Cox-2 via adenylate cyclase pathway and synergistic action with cAMP mimetics.

EP receptors). EP2 and EP4 receptors are Gs-coupled GPCRs and promote an increase in cAMP levels. EP3 receptor is Gi coupled and promotes a decrease in cAMP levels [2]. Cell-specific pattern of expression these receptors thus results in either an increase or decrease in cAMP levels when PGE_2 is present. This in turn will have an effect on COX-2 expression levels, as we will see later (see Fig. 1). While some signalling pathways might influence cAMP levels by interfering with its production, others may do so by affecting its degradation by stimulating or inhibiting phosphodiesterases (PDEs). Indeed, just as the receptor pattern governs the cell response to mediators from the point of view of COX expression, the cellular PDE expression pattern is also of great importance since it determines how a cAMP response is dampened and stopped (see Fig. 1).

1.2. Molecular aspects of COX-2 regulation

COX-2 was discovered in 1991 as a primary response gene [3,4]. COX-2 expression is regulated by both transcriptional and post-transcriptional mechanisms. The human COX-2 gene maps to chromosome 1 q25.2–q25.3 [5]. It is approximately 8.3 kb long and comprises 10 exons (see Fig. 2a). Three mRNA transcript variants have been reported, they are respectively 2.8, 4,0, and 4.6 kb in length. The largest exon in the COX-2 gene encodes the entire 3′-untranslated region (3′-UTR). The latter contains several copies of the "ATTTA" RNA instability element (see Fig. 2c). Sequence analysis of the 5′-flanking region reveals several potential transcription regulator elements (see Fig. 2b). However, in all studied species, only a limited number of elements, namely the cAMP response element (CRE), the C/EBP-NF-IL6 ("CAAT/enhancer binding protein"), NFAT ("nuclear factor of activated T cells") and NFkB ("nuclear factor kappa B cells") sites, and the E-box are known to be involved in the regulation of COX-2 gene expression (see Tables 1 and 2 for transcription factor binding sites and abbreviations). Differences exist between murine and human COX-2 promoters (see Fig. 2b): for example, the proximal promoter region of murine COX-2 has a single NFkB site, whereas the human gene has two putative NFkB sites [1].

1.3. Transcriptional regulation of COX-2

The CRE response element has been identified as one of the central regulatory elements in the COX-2 promoter region [1]. Several studies analyzed the effects of various signalling pathway, which do not activate cAMP generation, but do lead to gene transcription through the CRE site of the COX-2 gene. Homo- or hetero-dimers of c-fos, c-jun, ATF family members of blip proteins can bind to this CRE response element often leading to the activation of COX-2 expression. The following examples illustrate induction of COX-2 gene expression through various cAMP-independent pathways that ultimately signal through the CRE site.

Schroer et al. [6] mutated the CRE site in a −891/+9 human COX-2 promoter fragment from −59-TTCGTCA-53 (CRE-wild type, WT) to 59-TTgagCA-53 (CRE-mutant, M). Compared to CRE-WT basal promoter activity of CRE-M was significantly reduced. CRE-M abrogated the activity stimulated by PMA, IL-1β, or TNFα in human endothelial cells and human foreskin fibroblasts. Double mutants containing both CRE-M and C/EBP-M (due to the high sequence homology between the C/EBP and CRE response elements, C/EBP also serves as a binding site for cAMP-induced transcription) did not further reduce promoter activity, indicating the dominant role of CRE in COX-2 promoter activity (Table 2).

Subbaramaiah and co-workers [7] studied the induction of COX-2 by ceramides in 185B5/HER cells, a human mammary epithelial cell line. Ceramides (both C_2 and C_6– ceramide, 10 μM) induced COX-2 mRNA and protein expression. In order to identify the mechanism by which ceramide upregulates the transcription of the *COX-2* gene, wild-type "full-length" (−1432/+59) human COX-2 promoter constructs vs. mutagenized or truncated versions were transiently transfected into 185B5/HER cells. These promoter studies indicate that the CRE site is critical for transcriptional regulation of the *COX-2* gene by ceramides. Further work revealed that ceramide exposure increased MAPK signalling an activator of c-Jun. The latter induces *COX-2* gene transcription via the CRE site.

Kirtikara and co-workers [8] examined the transcriptional regulation of COX-2 in the human microvascular endothelial cell line HMEC-1 treated with IL-1β. In their experiments, they used a wild-type "full-length" (−1432/+59) human COX-2 promoter construct or, mutagenized or truncated versions. All truncated constructs exhibited similar activity as the wild type constructed, except for the most extensively truncated constructs were all three AP-2, NF-IL6, and CRE sites were missing thus demonstrating their requirement for IL-1β-induced COX-2 promoter activity.

Inoue and co-workers [9] analyzed the *COX-2* gene expression induced by fluid shear stress in human umbilical vein endothelial cells (HUVECs) and bovine arterial endothelial cells (BAECs). They could show that laminar shear stress induces COX-2 mRNA in HUVECs approximately 4-fold over controls. Using a transiently transfected human *COX-2* gene promoter (−327/+59) they could demonstrate that shear stress also increases COX-2 promoter activity in BAECs. Mutations in the CRE site but not the NFkB or NF-IL6 sites significantly reduced shear stress-induced human COX-2 promoter activity. Electrophoretic mobility shift assays (EMSA) showed specific binding of nuclear extracts to labelled oligos containing the CRE site which was enhanced following exposure to shear stress. Both approaches demonstrate the critical role of the CRE site in shear stress-induced COX-2 expression.

Wu and colleagues [10] investigated the expression of COX-2 in primary cultures of normal and endometriotic human stromal cells. Expression levels of COX-2 mRNA and protein levels in ectopic endometriotic implants obtained from patients with pelvic implant and ovarian endometrioma were much greater than in eutopic endometrium of endometriosis-free women. In contrast to stromal cells derived from normal endometrium, IL-1β not only increased COX-2 mRNA stability but also upregulated COX-2 promoter activity in ectopic endometriotic stroma. Induction of COX-2 promoter activity by IL-1β is mediated via mitogen-activated protein (MAP) kinase-dependent phosphorylation of cAMP-responsive element binding protein (CREB). Promoter activity and electrophoretic motility shift assays demonstrate that a CRE site located at −571/−564 of COX-2 promoter is critical for IL-1β-induced *COX-2* gene expression. The importance of the −571/−564 CRE site is unusual given that all other studies utilizing human COX-2 promoter constructs suggest that the −59/53 CRE site is the relevant site. However, two independent approaches support a role of CRE −571/−564. First truncated promoter constructs leaving −550/−49 were unresponsive to IL-1β. Second, site-directed mutagenesis of the CRE located at −571/−564 abrogates IL-1β-induced COX-2 promoter activity; and third, EMSA data (direct binding and specific competition) demonstrated that CREB directly binds to the CRE site of this region.

