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COMMENTARY

Co-expression of Prostaglandin Receptors with Opposite Effects

A MODEL FOR HOMEOSTATIC CONTROL OF AUTOCRINE AND PARACRINE SIGNALING

*Barrie Ashby**

DEPARTMENT OF PHARMACOLOGY, TEMPLE UNIVERSITY HEALTH SCIENCES CENTER, PHILADELPHIA, PA 19140, U.S.A.

ABSTRACT. Prostaglandins are ubiquitous autocrine mediators that exert their effects through a number of G protein-coupled receptors. Many organs and tissues express many of the prostaglandin receptors, and prostaglandins have diverse effects on individual organs and tissues. In some cases, several prostaglandin receptors are expressed on a single cell type. Co-expressed prostaglandin receptors frequently appear to have opposite actions, suggesting homeostatic control of prostaglandin effects. Co-expression of opposing receptors provides a molecular mechanism for weak or partial agonism and explains the action of a drug as a mixed agonist/antagonist. The physiological relevance of co-expressed opposing receptors for a single agonist perhaps can be explained in terms of the difference between endocrine and autocrine mediators. Endocrine hormones are generally produced by cells distant from their site of action so that they are diluted to an elevated but stable concentration by the time they reach their target cells. In contrast, autocoids are produced by the same cell type on which they act and may reach transiently high levels at their sites of action. The presence of a second type of receptor that negates the action of the first receptor would tend to buffer cellular responses to transient extremes of agonist concentration. The slow onset of inhibition would also allow for time-dependent buffering, providing additional control over autocoid release and effect. The mechanism is relevant to other autocrine and paracrine mediators including neurotransmitters, which reach transiently high concentrations in the synaptic cleft. BIOCHEM PHARMACOL **55**;3:239–246, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. prostaglandin receptors; cyclic AMP; adenylyl cyclase; desensitization

Prostaglandins are ubiquitous autocrine mediators involved in numerous physiological and pathological events [1]. Physiological effects on the cardiovascular system include vasodilation and regulation of platelet aggregation. Prostaglandins also lead to contraction or relaxation of smooth muscle in the bronchia and trachea. In the kidney, they regulate renal salt and water excretion in a variety of ways. They play a role in reproduction and parturition, they function in the central nervous system, and they can stimulate and inhibit bone resorption and formation. Prostaglandins are involved in inflammation and markedly enhance edema formation. They potentiate the pain-producing effects of bradykinin and other autocoids.

Prostaglandins exert their effects through a number of G protein-coupled receptors. Table 1 summarizes information on the prostaglandin receptors known to date and shows the major signal transduction system affected by each receptor. All of the receptors have been cloned from human sources [2–17].

The human EP3 receptor occurs as at least six isoforms that arise from alternative splicing of a single gene product. The isoforms are identical over the first 359 amino acids but differ in C-terminus, which varies in length from 6 to 65 amino acids (Table 2). They also differ [8, 11] in G protein specificity and susceptibility to desensitization. In addition, several of the isoforms of the human EP3 receptor are constitutively active [18], inhibiting cAMP† formation in the absence of agonist.

Many organs and tissues express many of the prostaglandin receptors including the isoforms of EP3, and prostaglandins have diverse effects on individual organs and tissues. In the kidney, low concentrations of PGE_1 inhibit arginine-vasopressin-induced water reabsorption through inhibition of adenylyl cyclase, whereas higher concentrations activate adenylyl cyclase, causing water reabsorption [19]. In this case, the receptors are localized in different regions of the organ. Kidneys show distinct cellular localization of mRNAs for three subtypes of prostaglandin E receptor [20, 21].

* Correspondence address: Department of Pharmacology, Temple University Health Sciences Center, 3400 North Broad St., Philadelphia, PA 19140. Tel. (215) 707-4404; FAX (215) 707-7068; E-mail: bashby00@nimbus.ocis.temple.edu

† *Abbreviations:* cAMP, adenosine 3′,5′-cyclic monophosphate; PG, prostaglandin; HEL, human erythroleukemia; GRK, G protein-coupled receptor kinase; PKA, protein kinase A; IBMX, 3-isobutyl 1-methylxanthine; PLC, phospholipase C; and TX, thromboxane.

