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THE BINDING OF [^{3}H]-PROSTACYCLIN TO MEMBRANES OF A NEURONAL SOMATIC HYBRID

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1 [^{3}H]-prostacylin bound to a washed membrane preparation of the NCB-20 neuronal hybrid cell line.

2 Kinetic analysis of [^{3}H]-prostacyclin binding suggested a simple, non-cooperative bimolecular interaction between the ligand and a single receptor population. The equilibrium dissociation constant was 16.6 nM, and binding at a saturating [^{3}H]-prostacyclin concentration enabled the receptor density of 2.57×10^5 receptor molecules per cell to be calculated.

3 At 20°C the rate constant for the forward reaction (k_{+1}) was 2.26×10^5 M^{-1} s^{-1}, and the rate constant for the dissociation of the ligand-receptor complex (k_{-1}) was 3.85×10^{-3} s^{-1}. Thus the dissociation constant (k_{-1}/k_{+1}) was 17.0 nM.

4 Prostaglandin E_1 and prostacyclin compete for a single receptor in these cells, and comparison of other prostaglandins as inhibitors of [^{3}H]-prostacyclin binding revealed some of the structural requirements for high-affinity occupation of prostacyclin receptors.

Introduction

Prostacyclin (PGI_2), a naturally occuring derivative of the prostaglandin endoperoxides (Moncada, Gryglewski, Bunting & Vane, 1976) has been shown to activate adenylate cyclase [ATP: pyrophosphate-lyase (cyclising); EC 4.6.1.1.] of the NCB-20 neuronal hybrid cell line (Blair, Hensby & MacDermot, 1980a). This cell line (Minna, Yavelow & Coon, 1975) is derived from a mouse neuroblastoma and brain of foetal Chinese hamster. It expresses many functions of highly differentiated nerve cells including acetylcholine synthesis, electrical excitability and competence to form stable, physiologically active synapses with myotubes in co-culture (MacDermot, Higashida, Wilson, Matsuzawa, Minna & Nirenberg, 1979). This system provides a convenient source of neuronal cells that are free from contamination with vascular, erthropoietic and other prostacyclin-sensitive tissues. There is a single population of prostacyclin receptors mediating activation of adenylate cyclase in this cell line (Blair *et al.*, 1980a), and the interaction between adenylate cyclase and the prostacyclin receptor is dependent on guanosine 5′-triphosphate. This suggests a mechanism of receptor-enzyme coupling similar to that observed in other hormonal (Lad, Welton & Rodbell, 1977; Levitzki, 1977) and neurotransmitter systems (Sabol & Nirenberg, 1979; MacDermot, 1979).

In a comparison of selected prostaglandins as activators of adenylate cyclase in the NCB-20 hybrid cell line, prostacyclin was observed to be 17 times more potent than prostaglandin E_1 (PGE_1), and over 1000 times more potent than PGE_2 (Blair *et al.*, 1980a). Prostaglandins $F_{1\alpha}$, $F_{1\beta}$, $F_{2\alpha}$ and D_2 were all inactive. Prostacyclin is rapidly hydrolysed to 6-oxo-$PGF_{1\alpha}$, even at neutral pH (Johnson, Morton, Kinner, Gorman, McGuire, Sun, Whittaker, Bunting, Salmon, Moncada & Vane, 1976); this stable product, however, mediated little or no increase in adenylate cyclase activity in this cell line (Blair *et al.*, 1980a).

These studies have now been extended in order to delineate the nature of prostacyclin binding to neuronal receptors expressed in this somatic cell hybrid. Having established previously the characteristics of the biological activity of these receptors, namely their interaction with adenylate cyclase, radio-ligand binding techniques have been employed to measure the binding directly. Analysis of the reaction rate and steady-state kinetics of [^{3}H]-PGI_2 binding provides evidence of receptor numbers and the appropriate binding constants. A number of prostaglandins have been compared for their ability to displace [^{3}H]-PGI_2 from its receptor. This allows further analysis of the structural requirements of these compounds for their high affinity interaction with the PGI_2 receptor.