There is a 5-fold increase of COX-2 mRNA expression levels upon treatment of normal human epidermal keratinocytes (NHEK) with TGF-alpha (50 ng/ml) [11]. Deletion and mutation analysis indicated the importance of the proximal CRE site in the transcriptional control of this gene by TGF-α. The increase in COX-2 mRNA by TGF-α requires activation of both the extracellular signal-regulated kinase (ERK) and p38 mitogen-activated protein kinase (MAPK) pathways. Inhibition of p38 MAPK decreases the stability of COX-2 mRNA, while inhibition of MAPK/ERK kinase (MEK) does not. These results suggest that the p38 MAPK signalling pathway controls COX-2 at the level of mRNA stability, while the ERK signalling pathway regulates COX-2 at the level of transcription.

1.4. Post-transcriptional regulation

The abovementioned mechanisms (regulation of mRNA stability and translation) have been reported to be crucially involved in the regulation of COX-2 expression [12]. Exon 10 of the cyclooxygenase-2 gene encodes the entire 3′-UTR (Fig. 2), and contains several copies of the pentameric ATTTA sequence. The AUUUA motif (also called AU-rich element — ARE) is found in the 3′-UTR of many unstable cytokine- and proto-oncogene-encoding mRNAs, and these motifs regulate mRNA stability and translation [13]. Involvement of these ARE in the post-transcriptional regulation of COX-2 expression has been reported [12,14]. Interestingly a small fragment of the human COX-2 3′-UTR (CR1; +1 to +123 of the 3′-UTR) containing 6 AREs was sufficient to destabilize a reporter mRNA in transfection assays. These AREs have been shown to regulate gene expression by interaction with different RNA-binding proteins [15]. Important RNA-binding proteins shown to bind to COX-2 AREs and to regulate COX-2 expression (mRNA stability or translatability) are AUF1, HuR, TIA-1, β-catenin, CUGBP2, TIAR and TTP [16–19]. Interestingly, the overexpression of COX-2 in most colon carcinoma cells seems to be promoted by a parallel enhanced expression of the ARE-mRNA stabilizing protein HuR [20].

ARES aside, other sequence elements of the human COX-2 mRNA have been shown to be involved in the post-transcriptional regulation of COX-2 protein expression. For instance, transfection experiments using the 3′-UTR of the human COX-2 mRNA with or without the AREs (ΔARE) demonstrated that the AREs play a role in the IGF-1-mediated enhancement of COX-2 mRNA stability [21].

Depending on the cell type analyzed, different signalling pathways (MAPK, AMPK, PKC, PI3K etc., alone or in combination) are involved in regulating COX-2 expression at the post-transcriptional level [22]. These signalling pathways directly or indirectly regulate the activity, localization or expression of several of the RNA-binding proteins discussed above.
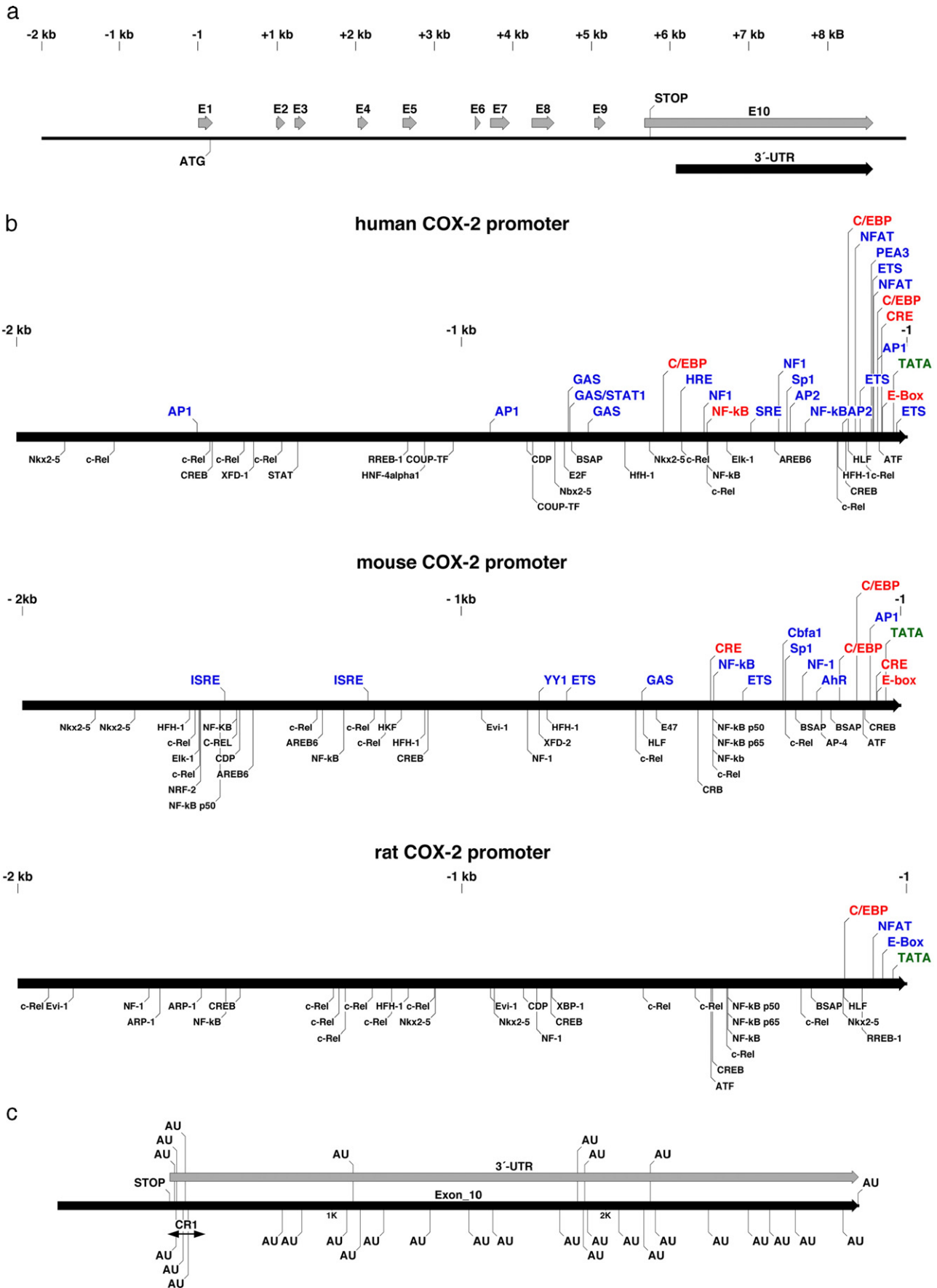
a