TABLE 1. Classification of prostaglandin receptor subtypes

Receptor type	Endogenous agonist	Rank order of potency	Signal transduction	Ref.
DP	PGD_2	$D_2 > E_2, F_{2\alpha}, I_2, TXA_2$	cAMP ↑	2
EP	PGE_2	$E_2 > I_2 \geq F_{2\alpha} > D_2$		
EP1			PLC	3
EP2			cAMP ↑	4, 5
EP3 (6 isoforms)			cAMP ↓	6–11
EP4			cAMP ↑	12
FP	$PGF_{2\alpha}$	$F_{2\alpha} > D_2 > E_2 > I_2$	PLC	13
IP	PGI_2	$I_2 > D_2, E_2, F_{2\alpha}, TXA_2$	cAMP ↑	14, 15
TP (2 isoforms)	TXA_2	$TXA_2 > D_2 > F_{2\alpha}, I_2, E_2$	PLC	16, 17

EP1 has been localized to the collecting ducts, EP4 to the glomeruli, and EP3 to the tubules in the outer medulla and in the distal tubules of the cortex.

In other cases, several prostaglandin receptors appear to be expressed on a single cell type. For example, osteoblasts express EP1 and EP4 subtypes [22]. In osteosarcoma cells [23], PGE_2 has been shown to stimulate cAMP formation with an EC_{50} of 0.073 μM and to stimulate calcium mobilization with an EC_{50} of 1.8 μM. cAMP is antiproliferative in osteosarcoma cells and calcium blunts the antiproliferative effect, indicating that prostaglandins act through distinct receptors to temper their own effects. B lymphocytes express mRNA for EP1, EP2, EP3, and EP4 receptors [24]. EP2 and EP4 agonists strongly inhibited expression of class II major histocompatibility complex.

Co-expressed prostaglandin receptors frequently appear to have opposite actions suggesting homeostatic control of prostaglandin effects, which may be important in buffering cellular response to transient changes in prostaglandin levels. Since prostaglandins are local mediators, their concentration might be expected to vary as the autocoid is released and diffuses away from its site of action.

This commentary will focus on co-expression of prostaglandin receptors and their role in the regulation of autocrine responses. The argument for homeostatic control through co-expressed, opposing receptors will be extended to other autocrine and paracrine mediators including neurotransmitters.

PROSTAGLANDIN REGULATION OF PLATELET cAMP METABOLISM

We have used prostaglandin regulation of cAMP metabolism in human platelets and HEL cells as a model to analyze regulation involving co-expression of stimulatory and inhibitory prostaglandin receptors coupled to adenylyl cyclase [25–28]. Platelets respond to prostaglandins in a variety of ways and appear to have a full repertoire of prostaglandin receptors [29]. TXA_2 causes platelet activation resulting in secretion and aggregation (TP receptor), whereas PGI_2, PGE_2, and PGD_2 inhibit platelet activation through stimulation of adenylyl cyclase and cAMP formation (DP, IP and EP2 or EP4 receptors). PGE_2 can also act in a proaggregatory manner, enhancing the activity of other agonists (EP1 receptor). In addition, we have shown that platelets possess a receptor linked to inhibition of adenylyl cyclase (EP3 receptor) that dampens the response to prostaglandins, giving rise to a novel form of desensitization [25–27]. Interestingly, Hirata and coworkers [30] have shown there are also two isoforms of the TP receptor in platelets that oppositely regulate adenylyl cyclase.

HEL cells possess some properties of megakaryocytes and platelets [31] and can be induced by dimethyl sulfoxide or phorbol ester to express more platelet-like characteristics [31], including an increase in both PGI_2 [32] and TXA_2 [33] receptor expression. We have shown the same patterns of cAMP metabolism in HEL cells as in platelets [28]. Using a HEL cell cDNA library, we have cloned the EP4 [4], IP and the EP3 receptor [6].

