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Bell-shaped curves for prostaglandin-induced modulation of adenylate cyclase: two mutually opposing effects

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Abstract

Each of the natural prostanoid is at least one order of magnitude more potent for its specific receptor (DP, EP, FP, IP and TP) than any of the other prostanoids. However, they are able to interact also with one or more of the other classes of prostanoid receptors. The concentration–response curves for modulation of adenylate cyclase activity in rabbit mesenteric artery smooth muscle cells by different prostaglandins are not always monotonic, i.e. simple sigmoidal curves in logarithmic scale, but they are often biphasic. Prostacyclin, iloprost and prostaglandin E_1 showed a convex bell-shaped curve, i.e. adenylate cyclase activity is stimulated at lower concentrations and inhibited at higher concentrations, while the curve of prostaglandin E_2 showed a concave bell-shaped curve, i.e. adenylate cyclase is inhibited at lower concentrations and stimulated at higher concentrations. By selectively inhibiting one of the transduction mechanisms present in mesenteric smooth muscle cells, we have demonstrated that the observed responses to these prostanoids are likely due to two mutually opposing effects. Thus, the data previously published by our laboratory on a prostacyclin analog, 5(*Z*)-carbacyclin, might be reinterpreted more correctly in the light of this new possibility.

Keywords: Prostaglandin; Prostanoid receptor; Bell-shaped curve; G-protein; Pertussis toxin; Partial agonist

1. Introduction

The relatively large number (five) of naturally occurring prostanoids (prostaglandin D_2, prostaglandin E_2, prostaglandin $F_{2\alpha}$, prostacyclin and thromboxane) and the variety of the responses elicited by them have always suggested heterogeneity of receptor subtypes. There is now evidence for the existence of five classes of prostanoid receptors (DP, EP, FP, IP and TP), one for each of the natural prostanoids, and at least four subtypes of the EP receptors, namely EP_1, EP_2, EP_3 and EP_4 (Coleman et al., 1994). Each prostanoid is at least one order of magnitude more potent for its specific receptor than any of the other prostanoids. However, all the five natural prostanoids are able to interact, albeit with different affinities, also with one or more of the other classes of prostanoids receptors. Therefore, cross-reactivity occurs, especially during in vitro experiments, depending on the range of concentrations used for each agonist.

Furthermore, for each of the receptor classes, the specific transduction mechanism has been identified and characterized (Coleman et al., 1994), and all the receptors identified so far are G-protein-coupled receptors. It is well known that prostacyclin, one of the most potent inhibitor of platelet aggregation (Moncada et al., 1976), exerts its effects by an increase in intracellular adenosine 3′:5′-cyclic monophosphate (cyclic AMP) levels (Tateson et al., 1977) through an interaction with the IP receptors (Lombroso et al., 1984) coupled to the adenylate cyclase system via a G_s protein (Siegl et al., 1979). It is also now known that cyclic AMP levels are regulated by different G-proteins, that either stimulate (G_s) or inhibit (G_i) adenylate cyclase (Gilman, 1984) and that these G-proteins are generally coupled to different receptors.

In this contribution, we will demonstrate that in rabbit mesenteric smooth muscle cells, bell-shaped concentration–response curve for prostacyclin, iloprost, prostaglandin E_1 and prostaglandin E_2 is the result of the interaction with two distinct receptors or, possibly, to a single receptor coupled to two different G-proteins, with opposite effects on adenylate

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[1] This paper is dedicated to the memory of Prof. Simonetta Nicosia who largely contributed to the eicosanoids biology and was a valuable mentor first and then a precious colleague for her collaborators.

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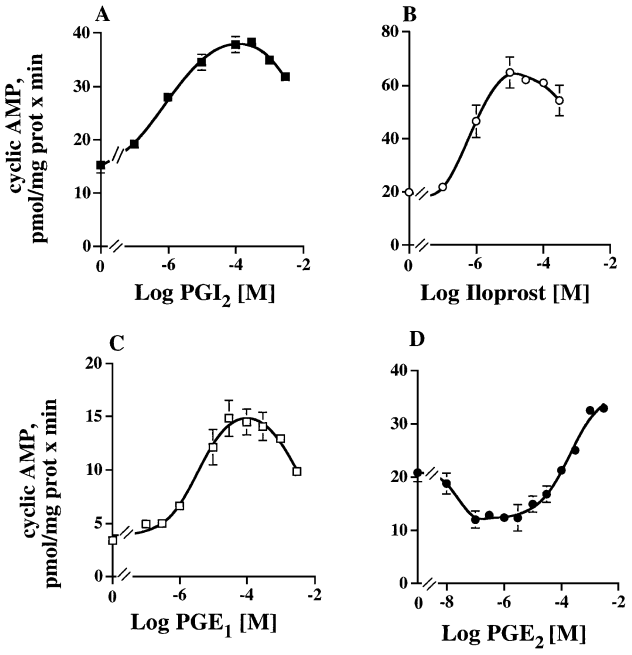