Methods

Cell culture

The NCB-20 hybrid cell line (Minna *et al.*, 1975) was obtained by Sendai virus-induced fusion of the N18TG2 mouse neuroblastoma clone (Minna, Glazer & Nirenberg, 1972), resistant to 6-thioguanine, and

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brain cells of embryonic Chinese hamster (18 days *in utero*). The cells were cultured and homogenates prepared for assay of adenylate cyclase activity as described previously (MacDermot *et al.*, 1979). Binding studies were performed on a washed particulate preparation of these cells: whole cells from 20 flasks (75 cm^2) were suspended in 25 mM Tris-HCl buffer, pH 8.5, containing 0.32 M sucrose and disrupted at 4°C by 20 strokes of a tightly fitting Dounce homogenizer. Undisrupted cells and nuclei were pelleted by centrifugation at 500 *g* at 4°C, for 10 min. The supernatant fraction was centrifuged at 100,000 *g* for 20 min at 4°C, and the pellet washed 3 times at 4°C by resuspension in 50 ml 50 mM Tris-HCl buffer pH 8.5, followed by centrifugation at 100,000 *g* for 20 min. The pellet was finally suspended in 10 ml 50 mM Tris-HCl buffer pH 8.5, and stored at −80°C. The protein concentrations of individual batches were determined by a modification of the method of Lowry, Rosebrough, Farr & Randall (1951).

Adenylate cyclase assay

Enzyme activity was determined by a modification (Sharma, Nirenberg & Klee, 1975) of method C of Salomon, Londos & Rodbell (1974). Reaction mixtures of 100 μl contained 50 mM Tris-HCl buffer pH 8.5; 5 mM magnesium chloride; 87 mM sucrose; 20 mM creatine phosphate, disodium salt (Sigma); 10 International Units creatine kinase, 150 iu/mg protein (ATP: creatine *N*-phosphotransferase; EC 2.7.3.2) from Sigma; 1 mM cyclic adenosine 3′, 5′-monophosphate, sodium salt (Sigma); 0.25mM Ro20-1724 (a phosphodiesterase inhibitor from Roche Products Ltd); 0.5% ethanol; 1 mM [α^{32}P]-ATP (3 μCi, Radiochemical Centre, Amersham; 1 Ci = 3.7×10^{10}Bq); and 100 to 200 μg of homogenate protein. Cell homogenates were stored at −80°C and then thawed and maintained at 4°C in an ice bath for no longer than 10 min before incubation. The production of [^{32}P]-cyclic AMP was proportional to protein concentration within the range 50–250 μg of homogenate protein per reaction mixture; similarly [^{32}P]-cyclic AMP synthesis increased linearly for 30 min. The results shown were obtained from experiments in which incubations were maintained at 30°C for 9 min, in order to minimize the spontaneous hydrolysis of PGI_2 to the inactive stable product 6-oxo-$PGF_{1\alpha}$.

Synthesis of [11β-^{3}H]-prostacyclin

Prostacyclin was labelled with tritium in the 11β position according to the method of Blair, Hensby & MacDermot (1980b). The method involved reduction of PGD_2 with sodium [^{3}H]-borohydride (32 Ci/mol, Radiochemical Centre, Amersham) yielding [11β-^{3}H]-$PGF_{2\alpha}$. This was followed by cyclic iodoether formation, dehydrohalogenation and saponification (Nicolaou, Barnette, Gasic, Magolda & Sipio, 1977) to give [11β-^{3}H]-PGI_2 as its sodium salt. The chemical identity of the stable hydrolysis product (6-oxo-$PGF_{1\alpha}$) was determined by gas chromatography-mass spectrometry, and the biological activity compared with that of authentic PGI_2 by adenylate cyclase activation in homogenates of the NCB-20 cell line (Blair *et al.*, 1980b).