TABLE 2. Isoforms of the human EP3 receptor with different carboxyl-terminal domains

Isoform	C-terminal sequence
EP3 I	. . .FCQIRYH**T**NNYA**SSST**SLPCQC**SST**LMW**S**DHLER
EP3 II	. . .FCQVANAV**SS**C**S**NDGQKGQPI**S**L**S**NEIIQ**T**EA
EP3 III	. . .FCQEEFWGN
EP3 IV	. . .FCQMRKRRLREQEEFWGN
EP3 V	. . .FCQMRKRRLREQLIC**S**LR**T**LRYRGQLHIVGKYKPIVC
EP3 VI	. . .FCQMRKRRLREQAPLLP**T**P**T**VIDP**S**RFCAQPRWFLDL**S**FPAM**SSS**HPQLPL**T**LA**S**FKLLREPC**S**VQL**S**

Nomenclature of the isoforms of EP3 differs among laboratories. We have used a roman numeral as indicated to describe the isoforms. The isoforms are identical for the first 359 amino acids up to the sequence FCQ. Serine and threonine residues, which are potential phosphorylation sites, are indicated in bold type.

HOMEOSTATIC CONTROL OF cAMP METABOLISM

In intact cells, cAMP is formed through the action of adenylyl cyclase and is removed by phosphodiesterases. In the continuous presence of a stimulatory agonist, the cAMP level might be expected to rise to a plateau, representing a steady state between formation and disappearance of the compound. In fact, the time–course of cAMP formation in most cells is generally observed as a rise to a peak followed by a decline to a stable plateau level [34]. This phenomenon reflects desensitization since a second challenge with the same agonist results in a blunted response. Desensitization most commonly results from time-dependent inhibition of adenylyl cyclase. Desensitization is a major mechanism of regulation of receptor signaling that typically involves phosphorylation of the receptor by protein kinases. Homologous or agonist-specific desensitization, mediated by GRKs, results in an attenuated response to only that particular desensitizing agent [35], whereas heterologous desensitization results in decreased responsiveness to a variety of stimuli [35] and is mediated by PKA. Prostaglandin receptors may undergo desensitization mediated by PKA and GRKs [36]. All the cloned prostaglandin receptor subtypes, except isoforms III and IV of EP3, contain serine and threonine residues in their carboxyl terminus that are potential targets for phosphorylation. We have shown that deleting 138 amino acids from the C-terminus of EP4, including 36 serine and threonine residues, abolishes desensitization of this receptor [37].

Apart from homeostatic control through classical desensitization mechanisms, we have suggested that platelet adenylyl cyclase is also regulated by a desensitization mechanism involving distinct stimulatory and inhibitory receptors because prostaglandins induce time-dependent inhibition with a different concentration dependency from their own activation of adenylyl cyclase [26, 27]. We modeled platelet cAMP metabolism using kinetic simulation [26, 27] and showed that complex patterns of cAMP metabolism can be explained by postulating rapid stimulation through a stimulatory receptor and slow inhibition through an inhibitory (EP3) receptor to give a form of desensitization that depends on the affinity of different prostaglandins for the two receptor types.

Figures 1 and 2 show actual data and simulations indicating the effect of prostaglandin concentration on the cAMP formation in intact platelets both in the presence and absence of the phosphodiesterase inhibitor IBMX. For these studies we used the stable PGI_2 analog Iloprost. In the presence of IBMX and at low concentrations of Iloprost, the rate of cAMP formation was linear (Fig. 1a). The rate of cAMP formation increased as a saturable function of prostaglandin concentration, reflecting binding to a stimulatory (IP) receptor. However, at higher concentrations of Iloprost, the rate of cAMP formation decreased with time and the extent of inhibition increased with Iloprost with a different concentration dependency from stimulation of