Fig. 1. Concentration–response curves for the activation of adenylate cyclase by different prostaglandins in membranes of myocytes from rabbit mesenteric artery. Panel A: prostacyclin (PGI_2); Panel B: iloprost; Panel C: prostaglandin E_1 (PGE_1); Panel D: prostaglandin E_2 (PGE_2). Data are expressed as mean ± S.D.

cyclase and, therefore, on cyclic AMP levels. We will also re-examine some of the data previously published by our laboratory (Corsini et al., 1987) on a prostacyclin analog, 5(*Z*)-carbacyclin, in the light of a new model (Rovati and Nicosia, 1994a) that explains the typical bell-shaped concentration–response curves obtained with this compound in mesenteric smooth muscle cells.

2. Materials and methods

2.1. Materials

[8-^{14}C]adenosine triphosphate ([8-^{14}C]ATP) and [2,8-^{3}H] cyclic adenosine monophosphate ([2,8-^{3}H]cyclic AMP) were

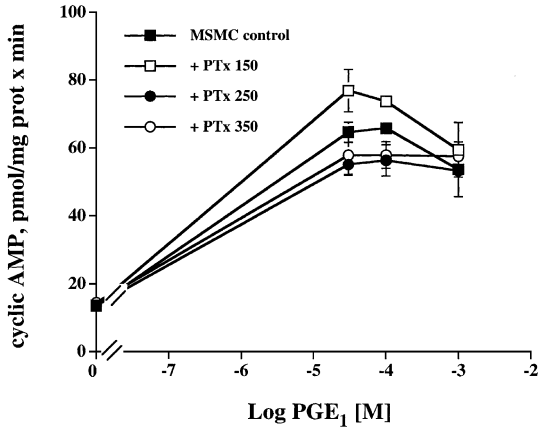

Fig. 2. Concentration–response curves for the activation of adenylate cyclase by prostaglandin E_1 (PGE_1) in mesenteric smooth muscle cells treated with increasing concentrations of pertussis toxin (PTx). Data are expressed as mean ± S.D.

from Amersham, Buckinghamshire, UK; prostacyclin, prostaglandin E_1, ATP, cyclic AMP, guanosine triphosphate (GTP), creatine phosphate, creatine phosphokinase and pertussis toxin were purchased from Sigma, St. Louis, MO, USA; iloprost was a generous gift from Schering, Milan, Italy. The solutions of prostacyclin, which was stored in ethanol at −20 °C, were freshly prepared immediately before use in 10 mM Tris–HCI buffer, pH 8. The other prostaglandins were dissolved in the same buffer. Eagle's minimum essential medium (MEM) F11, fetal calf serum, trypsin–EDTA, penicillin (10,000 U.I./ml), streptomycin (l0 mg/ml), tricine buffer (1 M) and nonessential amino acids (100 ×) were purchased from Grand Island Biological, Madison, WI, USA; disposable culture flasks and petri dishes were from Corning Glassworks, Amedfield, MA, USA.

2.2. Cell cultures

Cells were cultured as previously described (Oliva et al., 1984). Briefly: male white New Zealand rabbits (2–3 kg) were used. Cultures of smooth muscle cells from intima-medial layer of rabbit mesenteric arteries were prepared

Table 1
Parameters of the concentration–response curves for the modulation of adenylate cyclase activity in mesenteric smooth muscle cells by different prostaglandins

Parameter	PGI_2	Iloprost	PGE_1	PGE_2
E_1	1.3 ± 19	20 ± 6.3	3.8 ± 15	20.1 ± 4.8
$Slope_1$	0.55 ± 32	1 (imposed)	0.9 ± 28	−0.76 ± 21
EC_{50_1}	8.6×10^{-7} ± 52	8.3×10^{-7} ± 9.4	3.8×10^{-6} ± 52	1.9×10^{-8} ± 45
E_{max}	4.1 ± 11	67.6 ± 2.6	16 ± 15	11.9 ± 6
$Slope_2$	−0.76 ± 58	−0.6 ± 75	−0.8 ± 51	1.5 (imposed)
EC_{50_2}	7.1×10^{-3} ± 33	1.5×10^{-3} ± 36	3×10^{-3} ± 43	1.9×10^{-4} ± 36
E_2	1.3 ± 19	20 ± 6.3	3.8 ± 15	36 ± 7