E1 ATG E2 E3 E4 E5 E6 E7 E8 E9 STOP E10 3′-UTR

b

human COX-2 promoter

mouse COX-2 promoter

rat COX-2 promoter

c

Table 1
Transcription factor binding sites described in the literature

Binding site	Position	References
Human promoter		
AP1	−1596/−1576	[62]
GAS	−895/−877	[63]
GAS	−850/−831	[63]
HRE	−608/−603	[64]
SRE	−422	[65]
SP1	−270/−265	[1,66]
NFkB	−447/−438	[67]
NFkB	−223/−214	[1,67,68]
ETS	5× −170/+50	[69]
C/EBP (CAAT-box)	−132/−124	[1,6,8,68,70]
NFAT	−105/−97	[71–73]
AP2	−97/−89	[70]
NFAT	−76/−71	[71–73]
PEA3	−75/−73	[74]
AP1	−66/−58	[75]
CRE	−59/−53	[1,6,8,68,70]
E-box	−54/−49	[68,70]
Mouse promoter		
ISRE	−1541/−1522	[76,77]
ISRE	−1215/−1206	[76,77]
YY1	−796/−780	[78]
ETS	−725/−734	[21]
CRE	−433/−428	[79,80]
NFkB	−401/−393	[81,82]
ETS	−320/−329	[21]
Cbfa1	−267/−261	[83]
AhR	−167	[56]
AP2	−150/−142	[81]
C/EBP	−138/−132	[79,81,82,84–87]
C/EBP	−93/−85	[56,79,82]
AP1	−67/−62	[79,80,88]
CRE	−59/−52	[56,79,82,89,90]
E-box	−52/−47	[79,80,82,89,90]
Rat promoter		
C/EBP	−139/−131	[91]
NFAT	−75/−70	[92]
E-box	−54/−49	[91]

Several pro-inflammatory stimuli, which induce COX-2 gene expression, also stimulate MAPK. In particular, the activation of p38MAPK has been shown to be critically involved in the post-transcriptional regulation of several pro-inflammatory genes [23]. Furthermore, p38MAPK has been shown to regulate the localization and activity of several RNA-binding proteins involved in post-transcriptional control of COX-2 expression [15]. Accordingly, specific inhibitors of p38 block the accumulation of COX-2 mRNA in a variety of cells subjected to pro-inflammatory signals. In Hela cells stimulated with IL-1α

and β, and in primary human monocytes stimulated with LPS, inhibition of p38 results in a rapid and specific destabilization of COX-2 mRNA but has little effect on COX-2 transcription. Similar findings of p38 activation on mRNA stability were reported in human monocytes as well as myofibroblasts [24,25].

In addition to p38MAPK, ERK1/2 and JNK have also been shown to be involved in the post-transcriptional regulation of COX-2 expression in some cell types. The post-transcriptional regulation of COX-2 expression by bile acid and ceramide in rat intestinal epithelial cells [26] and IGF in human ovarian cancer cells [21] has been shown to depend on ERK1/2. However, the exact mechanisms (e.g., RNA-binding proteins involved) have not been determined. In murine J774 macrophages, specific inhibition of JNK by SP600125 resulted in reduction of LPS-induced COX-2 expression by destabilization of the COX-2 mRNA [27]. JNK activity has been shown to modulate Tristetraprolin (TTP) expression [28] and TTP destabilizes the COX-2 mRNA [17,18]. However, whether the SP600125-mediated modulation of TTP expression is involved in the SP600125-dependent inhibition of LPS-induced COX-2 expression in J774 macrophages is not known.

In addition to MAPK, other signalling pathways seem to be involved in the post-transcriptional regulation of COX-2 expression in different cell types. In human ovarian cancer cells IGF-1-induced stabilization of the COX-2 mRNA depends on the activation of PI3K and PKC in addition to the activation of ERK1/2 and p38MAPK [21]. Furthermore, in human mesangial cells the ATP-induced stabilization of the COX-2 mRNA is inhibited by PKC inhibitors or specific downregulation of PKC-α by siRNAs. Interestingly, in this cell type ATP incubation induces PKCα-dependent HuR phosphorylation, which results in enhanced nuclear to cytoplasmic shuttling of HuR and in turn in stabilization of COX-2 mRNA [29].

Activation of the AMP-activated kinase (AMPK) has been shown to reduce COX-2 expression [30]. AMPK has been shown to regulate the nucleo-cytoplasmic shuttling of HuR. Activation of AMPK reduces cytoplasmic HuR levels [31]. However, whether AMPK-activation reduces COX-2 mRNA stability has not been determined.

Most studies addressing the role of cAMP signalling in human cells used a combination of pro-inflammatory signals and various cAMP modifiers. The results of these studies are summarized in Table 3.

2. Effects of cAMP signalling on COX-2 expression

Modulation of COX-2 transcriptional regulation by cAMP in different cell types was investigated in various ways. On the one hand researchers have used endogenous mechanisms such as

Fig. 2. (a) Structure of the human *COX-2* gene. Shown are the exons (E1 to E10), the start codon (ATG), the top codon (STOP) and the 3′-untranslated region (3′-UTR) of the gene. (b) Comparison of transcription factor binding sites in the 5′-flanking sequences (promoter) of the human, murine and rat *COX-2* gene. Functional transcription factor binding sites described in the literature are shown in blue or red. Binding sites of transcription factor known to be regulated by cAMP are shown in red. Putative transcription factor binding sites found by P-MATCH (http://www.gene-regulation.com/cgi-bin/pub/programs/pmatch/bin/p-match.cgi) are shown in black. (c) Structure of the exon 10 and 3′-UTR of the human *COX-2* gene. Shown are the top codon (STOP), the AU-rich sequences (AU) and the CR1 fragment (CR1).

Table 2
Abbreviations used

Abbreviation	Description
3′-UTR	3′-Untranslated region of the human COX-2 mRNA
AhR	Aryl hydrocarbon receptor
AP-1	Activating protein-1
AP-2	Activating protein-2
AP-4	Enhancer binding factor AP-4
AREB6	Atp1a1 regulatory element binding protein 6
ARP-1	Apolipoprotein AI regulatory protein
ATF	Activating transcription factor
ATG	Start codon
AU	AU-rich element (AUUUA or AUUUUA)
BSAP	B-cell-specific activator protein
Cbfa1	Core binding factor a1
CDP	CCAAT displacement protein;
C/EBP (NF-IL6)	CAAT/Enhancer binding protein
COUP-TF	Chicken ovalbumin upstream promoter transcription factor
CR1	3′-UTR fragment of the human COX-mRNA with 6 ARE
CRE	cAMP response element
CREB	cAMP response element-binding protein
c-Rel	Nuclear factor kappa B c-Rel
c-Rel	Nuclear factor kappa B c-Rel
E1–E10	Exons of the human COX-2 gene
E2F	Adenovirus E2 promoter binding factor
E47	Helix–loop–helix protein
Elk-1	Ets-related protein
ETS	Avian E26 (E-twenty-six) retrovirus encoded transcription factor
Evi-1	Evi-1 zinc finger myeloid transforming gene
GAS	γ-Activated site
ISRE	Interferon stimulated response element
HFH-1	HNF-3/Forkhead-like protein 1
HLF	Hepatic leukemia factor
HNF-4alpha1	Hepatocyte nuclear factor 4alpha1
HRE	HIF-1-responsive element
NF-1	Nuclear factor-1
NF-IL6	Nuclear factor-1
NFAT	Nuclear factor of activated T cells
NFkB	Nuclear factor kappa B cells
NFkB p50	Nuclear factor kappa B p50
NFkB p65	Nuclear factor kappa B p65
Nkx2-5	Cardiac-specific homeobox protein
NRF-2	Nuclear respiratory factor 2
PEA3	Polyomavirus enhancer activator 3
RREB-1	Ras-responsive element binding protein 1
SP1	Stimulating protein-1
SRE	Sterol response element
STAT	Signal transducer and activator of transcription
STOP	Stop codon
XBP-1	X-box binding protein 1
XFD-1	*Xenopus* fork head domain factor-1
XFD-2	*Xenopus* fork head domain factor-2
YY1	Ying yang 1

stimulation of cell membrane receptors. On the other hand, the intracellular cAMP signalling pathway was manipulated via direct activation of adenylate cyclase (using forskolin or cholera toxin) and inhibition of cAMP degradation using selective and unselective phosphodiesterase inhibitors (PDEi, i.e., rolipram, IBMX, respectively). Membrane-permeable cAMP analogues such as dibutyryl cAMP (db-cAMP) and 8-bromo-cAMP and inhibition of phosphatases with okadaic acid have also been used (Fig. 1).