Binding of [^{3}H]-prostacyclin to washed membranes of NCB-20 cells

The binding assay was performed by a modification of previously described techniques (Pert & Snyder, 1973; MacDermot & Nirenberg, 1978). Reaction mixtures of 100 μl contained 50 mM Tris-HCl buffer pH 8.5; 10 mM magnesium sulphate, [^{3}H]-PGI_2 at selected concentrations (8 Ci/mmol); and 50 to 130 μg protein of a washed membrane preparation of NCB-20 hybrid cells. Reaction mixtures were prepared at 4°C in an ice bath, and then incubated for 20 min at 20°C. Incubations were terminated by the addition of 4 ml 50 mM Tris-HCl buffer pH 8.5 at 4°C, followed by rapid filtration under reduced pressure through a Whatman GF/B glass filter disc (24 mm diam.). The filters were then washed 3 times with 4 ml 50 mM Tris-HCl buffer pH 8.5 at 4°C under reduced pressure. The entire washing procedure was completed in about 20 s. The filter disc was counted in 10 ml Insta-Gel (Packard Instrument Co. Inc.) and 1 ml water. Counting at 28 to 32% efficiency was performed in a Packard liquid scintilation spectrometer. Specific binding was taken to be that displaced by 10 μM PGI_2 in parallel incubations. In time course experiments, single incubations of 1 to 2 ml, containing the same molar concentrations of reagents as described above, were performed. At selected time intervals, 100 μl of the reaction mixture was pipetted into a clean glass tube, and the reaction terminated as described previously. Under the conditions of this assay, binding of [^{3}H]-PGI_2 increased linearly with protein concentration within the range 50 to 1050 μg protein.

The following generous gifts were received: Ro20–1724 from Roche Products Ltd; 6β-PGI_1 and PGI_2 from the Wellcome Research Laboratories; other prostaglandins from Upjohn Co.; the NCB-20 cell line from Dr Marshall Nirenberg, National Institutes of Health, U.S.A.

Results

Analysis of the derivatized [11β-^{3}H]6-oxo-$PGF_{1\alpha}$ by gas chromatography-mass spectrometry demonstrated a chemically pure compound identical to the methoxime, tri-trimethylsilyl ether, methyl ester of

6-oxo-$PGF_{1\alpha}$ (Blair *et al.*, 1980b). It follows therefore that a mixture of no more than two compounds had been synthesized, namely [11β-^{3}H]-PGI_2 and [11β-^{3}H]-6-oxo-$PGF_{1\alpha}$. A comparison of this mixture with authentic PGI_2 as activators of adenylate cyclase in homogenates of the NCB-20 hybrid demonstrated that 42% of the radioactivity was [11β-^{3}H]-PGI_2 and the data from the GC-MS analysis above required that the remaining material was the stable hydrolysis product [11β-^{3}H]-6-oxo-$PGF_{1\alpha}$. In the experiments described here, the concentration of [^{3}H]-PGI_2 in the reaction mixtures was corrected according to its biological activity. This manipulation was validated by demonstration that basal adenylate cyclase activity in NCB-20 homogenates (5.4 ± 0.2 pmol cyclic AMP min^{-1} mg^{-1} protein) was not increased significantly by 6-oxo-$PGF_{1\alpha}$ at concentrations up to 10 μM (> 750 times the K_d of PGI_2) and furthermore PGI_2-activated adenylate cyclase (57.7 ± 1.3 pmol cyclic AMP min^{-1} mg^{-1} protein) was not inhibited by 6-oxo-$PGF_{1\alpha}$ within the same concentration range. In addition, in experiments presented in Table 1, 6-oxo-$PGF_{1\alpha}$ was shown to have no inhibitory effect on the binding of [^{3}H]-PGI_2 to NCB-20 cell membranes. Thus the contamination of [^{3}H]-PGI_2 by [^{3}H]-6-oxo-$PGF_{1\alpha}$ had no effect on PGI_2 binding.