E_1, $Slope_1$ and EC_{501} refer to interaction of the ligand with the higher potency receptor, and indicate the response when the concentration of ligand is zero, the slope and the concentration yielding 50% of the maximum response of the 1st component of the curve, respectively; E_2, $Slope_2$ and EC_{502} refer to interaction of a ligand with the lower potency receptor, and indicate the response for an infinite concentration of ligand, the slope and the concentration yielding 50% of the maximal response of the 2nd component of the curve, respectively; E_{max} indicates the maximum response. Parameters E_1 and E_2 are shared except for PGE_2. Parameters are expressed ± %CV. PGI_2 = prostacyclin; PGE_1 = prostaglandin E_1; PGE_2 = prostaglandin E_2.

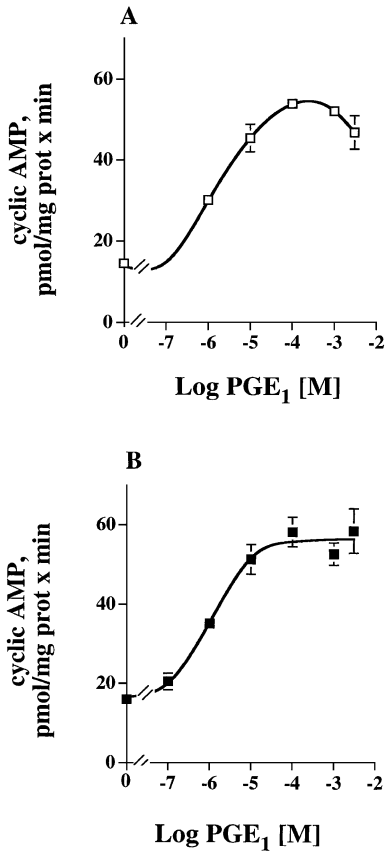

Fig. 3. Concentration–response curves for the activation of adenylate cyclase by prostaglandin E_1 (PGE_1). Panel A: control curve; Panel B: mesenteric smooth muscle cells treated with 350 ng/ml of pertussis toxin. Data are expressed as mean ± S.D.

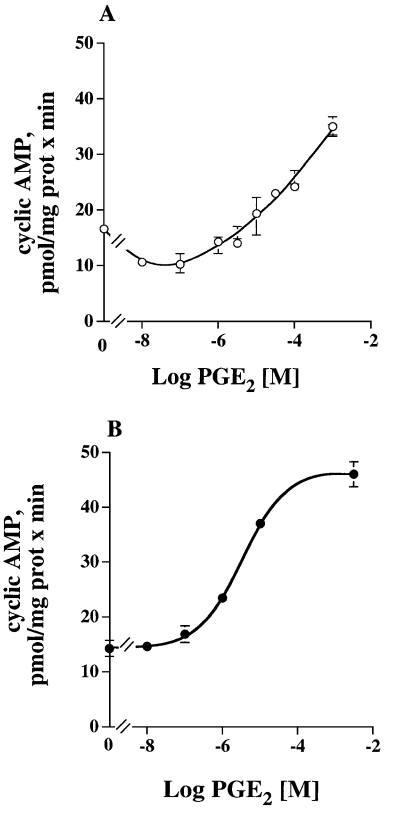

Fig. 4. Concentration–response curves for the activation of adenylate cyclase by prostaglandin E_2 (PGE_2). Panel A: control curve; Panel B: mesenteric smooth muscle cells treated with 350 ng/ml of pertussis toxin. Data are expressed as mean ± S.D.

according to the method of Ross (1971). Cells were grown in monolayers on petri dishes using MEM F11 as medium with the addition of penicillin, streptomycin, tricine and nonessential amino acids.

2.3. Membrane preparation

Smooth muscle cell from rabbit mesenteric artery was used from the 5th and 15th passages. Briefly, monolayers of cells were washed in 50 mM Tris–HCl buffer (pH 7.4), harvested by scraping, pooled and the membrane preparation (pellet at 15,000 g) was obtained as previously described (Oliva et al., 1984).