2.1. Leukocytes

There is consistent evidence that the cAMP pathway exerts a positive modulatory role on COX-2 induction in primary human cells (see Table 3). Hinz and colleagues [32] analyzed the effects of cAMP modifiers on the expression of cyclooxygenase-2 in human primary monocytes from healthy volunteers exposed to LPS. Alone, db-cAMP (100 μM) did not stimulate PGE_2 generation in monocytes, however, in combination with LPS (10 μg/ml) it led to a 2-fold increase of both PGE_2 levels and COX-2 mRNA levels. Likewise, cholera toxin (10 μg/ml) and IBMX (300 μM), respectively, led to 3-fold increase of both PGE_2 and COX-2 mRNA levels when given in presence of LPS. Furthermore, activation of Gs-coupled EP receptors (EP2/4) increased COX-2 mRNA levels (10 μM Butaprost, agonist for EP2; 10 μM 11-deoxy PGE_1, agonist for EP2/4) in monocytes stimulated with LPS. In contrast, sulprostone (10 μM) acting on EP1 and EP3 receptors did not further augment COX-2 mRNA levels. Furthermore, results reported by Juergens and colleagues indirectly confirm the positive modulatory role for cAMP on COX-2 regulation. They determined the effects of PDE inhibition by theophylline on cAMP and LTB_4 and PGE_2 production, in cultured monocytes in vitro obtained from healthy non-smoking donors [33]. LPS-stimulated generation (10 μg/ml) of intracellular cAMP increased significantly (+162%) in the presence of theophylline (18 μg/ml); production of LTB_4 was suppressed (−42%) compared to the baseline, whereas PGE_2 production increased significantly (+39%). Production of cAMP correlated with increased PGE_2 production and with suppression of LTB_4. These effects were mimicked by db-cAMP but not by dibutyryl-cGMP. Interestingly, these effects could be abolished by ibuprofen suggesting that the underlying mechanism is a feedback regulatory induction of COX-2 by a prostaglandin-driven cAMP-mediated process (possibly involving the EP2/4 receptors). These results provide evidence that the clinical efficacy of theophylline may also result from inhibition of leukotriene production and its ability to stimulate PGE_2 production. Results by Inoue and Tanabe [34] using the human U937 monocytic cell line could not confirm the positive modulatory role of cAMP on COX-2 transcriptional regulation. In U937 cells differentiated to a monocytic phenotype with PMA and LPS COX-2 mRNA levels were increased 18-fold as compared to undifferentiated controls. Db-cAMP (100 μM) alone or in combination with LPS did not further increase COX-2 mRNA levels, compared to their respective controls. This would tend to suggest that U937 is not completely suitable as a model for monocytes. Beusenberg and co-workers [35] analyzed the effect of 1 mM IBMX, 10 μM salbutamol and 1 mM sodium nitroprusside on PGE_2 and LTB_4 release as well as on cAMP and cGMP levels in human alveolar macrophages obtained from controls and COPD patients. In both populations IBMX increased cAMP level by 55 93% and salbutamol IBMX by 285 352%. Except for the 61% rise in LTB_4 release by salbutamol IBMX the drugs hardly affected PGE_2 and LTB_4 release from control macrophages. In COPD alveolar macrophages, however, IBMX and salbutamol IBMX largely reduced PGE_2 release (63 vs. 11 pg per 10^6 cells) but less efficiently

Table 3
Effects of cAMP-modifiers on COX-2 induction

Cell type	COX-2 inducer	cAMP modifier	COX-2 expression	References
Human leucocytes and macrophages				
hMonozytes	LPS	db-cAMP, IBMX, Cholera toxin	PGE_2 ↑, COX2-mRNA: ↑	[32]
	LPS	11-Deoxy-PGE1, butaprost	PGE_2 ↑, COX-2 mRNA: ↑	
	LPS	Sulprostone	COX-2 mRNA: ↔	
hMonozytes	LPS	Theophyllin	PGE_2 ↑	[33]
hMonozytes	LPS	db-cAMP	PGE_2 ↑	
hAlv. Macrophages		IBMX, salbutamol	PGE_2 ↔	[35]
COPD hAlv. Macrophages		IBMX, salbutamol	PGE_2 ↓	
U937		db-cAMP,	Promoter: ↔, mRNA: ↔	[34]
U937	LPS	db-cAMP,	Promoter: ↔, mRNA: ↔	
PMN	fMLP	forskolin+RO 20–1724	mRNA: ↑, protein: ↑	[36]
	fMLP	S-P-cAMP	mRNA: ↑, protein: ↑	
		S-P-cAMP	protein: ↔, mRNA: ↔	
Jurkat	α−CD3 & α−CD28,		wt-promoter: ↑, with NFAT site: ↔	[37]
	α−CD3 & α−CD28	Rolipram, etazolate, Ro 20–1724	wt-Promoter: ↓,	
	α−CD3 & α−CD28	db-cAMP, forskolin	wt-Promoter: ↑	
Mouse macrophages and related cell types				
RAW264.7		db-cAMP	PGE_2 ↑, COX-2 mRNA: ↑	[93]
	LPS	db-cAMP	PGE_2 ↑↑, COX-2 mRNA: ↑↑	
RAW264.7	LPS/TNF/IL-1β	Milrinone, 8br-cAMP	COX-2 mRNA: ↑	[59]
RAW264.7	LPS/TNF/IL-1β	Amrinone	COX-2 mRNA: ↓	
	LPS		mRNA: ↑, protein: ↑, PGE_2 ↑	[94]
	LPS	ANP	mRNA: ↓, protein: ↓, PGE_2 ↓	
Human endothelial cells and smooth muscle				
hPASMC	Bradykinin		Promoter: ↑, promoter with deleted CRE site: ↔	[39]
		EP4 (ONO-AE1 329)	Promoter: ↑, promoter with deleted CRE site: ↔	
		PGE_2, EP2 (ONO-AE1 259)	Promoter: ↑, promoter with deleted CRE site: ↔	
hASMC	PMA	Iloprost	mRNA: ↑↑, protein: ↑↑, PGI_2 ↑↑	[61]
hASMC		Forskolin, db-cAMP, iloprost	mRNA: ↑, protein: ↑, PGI_2 ↑,	
hBSMC	IL-1β	PGE_2, forskolin	mRNA: ↑, protein: ↑, PGE_2↑	[95]
RASF	IL-1β		Protein: ↑↑,	[42]
	IL-1β	Rofecoxib	Protein: ↑,	
	IL-1β	Rofecoxib, EP2 or EP4 agonist	Protein: ↑↑,	
Mesangial cells		PGE_2, db-cAMP	mRNA: ↑, protein: ↑, PGE_2↑,	[44]
HUVEC	IL-1β		Protein: ↑	[45]
	IL-1β	PGE_2	Protein: ↔	
	IL-1β	PGE_2, forskolin	Protein: ↑	
BAEC	LPS		Protein: ↑	[46]
	LPS	db-cAMP	Protein: ↑↑	
	LPS	db-cAMP, IBMX	Protein: ↑↑↑	
Miscellaneous cell types				
hKeratinocytes	UVB		Promoter: ↑	[47]
		Forskolin	promoter: ↑	
mKeratinocytes		PGE_2, forskolin, db-cAMP	protein: ↑	[48]
Rat granulosa cells	LH, FSH	db-cAMP, CTX, IBMX	Protein: ↑, PGE_2	[49]
		Forskolin	Protein: ↑, PGE_2	
Rat granulosa cells		Forskolin	Promoter: ↑	[50]
	LH, FSH		Promoter: ↑	
Bovine granulosa cells		Forskolin, db-cAMP	Promoter: ↑	[51]
Human chondrocytes	IL-1β	db-cAMP, foskolin, IBMX	PGE2 ↓, 6-keto-PGF1α ↓	[57]
MC3T3-E1	Shear stress		mRNA: ↑, promoter: ↑	[52]
	Shear stress	PKA inhibitor	mRNA: ↔, promoter: ↔	
		Forskolin:	mRNA: ↑, promoter: ↑	
MC3T3-E1	PTH		mRNA: ↑	[53]
	PTH	Forskolin, 8br-cAMP	mRNA: ↑↑	
JEG3, trophoblast		8br-cAMP	Promoter ↑: mRNA: ↑, PGE_2↑	[54]
hEGEC	hCG	8-Cl-cAMP	Protein: ↑,	[55]
		8-Br-cAMP	Protein: ↔	