In a time course experiment, the rate of association of [^{3}H]-PGI_2 and the membrane receptor was measured at 20°C (Figure 1a). The time required for half saturation was 210 s at a ligand concentration of 10 nM. From the pseudo-first order rate plot (Figure 1b), the observed rate constant (k_{obs}) was given by the slope, and found to be 2.26×10^{-3} s^{-1}. The calculated 2nd order rate constant (k_{+1}) for the ligand-receptor association is given by k_{obs}/[ligand], and this was found to be 2.26×10^5 M^{-1} s^{-1}. The dissociation of the ligand-receptor complex was determined in another time course experiment (Figure 2a). PGI_2 receptors were saturated with [^{3}H]-PGI_2 by incubation for 15 min at 20°C in the presence of 50 nM [^{3}H]-PGI_2. At zero time, a large excess (final concentration = 500 μM) of cold PGI_2 was added, and the remaining [^{3}H]-PGI_2 bound to the membrane preparation determined at selected times. Non-specific binding was subtracted from the total binding at each time point, and was determined by measuring the residual binding after the incubation had been allowed to proceed for 20 min after the addition of cold PGI_2. Figure 2b shows a semi-logarithmic plot of the same data, from which the half time ($T_{½}$) for the dissociation of the receptor-ligand complex was found to be 180 s. The first order rate constant (k_{-1}) for the reaction was calculated to be 3.85×10^{-3} s^{-1}. The true dissociation constant (K_d) was then calculated as the ratio of k_{-1} and k_{+1}, and was found to be 1.7×10^{-8} M (17 nM).

Table 1 The relative potencies of prostaglandins that inhibit the binding of [^{3}H]-prostacyclin ([^{3}H]-PGI_2) to NCB-20 membrane receptors, and activate adenylate cyclase

Prostaglandin	[^{3}H]-PGI_2 *binding* K_i (nM)	*Adenylate cyclase activation* K_{act} (nM)
PGI_2	16.6	24.6
PGE_1	137	415
13,14-dihydro-PGE_1	315	668
8-*iso*-PGE_1	735	2,200
5,6-*trans*-PGE_2	735	2,590
17-phenyl-PGE_2	4,370	–
6β-PGI_1	7,350	5,000
13,14-dihydro-PGE_2	8,410	60,000
PGE_2	13,370	31,500
13,14-dihydro-15-oxo-PGE_2	>100,000	>100,000
15-*epi*-PGE_2	>100,000	–
$PGF_{1\alpha}$	>100,000	>100,000
6-oxo-$PGF_{1\alpha}$	>100,000	>100,000
$PGF_{2\alpha}$	>100,000	>100,000
PGD_1	>100,000	>100,000

Results were obtained from experiments in which the specific binding of [^{3}H]-PGI_2 was measured in the presence of selected concentrations of these prostaglandins. All data points were determined in triplicate, and the effect of no fewer than 4 different concentrations of each prostaglandin on the binding of [^{3}H]-PGI_2 (15 nM) was determined. The inhibition constants (K_i) were determined from the formula : $K_i = IC_{50}/[1 + ([PGI_2]/K_d)]$, where IC_{50} is the concentration of prostaglandin producing a 50% decrease in specific [^{3}H]-PGI_2 binding, and K_d is the equilibrium dissociation constant of [^{3}H]-PGI_2. The data for the prostaglandin-dependent activation of adenylate cyclase of the NCB-20 hybrid were taken from Blair *et al.* (1980a).