2.4. Adenylate cyclase assay

Adenylate cyclase assays were performed as previously described (Corsini et al., 1987). Briefly, the standard assay mixture (final volume: 100 μl) contained: 10 mM Tris–HCl buffer (pH 8); 0.1 mM [8-^{14}C]ATP (50 dpm/pmol); 0.5 mM [2,8-^{3}H]cyclic AMP (approximately 360 dpm/nmol); 2 mM $MgSO_4$; 2 mM creatine phosphate; 34 U.I./ml creatine

Table 2
Effect of the pertussis toxin treatment on the parameters of the concentration–response curves for the modulation of adenylate cyclase activity in mesenteric smooth muscle cells

Parameter	PGE_1	PGE_1 + PTx	PGE_2	PGE_2 + PTx
E_1	13.4 ± 3.2	16.3 ± 16	17 ± 5.4	14.4 ± 1.9
$Slope_1$	0.63 ± 9.3	0.9 ± 29	− 0.37 ± 4.4	0.77 ± 4.7
EC_{50_1}	2.4×10^{-6} ± 18	1.1×10^{-6} ± 34	4×10^{-9} ± 17	3.2×10^{-6} ± 7
E_{max}	58.6 ± 4.4	56.4 ± 3	8.2 ± 8	46.3 ± 1.7
$Slope_2$	− 0.77 ± 26.8	–	1.53 ± 16	–
EC_{50_2}	1.2×10^{-2} ± 22	–	1.3×10^{-4} ± 11	–
E_2	13.4 ± 3.2	–	46 (imposed)	–

Parameters as in Table 1. Parameters E_1 and E_2 for PGE_1 are shared. Parameters are expressed ± %CV. PGE_1 = prostaglandin E_1; PGE_2 = prostaglandin E_2; PTx = pertussis toxin.

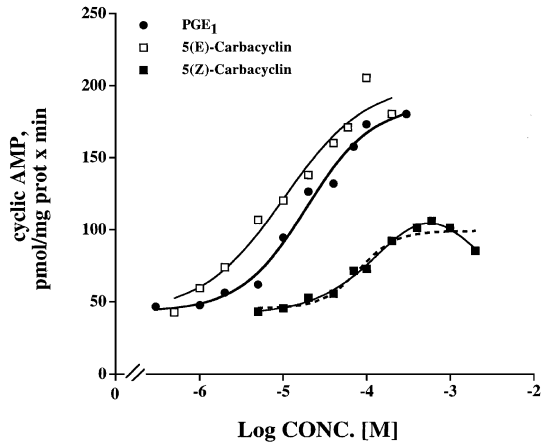

Fig. 5. Concentration–response curves for the activation of adenylate cyclase by different prostaglandins in membranes of myocytes from rabbit mesenteric artery (dashed line interaction with a single receptor, solid line interaction with two receptors with opposite effect).

phosphokinase; 10 μM GTP and the indicated prostaglandins. For solubility problems, it was more convenient to dissolve the prostaglandins in Tris–HCl buffer containing ethanol to yield a final concentration of 3.33% in the sample. The incubation, started with the addition of the membrane preparation (0.06–0.1 mg/protein per sample), was carried out at 30 °C for 8 min. [8-^{14}C, 2,8-^{3}H]cyclic AMP was isolated and detected according to Salomon et al. (1974).

2.5. Pertussis toxin treatment

Pertussis toxin treatment was carried out in intact cells for 24 h before membrane preparation, using 350 ng/ml of pertussis toxin (unless otherwise indicated), dissolved directly in MEM F11 without serum.

2.6. Data analysis

Concentration–response curves were analyzed using BELLFIT, a nonlinear least squares program for the analysis of bell-shaped concentration–response curves based on a modification of the four-parameter logistic equation as previously published (Rovati and Nicosia, 1994a,b). This model accounts for seven unknown parameters: E_1, Slope$_1$ and EC_{50_1} refer to interaction of a ligand with the higher potency receptor, and indicate the response when the concentration of ligand is zero, the slope and the concentration yielding 50% of the maximum response of the 1st component of the curve, respectively; E_2, Slope$_2$ and EC_{50_2} refer to interaction of a ligand with the lower potency receptor, and indicate the response for an infinite concentration of ligand, the slope and the concentration yielding 50% of the maximal response of the 2nd component of the curve, respectively; E_{max} indicates the maximum response. The program allows all the seven parameters to be fitted simultaneously, or one or more parameters to be set constant and, therefore, excluded by the analysis. The statistical principle of the "extra sum of squares" (Draper and Smith, 1966) was used to test different models and select the most appropriate one. Parameters are expressed ± % Coefficient of Variation (%CV). Data are expressed as mean ± S.D. of triplicates from representative experiments performed at least three times. Where S.D. is not shown, it is smaller than the symbol itself. All the curves shown are computer generated.