hEGEC: Human endometrial epithelial gland cells.

increased LTB_4. In both macrophage populations sodium nitroprusside (SNP) substantially increased (3- to 4-fold) cGMP level but did not affect eicosanoid production. These results indicate that drugs that enhance cAMP level decrease PGE_2 release from COPD macrophages and stimulate the release of LTB_4, a chemotactic mediator involved in bronchial inflammatory reactions.

Pouliot et al. [36] examined the effects of adenosine and fMLP (formyl methyl leucine proline) on the expression of COX-2 in human PMNs. Adenosine upregulates the expression of the COX-2 enzyme and mRNA. Production of PGE_2 in response to exogenous arachidonic acid was also increased by adenosine and correlated with COX-2 protein levels. This effect on COX-2 could be mimicked by pharmacological increases of intracellular cAMP levels, involving the latter as a putative second messenger for the upregulation of COX-2 by adenosine. Agents that raise intracellular cAMP levels (S-P-cAMP-acetoxymethyl: 50 μM; forskolin: 50 μM; Ro20–1724: 10 μM) potentiated the fMLP-stimulated expression of COX-2 protein. S-P-cAMP alone, however, did not stimulate COX-2 protein levels in PMNs. Thus, adenosine may promote a self-limiting regulatory process through the increase of PGE_2 generation, which may result in the inhibition of PMN functions.

Jimenez et al. [37] show that phosphodiesterase (PDE)-4 inhibitors (rolipram, Ro20–1724; >50 μM) block COX-2 induction and prostaglandin synthesis in activated human T cells. Cells were stimulated with anti-CD3 plus CD28 antibodies, resulting in activation of the NFAT (nuclear factor of activated T cells) pathway, which can be blocked by calcineurin inhibitors such as cyclosporine. COX-2 inhibition by PDE4 inhibitors occurs mainly at the transcriptional level. Two response elements for the nuclear factor of activated T cells (NFAT) in the COX-2 promoter were required for inhibition by these drugs. PDE4 inhibitors did not affect NFAT nuclear translocation upon T cell activation. Rather they prevented NFAT binding to DNA and induction of the transactivation function of GAL4-NFAT. These effects seem to be cAMP/PKA independent as they were not mimicked by db-cAMP or by forskolin, neither can be reverted by the PKA inhibitors H89 or KT-5720. These results may explain some of the anti-inflammatory properties of PDE4 inhibitors through the blockade of NFAT-mediated transactivation of pro-inflammatory genes such as *COX-2*.

2.2. Smooth muscle cells, smooth muscle derived cells, and fibroblasts

It was reported that bradykinin (10 μM) induces COX-2 and stimulated the release of PGE_2 from cultured human pulmonary artery smooth muscle cells (hPASMC) [38]. Using the same cells the authors investigated in a subsequent work the importance of CRE binding for bradykinin mediated COX-2 induction [39]. EMSA experiments revealed that oligonucleotides specific for CRE of the COX-2 promoter and consensus oligonucleotides showed strong specific binding. Furthermore BK increased p6CRE/luc-mediated luciferase expression. CRE activation occurred by BK inducing cytosolic phospholipase A_2-mediated arachidonic acid release and rapid PGE_2 production, thereby increasing cAMP. Indomethacin inhibited BK-induced PGE_2 production, cAMP accumulation, and CRE/luc reporter and COX-2 promoter luciferase activity. Exogenous PGE_2 (1 μM) and EP2 (ONO-AE1 259) and EP4 (ONO-AE1 329) PGE_2 receptor agonists (10 nM each) mimicked the effect of BK. Collectively these studies indicate that COX-2 induction by BK in human pulmonary artery smooth muscle cells is mediated by the CRE through a novel autocrine loop involving endogenous PGE_2.

Debey et al. [40] studied COX-2 expression in primary cultures of human arterial smooth muscle cells (hASMC), derived from coronary artery and aorta. HSMCs were challenged with PMA (100 nM), and iloprost (100 nM), which acts on the prostacyclin receptor stimulating adenylate cyclase. Both agents increased COX-2 mRNA levels. In combination, superinduction occurred, as evidenced by COX-2 protein levels and COX-2-dependent synthesis of endogenous prostacyclin. Forskolin (10 μM) and db-cAMP (1 mM) induced COX-2 mRNA, albeit to a smaller extent than iloprost. The authors did not rule out the possibility that iloprost besides increasing intracellular cAMP levels acts on COX-2 expression via activation of the ligand activated transcription factor peroxisome proliferator activated receptor delta (PPAR-delta) [41]. PPAR-delta promoter constructs have been shown to be activated by 10 μM iloprost, but not by cicaprost. Some weak indirect evidence that iloprost acts via cAMP signalling in human cells was provided by the fact that iloprost induces the expression of the transcriptional repressor ICER (inducible cAMP early repressor), whose expression is regulated by four CRE sites within the ICER promoter.