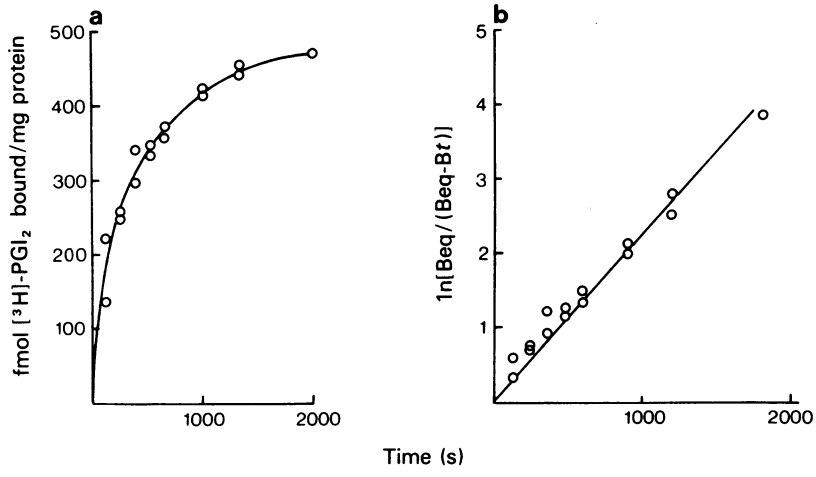

Figure 1 Association of [³H]-prostacyclin ([³H]-PGI_2) and the membrane receptor of the NCB-20 hybrid cell line. The experiment was performed as described in Methods, and each data point shows the result of a single measurement. The time-dependent specific binding of 10 nM [³H]-PGI_2 at 20°C is shown in (a), and the pseudo-1st order rate plot of the same data is shown in (b). Beq is the specific [³H]-PGI_2 binding at equilibrium, and Bt is the specific binding at any particular time (t).

In steady-state binding studies, the binding of [³H]-PGI_2 to membrane receptors was determined at selected concentrations of [³H]-PGI_2. Specific binding was saturable (Figure 3), and from the Scatchard plot the equilibrium dissociation constant is given by 1/slope, and was found to be 16.6 nM (Figure 3, inset). The maximum binding is given by the abscissa intercept and was found to be 1280 fmol/mg protein. The protein recovery in the membrane preparations was 33% of the protein concentration in the original cell homogenate, from which the receptor density in the whole homogenate

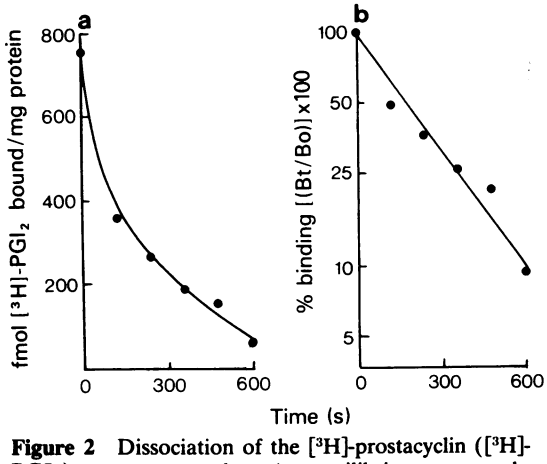

Figure 2 Dissociation of the [³H]-prostacyclin ([³H]-PGI_2)-receptor complex. An equilibrium was established in 15 min at 20°C between the membrane receptors and 50 nM [³H]-PGI_2. At time zero, cold PGI_2 was added to a final concentration of 500 μM. The time-dependent decrease in specific [³H]-PGI_2 binding to the receptor is shown in (a), and a semi-logarithmic plot of the same data is shown in (b), where Bt is the [³H]-PGI_2 bound at any particular time (t), and Bo is the [³H]-PGI_2 bound at the zero time point.

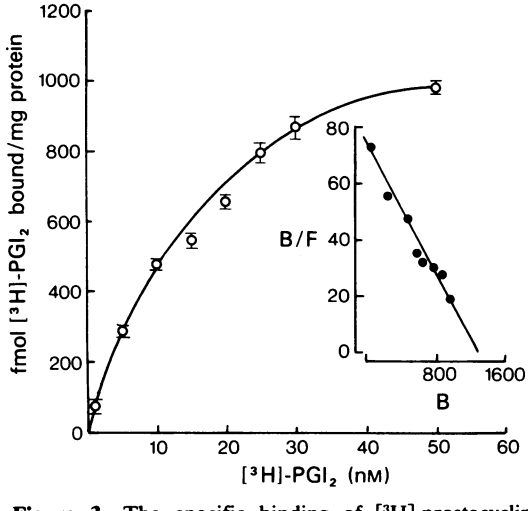

Figure 3 The specific binding of [³H]-prostacyclin ([³H]-PGI_2) to membrane receptors of the NCB-20 hybrid cell line. The effect of [³H]-PGI_2 concentration is shown, and each data point shows the result of the mean (vertical lines indicate s.e. means) of triplicate determinations of [³H]-PGI_2 binding. The inset shows a Scatchard plot, where B is fmol [³H]-PGI_2 bound/mg protein, and F is the free ligand concentration.