3. Results

3.1. Prostaglandin concentration–response curves on adenylate cyclase activity

We have investigated the effect of different prostaglandins on adenylate cyclase activity in rabbit mesenteric smooth muscle cells. Fig. 1 shows a concentration–response curve for prostacyclin, iloprost, prostaglandin E_1 and prostaglandin E_2 (Panels A, B, C and D, respectively). It is evident that all the concentration–response curves are not monotonic, i. e. simple sigmoidal curves on logarithmic scale, but biphasic. Prostacyclin, iloprost and prostaglandin E_1 showed a convex bell-shaped curve, i.e. adenylate cyclase activity stimulation at lower concentrations and adenylate cyclase inhibition at higher concentrations, while the curve of prostaglandin E_2 showed a concave bell-shaped curve, i.e. adenylate cyclase activity inhibition at lower

Table 3
Parameters of the concentration–response curves for the modulation of adenylate cyclase activity in mesenteric smooth muscle cells

Parameter	PGE_1	5(*E*)-Carbacyclin	5(*Z*)-Carbacyclin (1 receptor)	5(*Z*)-Carbacyclin (2 receptors)
E_1	42 ± 12	42 ± 12	42 ± 12	42 ± 12
Slope$_1$	1.1 ± 24	0.62 ± 44	1.8 ± 46	1.02 ± 28
EC_{50_1}	1.9×10^{-5} ± 23	7.9×10^{-6} ± 47	8.1×10^{-5} ± 24	2.4×10^{-4} ± 91
E_{max}	187 ± 7	253 ± 24	99 ± 4.5	199 ± 81
Slope$_2$	–	–	–	−1 ± ND
EC_{50_2}	–	–	–	1.3×10^{-3} ± 65
E_2	–	–	–	42 ± 12

Parameters as in Table 1. 5(*Z*)-Carbacyclin has been analyzed according to the single receptor model and to the two-receptor model. Parameters E_1 and E_2 for 5(*Z*)-Carbacyclin are shared. Parameters are expressed ± %CV. PGE_1 = prostaglandin E_1.

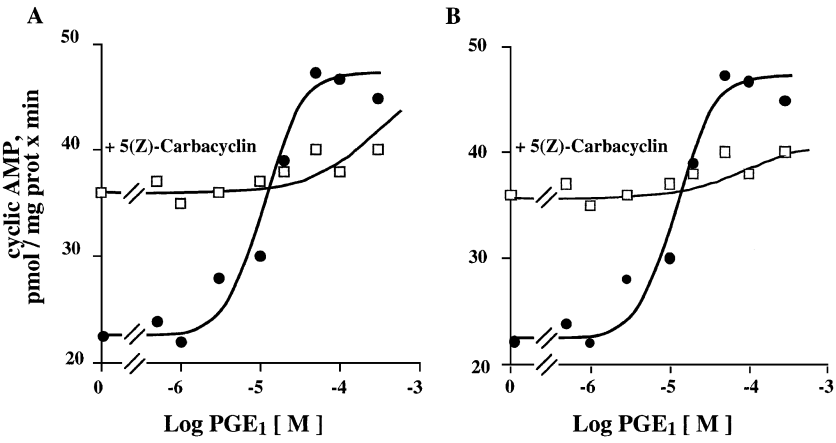

Fig. 6. Concentration–response curves for the activation of adenylate cyclase by a full agonist, prostaglandin E_1 (PGE_1), in the absence and in the presence of a fix concentration (0.2 mM) of 5(*Z*)-carbacyclin. Panel A: both curves have been analyzed assuming the model of interaction of a partial agonist with a single receptor. Panel B: the same curves have been analyzed assuming the model of interaction of a full agonist with two receptors with opposite effect on cyclic AMP production.

concentrations and adenylate cyclase stimulation at higher concentrations. The parameters and their %CV have been calculated using BELLFIT and presented in Table 1. For prostacyclin, iloprost and prostaglandin E_1, the parameters E_1 and E_2, i.e. the effect at the minimum and maximal concentrations respectively, are imposed to be equal (shared) due to the impossibility to obtain a plateau.

3.2. Pertussis toxin effect

We have investigated the effect of pertussis toxin on adenylate cyclase activity by pretreating intact mesenteric smooth muscle cells with increasing concentrations of pertussis toxin. As it is clear from Fig. 2, 350 ng/ml pertussis toxin was able to completely abolish the descending part of the curve of prostaglandin E_1 stimulation. Thus, the same concentration of pertussis toxin was used in all the subsequent experiments.