Kojima and colleagues [42] analyzed the expression of COX-2 (and microsomal PGE synthase) in IL-1β-stimulated human rheumatoid arthritis synovial fibroblast (RASFs) obtained from joint replacement surgery. IL-1β (1 ng/ml) strongly induced COX-2 protein expression in RASFs. Co-incubation with a COX-2-selective inhibitor rofecoxib (10 μM) resulted in modest attenuation of COX-2 expression suggesting that PGE_2 acts in positive feedback loop on its own synthesis. Co-incubation of RASFs with IL-1β and EP2 (ONO-AE1-259) or EP4 (ONO-AE1-329) selective agonists in the presence of rofecoxib increased COX-2 mRNA levels compared to IL-1β and rofecoxib alone. Thus, increased cAMP signalling elicited by EP2 or EP4 superinduces COX-2 in IL-1β-stimulated RASFs. The effects of cAMP elevating agents in the absence of IL-1β were not reported.

In a similar study, Faour and co-workers [43] explored a positive feedback, PGE_2-dependent stabilization of COX-2 mRNA mediated by the p38 MAPK cascade in rhIL-1β (100 pg/ml)-stimulated human synovial fibroblasts (HSF). HSF were from synovia harvested at necropsy from donors with no evidence of arthritis. IL-1 led to a rapid (5 min), massive (N30-fold), and sustained (N48 h) increase in COX-2 mRNA, protein, and PGE_2 release that was inhibited by NS-398 (100 ng/ml), a COX-2 inhibitor, and SB202190 (10 g/ml), a selective, cell-permeable p38 MAPK inhibitor. PGE_2 (100 nM) completely reversed NS-398-mediated inhibition but not SB202190-dependent inhibition. The eicosanoid did not

potentiate IL-1β-induced COX-2 expression nor did it activate *COX-2* gene expression in quiescent cells. Transfection experiments with a human COX-2 promoter construct ($-2390/+34$) revealed a minor element of p38 MAPK-dependent transcriptional control after IL-1β stimulation. p38 MAPK synergized with the cAMP/cAMP-dependent protein kinase cascade to transactivate the COX-2 promoter. When human synovial fibroblasts were activated with IL-1β for 3–4 h (steady state) followed by washout, the elevated levels of COX-2 mRNA declined rapidly (<2 h) to control levels. If PGE_2, unlike EP2/3 agonists butaprost and sulprostone (up to 10 μM each), was added to fresh medium, COX-2 mRNA levels remained elevated for up to 16 h. SB202190 or anti-PGE_2 monoclonal antibody compromised the stabilization of COX-2 mRNA by PGE_2. Deletion analysis using transfected chimeric luciferase–COX-2 mRNA 3′-untranslated region reporter constructs revealed that IL-1β increased reporter gene mRNA stability and translation via AU-containing distal regions of the untranslated region. This response was mediated entirely by a PGE_2/p38 MAPK-dependent process. The authors concluded that the magnitude and duration of the induction of COX-2 mRNA, protein, and PGE_2 release by rhIL-1β are primarily the result of PGE_2-dependent stabilization of COX-2 mRNA and stimulation of translation, a process involving a positive feedback loop mediated by the EP4 receptor and the downstream kinases p38MAPK and, perhaps, cAMP-dependent protein kinase.

Nusing and colleagues analyzed the effect of dibutyryl cAMP (db-cAMP) on the regulation and expression of cyclooxygenase-2, inducible NO synthase and argininosuccinate synthase in rat renal mesangial cells [44]. Low concentrations of db-cAMP in the range of 10–50 μM elevated levels of cyclooxygenase-2 mRNA, protein and activity. Prostaglandin E_2 stimulated cyclooxygenase-2 expression and activity at a concentration of 10 μM.

2.3. Endothelial cells

The effects of PGE_2 on the induction of COX-2 were studied in HUVECs treated with IL-1β (1 ng/ml) [45]. COX activity was measured by the production of 6-keto-$PGF_{1\alpha}$, PGE_2, $PGF_{2\alpha}$ and thromboxane B_2 (TxB_2) in the presence of exogenous arachidonic acid (10 μM for 10 min). Untreated HUVECs contained only COX-1 protein while IL-1β-treated HUVEC contained COX-1 and COX-2 protein. PGE_2 (3 μM for 24 h) did not affect COX activity and protein levels in untreated HUVEC. PGE_2 inhibits COX-2 protein, in HUVEC treated with IL-1β. This inhibition was reversed by co-incubation with forskolin (100 μM), suggesting that cAMP induces COX-2 in IL-1β-stimulated HUVECs.

Samokovlisky et al. [46] investigated the effects of cAMP-elevating agents on COX expression in BAECs. Treatment of resting BAECs with cAMP-elevating agents inhibited prostacyclin production and COX activity, without affecting arachidonic acid release. No change was detected in COX-1 protein expression. In LPS-treated COX-2 expressing bovine aortic endothelial cells, COX-2 protein was induced upon treatment with db-cAMP (1 mM) and further induction of COX-2 protein

was effected by IBMX (1 mM). These results suggest that increased cellular cAMP selectively inhibits COX-1 activity without altering COX-1 protein expression, and at the same time, upregulates COX-2 protein. This complex regulation of COX activity and protein expression by cAMP may represent a prostacyclin-induced autoregulatory mechanism in bovine aortic endothelial cells.

2.4. Keratinocytes

Tang and co-workers analyzed the effect of UVB irradiation on the expression of COX-2 in immortalized "normal" human keratinocytes [47]. Analyses of the COX-2 promoter ($-327/+59$) revealed that the CRE site was essential for both basal and UVB-induced COX-2 expression. Dominant negative mutant of CREB strongly inhibited the activity of COX-2 promoter. EMSA indicated that CREB and ATF-1 were the major proteins binding to the COX-2 CRE. CREB and ATF-1 were phosphorylated upon UVB treatment, and SB202190, a p38MAPK inhibitor, decreased the phosphorylation of CREB/ATF-1 and suppressed COX-2 promoter activity. Likewise, treatment with 10 μM forskolin led to phosphorylation of CREB and ATF-1 and activation of COX-2 promoter.

Maldve et al. [48] demonstrate autoregulation of prostaglandin (PG) production by the PGs themselves and their precursor, AA. 10 μM AA and PGs induced COX-2, as well as COX-1 mRNA, expression in cultured murine keratinocytes approximately 3 h after treatment. 10 μM forskolin, 100 μM db-cAMP induced COX-1 and COX-2 mRNA, suggesting that cAMP is a second messenger for COX expression. 1 μM SQ 22536, an adenylate cyclase inhibitor, inhibited COX-2 mRNA induction by PGE_2 in a dose-dependent manner suggesting that PGE_2-induced expression may be through one of the cAMP-linked PGE_2 receptors. Further, both COX isoforms can be up regulated by their products, the PGs, and this autoregulation probably occurs via prostaglandin receptors linked to a cAMP signal transduction pathway.