was calculated to be 427 fmol/mg protein. Cell counts of NCB-20 hybrid cells were performed in a haemocytometer on cell suspensions of known protein content, and it was found that 1 mg protein was contained in approximately 10^6 cells. From Avogadro's number (6.02×10^{23}), it follows that there are 2.57×10^5 PGI_2 receptor molecules per cell. In a Hill plot of the same data (Figure 4), the interaction coefficient (n) was found to be 1.0.

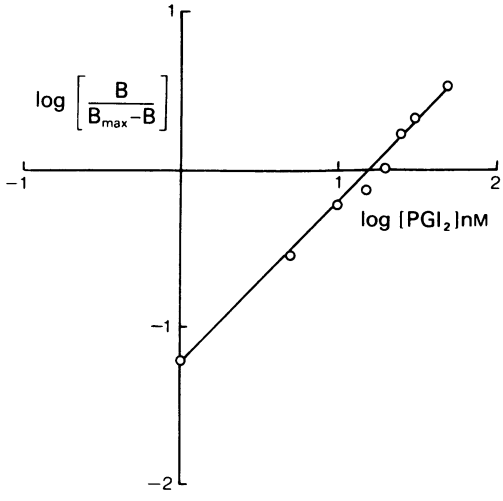

Figure 4 The specific binding of [^{3}H]-prostacyclin ([^{3}H]-PGI_2) to membrane receptors of the NCB-20 hybrid cell line. The data from Figure 3 are shown as a Hill plot. *Bmax* is [^{3}H]-PGI_2 binding at a saturating ligand concentration, and was determined from the abscissa intercept of the Scatchard plot shown in Figure 3 (inset). B is the [^{3}H]-PGI_2 binding at any particular [^{3}H]-PGI_2 concentration.

In preliminary experiments, PGE_1 was found to inhibit [^{3}H]-PGI_2 binding, and the characteristics of this interaction were analysed. In three concentration curves of PGI_2 binding to membrane receptors, binding was compared in the absence or presence of two PGE_1 concentrations (100 and 700 nM). In a double reciprocal plot of these data (Figure 5) it is apparent that the ordinate intercept of each line is identical, suggesting competitive inhibition of [^{3}H]-PGI_2 binding by PGE_1. The inhibition constant (K_i) of PGE_1 was determined from the abscissa intercept of the [^{3}H]-PGI_2 binding curve in the presence of 700 nM PGE_1 [− intercept] = $[-K_d/(1 + i/K_i)]$, where i is the inhibitor concentration and K_d is the equilibrium dissociation constant of [^{3}H]-PGI_2. The K_i value was calculated to be 61 nM.

In a comparison of numerous prostaglandins as inhibitors of [^{3}H]-PGI_2 binding (Table 1), experiments were performed with selected concentrations of these prostaglandins in the presence of a