Fig. 3 shows a complete concentration–response curve for prostaglandin E_1 performed on control (Panel A) and pertussis toxin treated (Panel B) mesenteric smooth muscle cells. The control curve is a bell-shaped curve as already discussed, while the curve performed on pertussis toxin treated cells is monotonic with a straight upper plateau without the descending component. The parameters and their %CV are shown in Table 2. The same experiment has been performed using also iloprost with similar results (data not shown). In the same way, pertussis toxin treatment was also able to abolish the descending component of the prostaglandin E_2 concentration–response curve, leaving the ascending component untouched (Fig. 4, Panels A and B and Table 2).

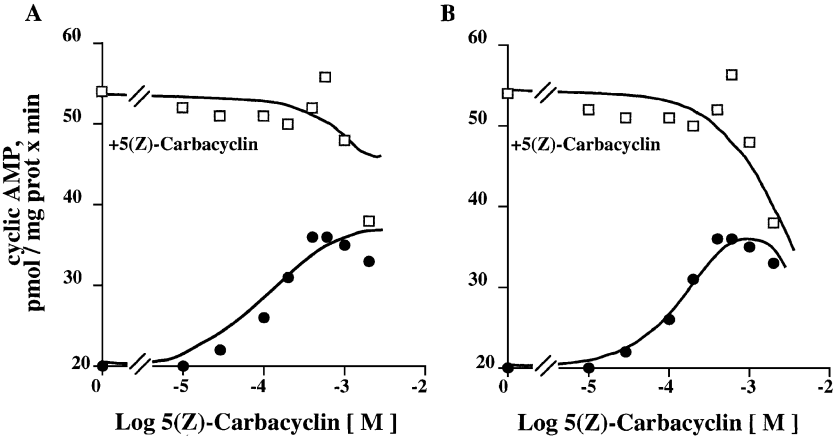

Fig. 7. Concentration–response curves for the activation of adenylate cyclase by 5(*Z*)-carbacyclin in the absence and in the presence of a fix concentration (0.3 mM) of the full agonist prostaglandin E_1 (PGE_1). Panel A: both curves have been analyzed assuming the model of interaction of a partial agonist with a single receptor. Panel B: the same curves have been analyzed assuming the model of interaction of a full agonist with two receptors with opposite effect on cyclic AMP production.

3.3. Reinterpretation of 5(Z)-carbacyclin data

In Fig. 5, we present the concentration–response curves for prostaglandin E_1 and the prostacyclin analogs 5(*E*)-carbacyclin and 5(*Z*)-carbacyclin, on adenylate cyclase activity in mesenteric smooth muscle cells as previously published by our laboratory (Corsini et al., 1987). 5(*Z*)-Carbacyclin data have been now analyzed either assuming the classical model of interaction of a partial agonist with a single receptor (Fig. 5, dashed line) or assuming an interaction with both a stimulatory plus a second inhibitory receptor (Fig. 5, solid line). The parameters for both models are presented in Table 3. The model involving the interaction with a second receptor with opposite effect gives a significantly ($P < 0.05$, *F* test) better fitting than the simpler one.

We have also reanalyzed the two experiments (Corsini et al., 1987) that are usually performed to study the apparent partial agonistic activity of a compound, i. e. the concentration–response curve of a full agonist in the presence of a fixed concentration of the putative partial agonist (Fig. 6) and, vice versa, the concentration–response curve of the putative partial agonist in the presence of a fixed concentration of a full agonist (Fig. 7). In Figs. 6 and 7, in Panel A, both curves have been analyzed assuming that 5(*Z*)-carbacyclin behaves as a partial agonist, thus interacting with a single receptor, while in Panel B, the same curves have been analyzed assuming that 5(*Z*)-carbacyclin is a full agonist interacting with two receptors with opposite effect on cyclic AMP production.

4. Discussion

The occupancy theory states that the response is linearly dependent on receptor occupancy, and the maximal response is reached when the total number of receptors is occupied (Clark, 1937). This predicts that the plot of the response versus the agonist concentration is a hyperbolic curve starting from the origin and approaching the maximal response (E_{max}) asymptotically. It is known that in many systems, the response is not directly proportional to receptor occupancy and, therefore, many modification to the classical Clark's theory have been published (Ariëns, 1954; Furchgott, 1966; Stephenson, 1956) to explain some of the practical observations that many scientists have encountered in the real word. However, all these variations to the occupancy theory still predict that a concentration–response curve will be monotonic, i.e. it will be an "S"-shaped (sigmoidal) curve on a semilogarithmic plot, approaching the maximal response asymptotically and, therefore, with a straight upper plateau.