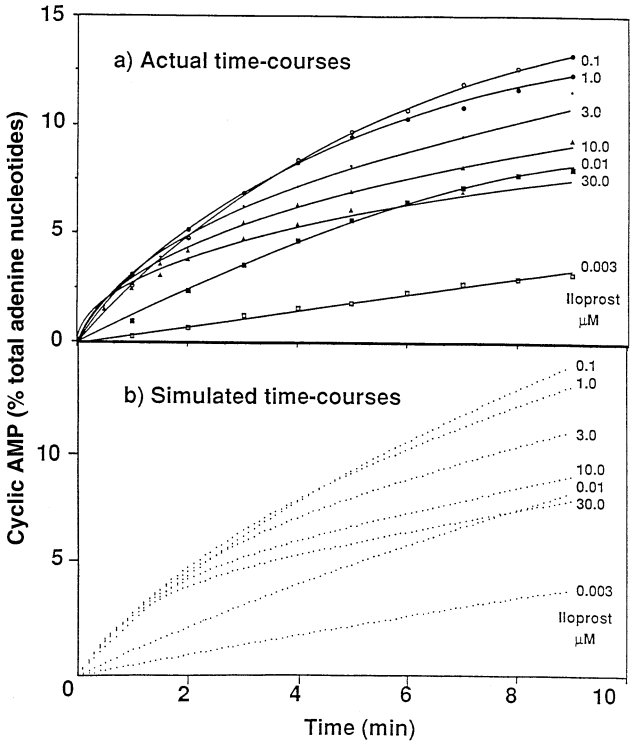

FIG. 1. Actual (a) and simulated (b) time–courses of Iloprost-stimulated cAMP formation in intact platelets in the presence of IBMX. Simulations were performed as indicated in Ref. 26 using a simple two-receptor model.

cAMP formation. The simplest way to account for the difference in prostaglandin concentration-dependency of stimulation and inhibition is to propose that there are separate stimulatory (IP) and inhibitory (EP3) receptors, and it is possible to determine EC_{50} values for the two processes by measuring the initial and final rates as a function of prostaglandin concentration.

Simulation of cAMP formation in intact platelets as a function of prostaglandin concentration is shown in Fig. 1b. Barber and co-workers [34] modeled cAMP formation and desensitization in intact cells using an integrated rate equation that included slow, agonist-mediated, reversible transition of adenylyl cyclase to an inactive form. We modified this equation to include separate receptors for stimulation and desensitization of adenylyl cyclase. For the simulations, we used KINSIM, a kinetic simulation program that employs numerical integration [38]. We were able to simulate cAMP formation over a wide range of prostaglandin concentrations.

Figure 2a shows time–courses of cAMP formation in the absence of phosphodiesterase inhibitors. In this case, low concentrations of Iloprost gave rise to a steady state with little subsequent desensitization, whereas at high concentrations of Iloprost peak and plateau effects were observed, indicating prostaglandin concentration-dependent desensitization. Curves were modeled using KINSIM by including phosphodiesterase activity in the simulation (Fig 2b). The simulated curves retain the characteristics of the real data

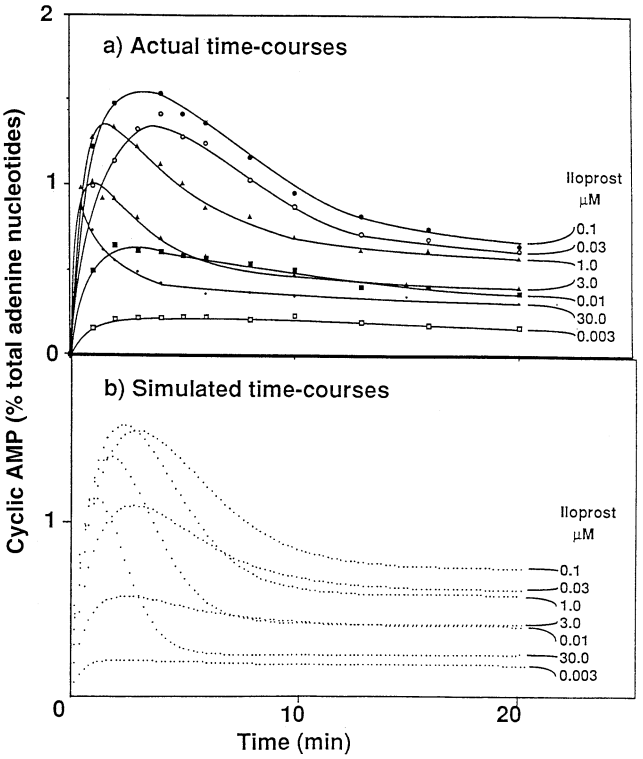

FIG. 2. Actual (a) and simulated (b) time–courses of Iloprost-stimulated cAMP formation in intact platelets. Simulations were performed as indicated in Ref. 26 using a simple two-receptor model.

in that the shape of the time–course varies with prostaglandin concentration, and peak and plateau effects increase with prostaglandin concentration to the point that the curves cross.