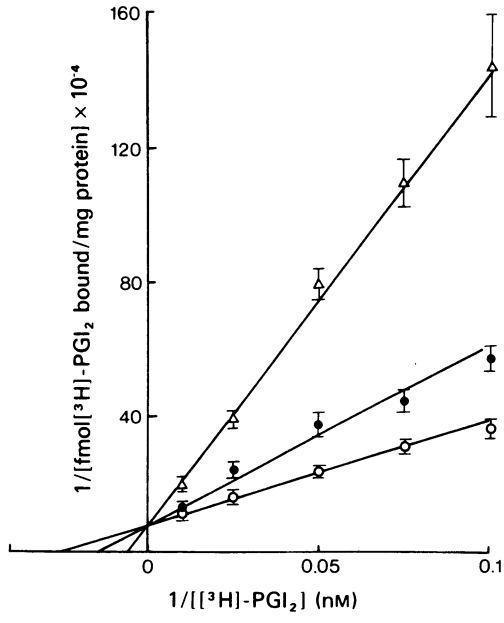

Figure 5 The prostaglandin E_1 (PGE_1)-dependent inhibition of specific [^{3}H]-prostacyclin ([^{3}H]-PGI_2) binding to membrane receptors of the NCB-20 hybrid cell line. Three [^{3}H]-PGI_2 concentration curves (10 to 100 nM) are presented as a double reciprocal plot. Measurements of [^{3}H]-PGI_2 binding were made in the absence (○) or presence of 100 nM (●) or 700 nM (△) PGE_1. Results show means (vertical lines indicate s.e. means) of triplicate determinations.

single (15 nM) concentration of [^{3}H]-PGI_2. The K_i values were determined according to the equation $K_i = IC_{50}/[1 + ([PGI_2]/K_d)]$, where IC_{50} was the concentration of any particular prostaglandin which diminished specific [^{3}H]-PGI_2 binding by 50%, and K_d was the equilibrium dissociation constant of [^{3}H]-PGI_2 for the membrane receptor (16.6 nM).

Discussion

In previous studies employing the NCB-20 neuronal hybrid cell line, kinetic analysis of the activation of adenylate cyclase by PGI_2 (Blair *et al.*, 1980a) has suggested a simple non-cooperative, bimolecular interaction between PGI_2 and a single receptor population. This interpretation of the data is supported by the present work, where Scatchard analysis of specific [^{3}H]-PGI_2 binding shows a linear relationship between 'bound/free' and 'bound' with increasing [^{3}H]-PGI_2 concentrations. The result suggests a simple mass-action interaction between PGI_2 and a single receptor species. From the intercept on the abscissa, the receptor density was

determined, and this led to the calculated number of receptor molecules per cell. The Hill plot was linear and revealed an interaction coefficient of 1.0, suggesting a non-cooperative mechansim of PGI_2 binding. The equilibrium dissociation constant was given by 1/slope in the Scatchard plot (16.6 nM), and this approximated closely to the true dissociation constant determined from the ratio of the rate constants for the back and forward reactions (17.0 nM).

Putative PGE_1 receptors have been identified in several cultured neuronal cell clones (Brunton, Wiklund, Van Arsdale & Gilman, 1976). The results presented here suggest the possibility that neuronal PGE_1 receptors should be considered as PGI_2 receptors, since both ligands compete for a single receptor species in the NCB-20 hybrid cell line. The highest affinity interaction indentified for this receptor is with PGI_2 which is 8 and 800 times greater than the affinity of PGE_1 and PGE_2 respectively. Studies of the desensitization of adenylate cyclase activation by several prostaglandins in platelets have suggested that E-prostaglandins and PGI_2 may interact at a single receptor species (Miller & Gorman, 1979). Furthermore, employing a specific inhibitor of the PGD_2-dependent inhibition of platelet aggregation, Whittle *et al.* (1978) have also suggested that PGE_1 and PGI_2 mediate their effects on similar receptor sites, which are distinct from PGD_2 receptors. The results presented here confirm a similar interaction at the PGI_2 receptor of the NCB-20 cell line.

The affinity of [^{3}H]-PGI_2 binding to the NCB-20 membrane receptor approximated closely to that determined for high affinity binding to platelets (12.1 nM (Siegel, Smith, Silver, Nicholaou & Ahern, 1979). A lower affinity receptor species was also identified in platelets (909 nM), and in this respect [^{3}H]-PGI_2 binding to platelets differed significantly from the binding to NCB-20 membranes, in which only one receptor affinity was observed.

Hydrolysis of PGI_2 to 6-oxo-$PGF_{1\alpha}$ resulted in total loss of biological activity in the NCB-20 hybrid cells (Blair *et al.*, 1980a), in platelet aggregation (Tateson, Moncada & Vane, 1977), in gastric acid secretion (Whittle, Boughton-Smith, Moncada & Vane, 1978), and in the inhibition of noradrenergic transmission (Hedqvist, 1979). Confirmation of the mechanism for this loss of activity is provided by the insignificant displacement of [^{3}H]-PGI_2 from its receptor by 6-oxo-$PGF_{1\alpha}$. This result was predicted by the finding that 6-oxo-$PGF_{1\alpha}$ neither inhibited the PGI_2-dependent activation of adenylate cyclase, nor itself activated the enzyme in these cells.