However, it is very common for experimentalists to encounter in practice some concentration–response curves that are "bell shaped" (Szabadi, 1977). This is also the case with some classes of prostanoid receptors. Based on kinetic studies, Ashby, 1989, 1990 has already postulated that adenylate cyclase in platelets is controlled through separated stimulatory and inhibitory prostaglandin receptors, suggesting a model of heterologous desensitization for homeostatic control of prostaglandin effects. However, this model gives rise to bell-shaped curves only as a function of a time. While bell-shapes curves might, sometimes, be due to desensitization produced by a cumulative procedure, in our experimental approach, each sample was exposed to a single stimulus concentration for a constant time. Therefore, under our conditions, it is unlikely that desensitization will produce bell-shaped dose–response curve (Rovati and Nicosia, 1994b); presumably, desensitization would yield a dose–response curve with reduced efficacy, but with a straight upper plateau (De Lean et al., 1979).

Thus, in this contribution, we have demonstrated that, independently of time-related phenomena, concentration–response curves for adenylate cyclase modulation for prostacyclin, iloprost and prostaglandin E_1 are not simple sigmoidal curves, but their upper plateau is bending at high concentrations (Fig. 1, Panels A, B and C), thus suggesting a more complicated model than a simple one ligand–one receptor interaction. The same is also true for prostaglandin E_2 concentration–response curve (Fig. 1, Panel D); in this case, however, we have a concave bell-shaped curve. We have previously formally presented a model of interaction of a single ligand with two distinct receptors with opposite effect and elucidated its intrinsic characteristics (Rovati and Nicosia, 1994a). We have now applied this model to the prostaglandin-induced modulation of adenylate cyclase in rabbit mesenteric smooth muscle cells demonstrating that the difference in cyclic AMP synthesis is the result of algebraic summation of positive and negative stimuli.

It is well known that, despite the fact that each prostanoid is at least on order of magnitude more potent for its specific receptor than any of the other prostanoids, all of them show some degree of cross-reactivity, i.e. they are able to interact, albeit with different affinities, also with one or more of the other prostanoid receptors (Coleman et al., 1994). This situation is the rule rather than the exception for substances with closely related structures. In particular, there are several systems regulated by both stimulatory and inhibitory receptors and the adenylate cyclase system is one of the best-studied examples. Thus, we have hypothesized that the bell-shaped curves of these prostanoids could be the result of the interaction with two distinct receptors (Rovati and Nicosia, 1994a) or, possibly, with a single receptor coupled to two different G-proteins (a stimulatory G_s and an inhibitory G_i) with opposite effects on adenylate cyclase and thus on cyclic AMP levels. It is in fact known that G-protein-coupled receptors, including IP (Katsuyama et al., 1994) and EP_3 (Namba et al., 1993) receptors, are capable of interacting with more than one G-protein, thus resulting in multifunctional signaling (Milligan, 1993). Therefore, beside the cross-reactivity of each ligand with more than

one receptor, it is also possible that the same agonist interacting with the same receptor might elicit different responses by interacting with distinct G-proteins at different concentrations (Kenakin, 1995, 1997).

We have selectively inhibited one of the transduction mechanisms present in mesenteric smooth muscle cells, namely the one coupled to a G_i, treating the cells with pertussis toxin. This toxin is know to selectively ADP-ribosylate the α subunit of the G_i class of protein, thus preventing adenylate cyclase inhibition by a G_i. As it is clear from the result presented in Figs. 3 and 4, the pretreatment with pertussis toxin, knocking out the inhibitory component of the system, has completely abolished the bell shape of the curves. Under this conditions, prostacyclin, iloprost and prostaglandin E_1, agonists of the IP receptor are only able to stimulate adenylate cyclase, presumably via the IP receptor that is coupled to a stimulatory G-protein, G_s, that is not affected by pertussis toxin treatment. Indeed, cicaprost, a more selective IP receptor agonist, gives rise to a simple monotonic concentration–response curve also in the absence of pertussis toxin treatment (data not shown).

On the other hand, prostaglandin E_2, agonist of the EP series of receptors, is no more able to inhibit adenylate cyclase activity in pertussis toxin-treated cells. This inhibition of adenylate cyclase is elicited presumably via the EP_3 receptor (Ortiz-Vega and Ashby, 1997) coupled to an inhibitory G-protein, G_i. However, prostaglandin E_2 is still able to stimulate adenylate cyclase activity via a receptor coupled to a G_s. Interestingly, the lack of inhibitory effect on adenylate cyclase due to pertussis toxin treatment causes an almost 2 log unit shift in the EC_{50} of the stimulatory component of prostaglandin E_2.