2.5. Granulosa cells

So far only non-human cells have been studied. To understand the molecular mechanisms by which LH regulates the induction of COX in rat preovulatory (PO) follicles, Wong et al. [49] established an in vitro system which mimics in vivo induction of the enzyme. This study was carried out prior to the discovery of COX-2; most likely the induced COX-isoform, which migrated slower than "constitutive" COX in granulosa cells, is COX-2. A rapid increase in COX enzyme (1) is stimulated by LH, FSH, and 10 μM forskolin in a time- and dose-dependent manner that is distinct from changes in steroidogenic enzymes analyzed in the same follicles; (2) is unaltered by end products (PGE_2 and PGF_2) of the reaction or inhibitors (indomethacin) of enzyme activity; (3) is blocked by inhibitors of transcription (alpha-amanitin) and translation (cycloheximide) at a step distal to production of cAMP and activation of PKA. In summary, the LH-stimulated appearance of COX in granulosa cells of PO follicles before ovulation is mediated by

cAMP in a complex manner involving transcriptional regulation (*COX* gene) and translational control of COX mRNA. The transient appearance of the COX enzyme represents a unique pattern of response by granulosa cells of PO follicles to LH/cAMP and thereby may involve novel intracellular factors and regulatory processes.

Sirois and co-workers [50] studied the effects of LH and FSH on the regulation of a rat COX-2 promoter construct (−2698/+32), as well as a series of 5′-deletion mutants in primary cultures of rat granulosa cells. 7.5 μM Forskolin, FSH (500 ng/ml), and LH (500 ng/ml) induced CAT activity following transfection with the −2698/+32 COX-2 construct. Gonadotropin-releasing hormone (10 μM) and 30 ng/ml IL-1β had no effect. Deletion mutants delineated the region spanning from −192 to −54 of the transcription start site to be essential for both basal and forskolin-regulated expression of the reporter gene. The same DNA fragment (−192/−54) exhibited specific binding to granulosa cell nuclear extract proteins as analyzed by EMSA. Collectively, these results provide the first structural and functional evidence that the transcriptional regulation of the rat *COX-2* gene by gonadotropins and forskolin in granulosa cells involves 5′-flanking DNA sequences, specifically a region between −192 and −54 of the transcription initiation site (CRE sites).

Wu and Wiltbank [51] elucidated the molecular mechanisms mediating Cox-2 regulation during differentiation of the bovine granulosa cell. A −1500/+59 bovine COX-2 promoter construct vector was transfected into freshly isolated bovine granulosa cells or granulosa cells after culture with or without 10 μM forskolin to induce luteinization in vitro. The −1500/+59 COX-2 promoter was inducible by 1 mM 8-bromo cAMP (but not by phorbol ester: 10 nM PDD) in fresh granulosa cells, and maximal expression by cAMP was delayed until 48 h after treatment. In contrast, after luteinization of granulosa cells by 8-day treatment with forskolin, the COX-2 promoter was immediately inducible by phorbol esters but not by cAMP. In granulosa cells cultured for 8 days without forskolin, the COX-2 promoter continued to be inducible only by cAMP and not by phorbol esters. Unexpectedly, no delay was observed in the induction of COX-2 by cAMP in granulosa cells that were cultured without forskolin, compared with a 1-day delay in COX-2 induction by cAMP in fresh granulosa cells. Time-course experiments showed that only 2 days of forskolin treatment could induce PKC responsiveness of the COX-2 promoter, although maximal responsiveness was not observed until 10 days of luteinization. Promoter activity was also analyzed in a series of deletion mutants as well as site-directed mutants of C/EBP, CRE, and E-box. A 282-bp sequence in the COX-2 59 flanking region containing a C/EBP, CRE, and E-box maintained full inducibility by PKA in granulosa cells and by PKC in luteinized granulosa cells. The E-box element was found to be the critical regulatory element for COX-2 induction by either PKA in granulosa cells or by PKC in luteinized granulosa cells. In fresh granulosa cells, mutation of the E-box eliminated the 8-bromo-cAMP-stimulated increase in luciferase activity. However, mutation in C/EBP, in CRE, or in random sites did not alter basal or cAMP-stimulated promoter activity. EMSA were performed on nuclear extracts from fresh or luteinized granulosa cells. Upstream stimulatory factor (USF)-1 and USF-2 bound to the E-box of the *COX-2* gene, and binding was similar for nuclear extracts from fresh, cultured, or luteinized granulosa cells. Thus, although luteinization changes transcriptional regulation of COX-2 from PKA to PKC dependence, the crucial role of the E-box element in this transcriptional activation is conserved.

2.6. Miscellaneous cell types

Fluid shear stress (FSS) at cell membranes induces new transcription of cyclooxygenase-2 in murine MC3T3-E1 osteoblasts, with peak effects at 4–5 h [52]. Using MC3T3-E1 cells stably transfected with the murine COX-2 promoter (−370/+70) fused to a luciferase reporter, Wadhwa et al. examined involvement of the protein kinase A (PKA) and protein kinase C (PKC) signalling pathways in the peak COX-2 mRNA and luciferase responses to FSS (10 dyn/cm^2). Neither inhibition nor downregulation of the PKC pathway affected the FSS stimulation of COX-2 mRNA or luciferase activity. In contrast, inhibitors of the PKA (PKI: 1 μM; and H-89: 30 μM) pathway used at doses, which inhibited forskolin-stimulated (forskolin: 10 μM) luciferase activity by 70–80%, reduced FSS-stimulated COX-2 mRNA expression and luciferase activity by 50–80%. Hence, peak FSS induction of COX-2 expression in MC3T3-E1 osteoblastic cells is largely dependent on the PKA signalling pathway.

Tetradis et al. [53] studied the regulation of *COX-2* gene expression by parathyroid hormone (PTH) and its possible signalling pathways in osteoblastic MC3T3-E1 cultures. Bovine PTH (1–34) at 0.01–10 nM increased COX-2, but not COX-1, messenger RNA (mRNA) levels. The effect of PTH was maximal at 1 h and decreased almost to control levels by 6 h. 0.1 μM PMA, 10 μM forskolin, and 1 mM 8-bromo-cAMP increased COX-2 mRNA levels, whereas 1 μM ionomycin had no effect. PTH, forskolin, and PMA increased the release of PGE$_2$ into the culture medium. Pre-treatment of cells with 0.1 μM PMA for 16 h blocked the induction of COX-2 mRNA levels by PMA, but did not alter the effects of PTH and forskolin. However, treatment of cells with 30 μM H-89, a protein kinase A inhibitor, significantly reduced the ability of PTH and forskolin to induce COX-2 mRNA levels. Moreover, PTH (3–34) at 0.1–100 nM did not induce COX-2 mRNA levels. These results show that PTH can rapidly and transiently induce COX-2 mRNA levels in osteoblastic MC3T3-E1 cells, primarily via the cAMP-protein kinase A signal transduction pathway.

Anteby et al. [54] examined the regulation of COX isozymes in human trophoblasts. To this end, primary trophoblasts and JEG3 cells were transfected with promoter constructs of either human COX-1 (789/+9) or COX-2 (891/+9) genes. In both cell systems, the basal activity of COX-2 promoter was 10- to 30-fold higher than the activity of COX-1 promoter. In response to either 25 ng/ml TPA, or 1 mM 8-bromo-cAMP, an increase in COX-2 promoter activity but no change in activity of COX-1 promoter was observed. Similarly, both agents enhanced COX-2

expression, as well as PGE_2 production. The activity of COX-2 promoter was potentiated by co-expression of protein kinase A and inhibited by co-expression of kinase A inhibitor. Aspirin attenuated the stimulatory effect of TPA on COX-2 promoter. The activity of COX-2 promoter is stimulated by either TPA or cAMP, and the stimulatory effect of TPA is attenuated by aspirin. These pathways may play a role in modulation of prostanoid synthesis by trophoblasts.