We compared the effects of Iloprost with PGE_1 and PGD_2 [27]. The time–course of cAMP formation in intact platelets was different for each prostaglandin. In contrast to Iloprost, peak and plateau effects were observed at all concentrations of PGE_1, indicating that PGE_1 binds tightly to the inhibitory (EP3) receptor. PGD_2 produced a pattern of cAMP formation similar to that of PGI_2 except that the highest level of cAMP attained was much lower in the case of PGD_2. The two-receptor model explains the difference in response in terms of differences in affinity of the stimulatory and inhibitory receptors for each prostaglandin, and the model is borne out by studies with cloned EP4, IP, and EP3 receptors which confirm the rank order of potency for each prostaglandin at each receptor.

In other work [39], we showed that PGE_2 can both stimulate and inhibit cAMP formation on platelets, indicating the presence of EP2 or EP4 receptors, co-expressed with the EP3 receptor.

CONSTITUTIVE ACTIVITY OF G PROTEIN-COUPLED RECEPTORS

Constitutive activity of some EP3 isoforms adds another dimension to the control of prostaglandin regulation of cAMP metabolism. Constitutive activity, which is receptor activity in the absence of agonists, has been observed in G protein-coupled receptor systems of all types. In the rhodopsin [40] and thyroid-stimulating hormone receptor [41] systems, naturally occurring activating mutations lead to pathological manifestations such as retinitis pigmentosa and hyperthyroidism, respectively. There is clear evidence for constitutive activity of native opioid receptors linked to inhibition of adenylyl cyclase in NG108-15 cell membranes [42], and the D1B dopamine receptor displays constitutive stimulation of adenylyl cyclase [43].

PGE receptor EP3 isoforms have been observed to display constitutive activity. Following a report by Hasegawa *et al.* [44] that truncation of the mouse EP3 receptor at the splice variant site resulted in constitutive activity, we prepared a truncated form of the human EP3 receptor and showed that it, too, is constitutively active. We also examined the activity of four isoforms of human EP3 [18] and showed that EP3 III and EP3 IV are markedly constitutively active, whereas EP3 I and EP3 II show little or no constitutive activity. Constitutive activity was abolished by pertussis toxin.

Our results can be compared with those obtained with the mouse EP3 isoforms. The α isoform of the mouse EP3 receptor is constitutively active compared with the β isoform, displaying 50% inhibition in the absence of prostaglandins [44], whereas the γ form is fully constitutively active [45]. Comparison of the C-terminal domains of the human (Table 2) and mouse EP3 receptors reveals that human EP3 I is homologous (but not identical) to mouse EP3α and that human EP3 II is homologous (but not identical) to mouse EP3γ. Curiously, human EP3 II shows no constitutive activity, whereas its mouse homologue, EP3γ, is almost completely constitutively active. Human EP3 I shows about 25% constitutive activity, whereas its mouse homologue, EP3α, shows about 50% constitutive activity.

Although it is hard to imagine the physiological significance of a receptor that couples to inhibition of adenylyl cyclase in the absence of agonist, constitutive activity may be important in systems involving co-expression of opposing receptors for the same agonist. In this case, differential expression of EP3 isoforms with different levels of constitutive activity in different tissues would lead to varying degrees of homeostatic regulation and effectively alter the activity of prostaglandin receptors coupled to stimulation of adenylyl cyclase. In fact, the isoforms of EP3 show differential distributions in different tissues; for example, EP3 V is the major isoform expressed in placenta, whereas EP3 I is the major isoform in skeletal muscle. Similarly, EP3 II is the major isoform in HEL cells, whereas EP3 I is the major isoform in peripheral blood mononuclear cells [10]. Constitutive activity would also be important in damping out minor, transient increases in cAMP, effectively eliminating noise from the system.