Thus, the results obtained with pertussis toxin treatment confirm our hypothesis that the bell-shaped curves result from the interaction with two distinct receptors or, possibly, with single receptor coupled to two different G-proteins with opposite effects on cyclic AMP levels. The concave bell-shaped curve of prostaglandin E_2, compared to the bell-shape curves of the other prostaglandin tested, is as expected due to the fact that prostacyclin, its stable analog iloprost and prostaglandin E_1 have a higher affinity for the IP receptor, while prostaglandin E_2 has a higher affinity for the EP class of receptors (Coleman et al., 1994). Thus, the order of selectivity determines the shape of the concentration–response curves.

It is interesting to notice that some slope parameters of the bell-shaped curves are statistically less than unity, but this is not an unexpected finding. Rather, slope parameters different from unity are expected when the response is due to an interaction with more than one receptor. Indeed, after the pertussis toxin treatment, the slopes of the monotonic curves did not statistically differ from unity.

As we have previously demonstrated, the phenomenon of bell-shaped concentration–response curves influences also the apparent maximum response of the curve of an agonist, that can be, therefore, mistaken for a partial agonist (Rovati and Nicosia, 1994a). These compounds are characterized by a lower efficacy as compared with a full agonist, still with a straight upper plateau of the concentration–response curve tending to the intrinsic activity value α. Our laboratory had previously shown that 5(*E*)- and 5(*Z*)-carbacyclin, two prostacyclin analogs, displayed the same efficacy as prostaglandin E_1, and hence prostacyclin, in stimulating adenylate cyclase activity in membranes from human platelets (Corsini et al., 1987). On the contrary, 5(*Z*)-carbacyclin failed to produce the same maximal degree of enzyme stimulation in mesenteric smooth muscle cells (Fig. 5). It was, therefore, concluded that prostacyclin receptors in platelets and vascular smooth muscle cells differ because 5(*Z*)-carbacyclin could discriminate between them and that 5(*Z*)-carbacyclin displayed partial agonist properties (Corsini et al., 1987). However, at that time, the fact that 5(*Z*)-carbacyclin curve was bell-shaped was not taken into account and regarded as a possible artifact of the system.

On the basis of the data discussed above on the involvement of multiple prostanoid receptors on cyclic AMP modulation and in the light of the model we previously published (Rovati and Nicosia, 1994a), we have reinterpreted 5(*Z*)-carbacyclin data (Fig. 5). The reinterpretation of these data explains both the curvature of the upper plateau and the apparent reduction in efficacy for 5(*Z*)-carbacyclin. As it is clear from Table 3, the efficacy of 5(*Z*)-carbacyclin is apparently lower than that of the reference compound prostaglandin E_1, when the data are analyzed with the simpler model of interaction with a single receptor. On the contrary, when we simulated the interaction with a second inhibitory receptor to the model, the efficacy of 5(*Z*)-carbacyclin becomes not significantly different from that of prostaglandin E_1 (199 and 187 pmol/mg prot x min, respectively; $P>0.05$). Thus, the difference in behavior between platelets and mesenteric smooth muscle cells can simply be explained with differences in the density of each class of receptors present in the two cell types; on the other hand, the apparent different efficacies of the various agonists within the same tissue can be explained with differences in affinities of each agonist for each class of prostanoid receptor present.

Furthermore, we have also reanalyzed the data of the two experiments presented in Figs. 6 and 7. The data of the curve of the full agonist in the presence of a fixed concentration of the supposed partial agonist can be fitted equally well either assuming that 5(*Z*)-carbacyclin is a partial agonist interacting with a single receptor, or assuming that 5(*Z*)-carbacyclin is a full agonist interacting with a second inhibitory receptor. The situation is somewhat different for the data of Fig. 7. The downward curvature of the upper plateau of the curve of 5(*Z*)-carbacyclin alone reveals that the model hypothesizing a partial agonist behavior might not be the correct one. Therefore, caution must be used when the data of such experiments are analyzed. Very often,

lack of fit is interpreted as presence of experimental error, while in reality, it is evidence of an erroneous theoretical model.

In conclusion, cross-reactivity between closely related substances might explain some previously unexpected and disregarded biological phenomena and experimental observation. This is the case of prostaglandin bell-shaped concentration–response curve in rabbit mesenteric smooth muscle cells. Furthermore, co-expression of different receptors or activation of different G-proteins with opposite effects might account for some of the data present in the literature previously interpreted as due to partial agonist behavior. The design of the experiment is, in this case, a crucial step of the modeling procedure. Only performing experiments over a wide range of concentrations and considering the presence of more complex models will help to interpret experimental data in a more meaningful and accurate way.

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