Comparison of selected prostaglandins as inhibitors of [^{3}H]-PGI_2 binding, revealed a rank order of potency that was similar to that observed in a comparison of the same prostaglandins as activators of adenylate cyclase in NCB-20 cell homogenates (Blair *et al.*, 1980a). From Table 1 it is apparent however that for each prostaglandin, with the exception of 6β-PGI_1, the K_{act} value for adenylate cyclase activation is numerically greater than the K_i value for inhibition of [11β-^{3}H]-PGI_2 binding. The reduced affinity of ligand-receptor interactions under the conditions of the adenylate cyclase assay may be attributed to the presence of GTP. Occupation of the GTP binding protein (N), that is coupled to the receptor (R), but not the catalytic subunit (C) of adenylate cyclase, results in a decrease in the affinity of the ligand-receptor complex (Rodbell, 1980). The significance of the different structural substitutions on the parent compound (prostanoic acid) has already been discussed at length in relation to adenylate cyclase activation (Blair *et al.*, 1980a). The results presented here confirm that the differences in the biological potencies of the prostaglandins tested (K_{act} values in Table 1) are due to differences in their affinities for the PGI_2 receptor. The data in both studies confirm the requirement of the Z-5,6-double bond in PGI_2 (cf. PGI_2 and 6β-PGI_1), and illustrate the spacial similarity of the *trans* double bond of 5,6-*trans*-PGE_2 to the Z-5,6 double bond of PGI_2. The 5,6-*trans* double bond of 5,6-*trans*-PGE_2 conferred a potency 18 times greater than that of the *cis*-isomer (PGE_2). Reduction of the Z-5,6 double bond of PGI_2 to form PGI_1 resulted in significant loss of activity. Conversely, reduction of the 5,6-*trans* double bond of 5,6-*trans*-PGE_2 to form PGE_1 increased significantly the binding to the membrane receptor. The finding that PGE_1 and PGI_2 bind competitively to the same receptor (Figure 5), reduces the likelihood of there being two receptor populations to explain this anomaly. It has been suggested previously (Blair *et al.*, 1980a) that the α-side chain of PGE_1 takes up a conformation that is different from PGE_2, and more closely resembles PGI_2.

The 13, 14-*trans* double bond is of little significance in determining the binding characteristics of these prostaglandins (cf. PGE_1 and 13,14-dihydro-PGE_1), whereas an hydroxyl group at C-15 is an absolute requirement for high affinity binding (cf. PGE_2, 13,14-dihydro-PGE_2 and 13,14-dihydro-15-oxo-PGE_2). Furthermore, the absolute configuration of the 15S-hydroxyl group is critical (cf. PGE_2 and 15-*epi*-PGE_2). The configuration of the α-side chain is not an absolute requirement for the binding of PGE_1. Significant activity is retained by 8-*iso*-PGE_1 in which the two side chains now possess a *cis* relationship to each other. Reduction of the 9-oxo-function of the E-prostaglandins to form the F-prostaglandins (cf. PGE_1, PGE_2, $PGF_{1\alpha}$ and $PGF_{2\alpha}$) resulted in loss of activity. Subsequent oxidation of the 11-hydroxyl group did not restore activity (cf. PGE_1, PGD_1).

In conclusion, the results provide evidence for a high affinity PGI_2 receptor which is expressed in a

neuronal somatic cell hybrid. The receptor mediates activation of adenylate cyclase, and interacts with the GTP binding protein and adenylate cyclase in a manner similar to that observed in other neurotransmitter and hormonal systems. Adenylate cyclase activation and [^{3}H]-PGI_2 binding have delineated the characteristics of the interaction of PGI_2 and its receptor. Structure-activity relationships have been identified for the interaction of several prostaglandins with this PGI_2 receptor.

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