Zhou and colleagues [55] examined the effects of recombinant hCG (human chorionic gonadotropin) on primary cultures of human endometrial epithelial gland cells. hCG dose dependently (10–100 ng/ml) induced the expression of COX-2 mRNA (200%), protein (150%), and stimulated PGE_2 (200%) secretion. Since hCG is known to increase intracellular cAMP levels and thus activates PKA-dependent pathways, the effects of the PKA inhibitor H89 (50 nM), and Bis, a PKC inhibitor (50 nM), were studied. In contrast to Bis, H89 almost completely blunted induction of COX-2 mRNA and protein by hCG. In the absence of hCG induction of COX-2 protein was also observed with 8-Br-cAMP (0.1 mM), but did not occur with the PKA inhibitor 8-Cl-cAMP (0.1 mM).

Results from experiments done in-house using cytokine-stimulated A549 (human epithelial cells) demonstrated no effect of forskolin (10 μM), RP73401 (1 μM) or db-cAMP (1 mM) on PGE_2 synthesis by its own or in combination with cytokines (Mais and Klein 2004, unpublished observation). Yang and Bleich [56] used murine pancreatic β-cells (RINm5F) to explore the molecular mechanisms regulating COX-2 promoter activity. Through deletion analysis of a −907/+70-bp 5′ upstream region of the mouse *COX-2* gene, they identified an inhibition domain (−804/−371) and an activation domain (−371/+70). The highest promoter activity was seen when the promoter was reduced to 371 bp. Three sites were identified in the 371 construct that were essential for basal COX-2 promoter activity: (1) C/EBP, (2) aryl hydrocarbon receptor (AhR), and (3) CREB. Single mutation of each individual site inhibited 70 80% of basal COX-2 promoter activity. Double mutation of the AhR and CREB-binding sites showed synergy in repressing

Fig. 3. Inhibitory effect of cAMP mimetics on IL-1β (1 nM) stimulated 6-keto $PGF_{1\alpha}$ synthesis in primary human chondrocytes. Compounds were pre-incubated 30 min prior IL-1β stimulation and incubated for 24 h. 6-keto $PGF_{1\alpha}$ was analyzed via GC/MS spectrometry. Values represent means (ng/ml)±S.E.M. of 3 different donors.

COX-2 promoter activity, as did mutation of all three sites. Transcription factors from RINm5F nuclear extracts specifically bound to oligonucleotides containing C/EBP, AhR, or CREB consensus sites. 10 μM forskolin increased COX-2 promoter activity via the CREB site. COX-2 promoter activity was also increased by 10 nM 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, an AhR activator, through the AhR site.

In in-house experiments, we could show that PG synthesis in human chondrocytes is regulated by cAMP: using various modulators of cAMP signalling we could achieve a down-regulation of 6-keto-$PGF_{1\alpha}$ and PGE_2 synthesis. RP-73401, a selective PDE4i (1 μM), IBMX (300 μM), forskolin (10 μM) and db-cAMP (1 mM) lowered the IL-1β evoked 6-keto-$PGF_{1\alpha}$ production to about 50% (Fig. 3). Similar effects on human chondrocytes were described from Tenor et al. [57] on the IL-1β induced PGE_2 production.

3. Summary and concluding remarks

Analysis of the human COX-2 promoter in various cell types revealed the critical importance of the CRE site for *COX-2* gene expression. This response element is the downstream endpoint for a large variety of stimuli, encompassing classical inflammatory signals (i.e., IL-1β, LPS), shear stress, ceramide, and others at the transcriptional level. However, there is considerably less evidence that cAMP signalling plays a major role in the post-transcriptional regulation of COX-2 protein.

Various studies showed that activation of the cAMP signalling pathway alone may increase COX-2 mRNA and/or protein expression, albeit mostly at a modest level compared to a combined exposure: alone, cAMP modifiers induced COX-2 expression in the following cell types: RAW264.7 cells (db-cAMP [58]); human coronary artery endothelial cells, primary neonatal rat cardiomyocyte cultures, and RAW264.7 mouse macrophages (milrinone [59]); rat microglia (db-cAMP, forskolin [60]); human coronary arterial smooth muscle cells (forskolin, db-cAMP [61]), rat renal mesangial cells (db-cAMP [44]), murine keratinocytes (forskolin, db-cAMP [48]), osteoblastic MC3T3-E1 cultures (forskolin, 8br-cAMP [53]), human trophoblasts (8br-cAMP [54]), human endometrial epithelial gland cells (8br-cAMP [55]), rat preovulatory follicles (forskolin [49]). Transcriptional activation of COX-promoter constructs by cAMP elevating agents was observed in murine pancreatic β-cells (forskolin [56]), rat granulosa cells (forskolin [50]), bovine granulosa cell (8br-cAMP, forskolin [51]).

An attenuation of COX-2 expression following treatment with cAMP modifiers was observed in three studies [35,37] and was also evident from our own studies using human chondrocytes. In the Jimenez study, NFAT-dependent COX-2 gene expression was probably unrelated to cAMP levels increased by either rolipram, etazolate, or Ro20−1724.

Collectively, the majority of studies indicate that COX-2 expression is induced by cAMP elevating agents. This effect is observed in all species, and with very few exceptions in all cell types studied. The majority of data indicate that cAMP in vitro mainly superinduces COX-2 expression when a given (inflammatory) stimulus is already present. cAMP must
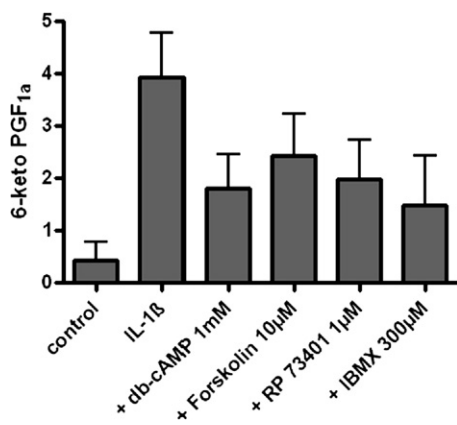
therefore be considered as a positive modulator of COX-2 expression, rather than an inducer. This may indicate that clinical use of, e.g., β_2-mimetics or PG receptors agonists in the absence of active inflammatory processes will not impact significantly on COX-2-dependent production of prostanoids. However, it may be conceivable that these pharmacological agents may augment COX-2 activity in patients with active inflammatory diseases. Nonetheless COX-2 derived prostanoids show protective (e.g., acceleration of ulcer heeling) as well as detrimental (e.g., rheumatic arthritis) effects on the organism. The therapeutics pros and cons of elevations of COX-2 levels will therefore have to be established on a case-to-case basis.

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