IMPLICATIONS OF THE MODEL

The idea of co-expressed stimulatory and inhibitory receptors provides an explanation for a number of pharmacological phenomena. The model gives rise to heterologous desensitization among all prostaglandins, which is a manifestation of binding of prostaglandins to the inhibitory site. Hence, because PGE_1 binds most tightly to the inhibitory site, it gives rise to the most striking degree of desensitization. In contrast, PGD_2 binds weakly to the inhibitory site and gives little desensitization.

The model provides a molecular mechanism that explains weak or partial agonism. PGI_2, PGE_1, and PGD_2 all appear to stimulate adenyl cyclase with different efficacies in the order $PGI_2 > PGD_2 > PGE_1$. From a pharmacological point of view, PGE_1 and PGD_2 are apparently weaker agonists that PGI_2. We propose that all three prostaglandins are full agonists and that apparent differences in efficacy result from different degrees of interaction with the inhibitory EP3 receptor. Hence, PGI_2 binds tightly to IP and weakly to EP3, whereas PGE_1 binds weakly to IP and tightly to EP3, so that the net effect is that PGE_1 appears to be a partial agonist. Rovati and Nicosia [46] have presented a formal analysis of the difference between partial agonism and dual regulation by the same agonist, using a similar model of co-expressed stimulatory and inhibitory receptors.

The model also provides a molecular mechanism that explains the action of a drug as a mixed agonist/antagonist. This is because the ratio of active to inactive adenylyl cyclase depends on the fractional occupancy of the stimulatory and inhibitory receptors. At saturation of both types of receptor, all prostaglandins give rise to the same level of adenylyl cyclase activity that is reflected in the steady-state level of cAMP [27]. At other concentrations of prostaglandins, the final steady-state level of cAMP depends on the affinity of individual prostaglandins for the stimulatory and inhibitory receptors. When two prostaglandins are added to the same reaction mixture, either at the same time or at separate times, the final steady-state level of cAMP represents contributions from both. Hence, incubation of platelets with a prostaglandin that binds tightly to the inhibitory receptor will lead to apparent desensitization to a second prostaglandin because adenylyl cyclase is already inhibited.

The idea that cellular responsiveness may be homeostatically controlled by receptors for the same agonist acting in opposition points to novel therapeutic strategies. In the case of co-expressed prostaglandin receptors coupled to stimulation and inhibition of adenylyl cyclase, agonists such as sulprostone, which act through the inhibitory EP3 receptor, would be functional antagonists of prostaglandin stimulatory receptors. In the case of prostaglandins, this is an important consideration because there are few effective receptor antagonists.

Prostaglandins are potent stimulators of cAMP formation in both platelets and vascular smooth muscle cells, leading to inhibition of platelet aggregation and relaxation of vascular smooth muscle so that therapeutic use of prostaglandins as antiplatelet agents may be offset by unwanted vasodilation. Based on pharmacological evidence, Corsini *et al.* [47] suggest that platelets and smooth muscle cells possess different prostaglandin receptors that may be targeted selectively by different drugs. We have suggested that the cells differ in their populations of inhibitory receptor, leading to differences in net cAMP synthesis [48]. The suggestion is supported by our preliminary data which show that platelets express more EP3 I than EP3 III, whereas vascular smooth muscle cells express more EP3 III than EP3 I.

GENERAL CASE OF THE MODEL

The physiological relevance of dual regulation of adenylyl cyclase by a single agonist acting on the same cell type perhaps can be explained in terms of the difference between endocrine and autocrine hormones (autocoids). Endocrine hormones are generally produced by cells distant from their site of action so that they are diluted in the blood stream to an elevated but stable concentration by the time they reach their target cells. In contrast, autocoids are produced by the same cell type on which they act (or, in the case of platelets, by the intimately related endothelial cells), forming a localized feedback mechanism. Consequently, autocoids may reach transiently high levels at their sites of action. The presence of a second type of receptor that negates the action of the first receptor would tend to buffer cellular responses to transient extremes of agonist concentration. The slow onset of inhibition would also allow for time-dependent buffering, providing additional control over autocoid release and effect. The rapid desensitization of adenylyl cyclase caused by prostaglandins can then be distinguished from slower and more complex forms of desensitization observed with, for example, β-adrenergic agonists [35]. Hence, prostaglandin or autocoid desensitization may simply represent a relatively rapid response to localized transient concentrations of agonist, maintaining cellular responsiveness within reasonable bounds, whereas desensitization to endocrine hormones represents true desensitization and down-regulation to long-term agonist exposure.

We postulate that the role of the inhibitory receptor is to provide homeostatic control of the cAMP level, buffering against rapid variations in agonist concentration when agonists are produced close to their sites of action. The mechanism would apply to circulating platelets responding to localized increases in prostaglandin synthesis caused by inflammatory agents. While circulating prostaglandin may be important in maintaining platelets in a non-thrombogenic state, the ability to buffer against excessive prostaglandin-stimulated cAMP formation would prevent platelets from becoming refractory to appropriate stimulatory challenges.

The mechanism is relevant to other autocrine and paracrine mediators including neurotransmitters. D_1- (stim-

ulatory) and D_2- (inhibitory) dopaminergic receptors are co-localized on the same postsynaptic membrane of striatal cells [49], and β- (stimulatory) and α_2- (inhibitory) adrenergic receptors are co-localized on other neurons [50] and on glial cells. We propose that D_1 and D_2 receptors work together to regulate adenylyl cyclase activity on the postsynaptic membrane so that cAMP is maintained at an elevated but essentially constant level despite sharp fluctuations in agonist concentration that must occur when neurotransmitter is released into the tiny volume of the synaptic cleft. Interference with this protective buffering mechanism that may occur through a reduction or absence of the inhibitory receptor would result in fluctuations in the level of cAMP, reflecting transient but physiological changes in neurotransmitter concentration. The effect of loss of control of dopamine-regulated cAMP level may affect neuronal firing, leading to manifestations such as those observed in schizophrenia.

We have shown that activation of the inhibitory EP3 receptor results in slow inhibition (desensitization) of adenylyl cyclase. We postulate that slow inhibition results from changes in the equilibrium between the protein components of the adenylyl cyclase system, mediated through the common βγ subunits of G_s and G_i, rather than phosphorylation. The mechanism of cross-talk between stimulatory and inhibitory receptors is identical to that proposed by Seeman *et al*. [51], who showed that D_1 and D_2 dopamine receptors are linked. Binding of a D_2 agonist to striatal tissue was affected by a D_1 antagonist and vice versa, suggesting cross-talk between the receptors which they suggest is mediated by interactions involving βγ. The link was missing in postmortem striata from schizophrenic patients but was present in control striata [51]. The parallel between prostaglandin and dopamine receptor regulation emphasizes the general nature of our model [52].

Desensitization by mechanisms involving receptor phosphorylation may be superimposed on control through a distinct inhibitory receptor. Atkinson and Minneman [53] analyzed a similar system in glial cells in which β- (stimulatory) and α_2- (inhibitory) adrenergic receptors are co-localized. In this case, chronic agonist exposure desensitizes β-receptors more rapidly than α_2-receptors so that continuing α_2 inhibition functionally accelerates the loss of agonist response.

It is not necessary that the co-expressed receptors act on the same second messenger system in order to homeostatically regulate cellular response. For example, human intestinal muscle cells have been shown to express both 5-HT2A serotonin receptors, mediating contraction through activation of PLC, and 5-HT4 serotonin receptors, mediating relaxation through activation of adenylyl cyclase [54]. Similarly, in osteosarcoma cells [23], PGE_2 stimulates cAMP formation and calcium mobilization. cAMP is antiproliferative in osteosarcoma cells, and calcium blunts the antiproliferative effect.

CONCLUSION

We have presented evidence for co-expression of prostaglandin receptors on the same cell that leads to opposite effects, effectively damping cellular response to transient extremes of agonist concentration. Constitutively active receptors may be important in this type of regulation, eliminating noise from the system. There are other examples of autocoids and neurotransmitters that act on co-expressed receptors, suggesting that the mechanism is a general one that applies when agonists are produced close to their sites of action and agonist concentration can fluctuate.

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