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DESENSITIZATION OF ADENYLATE CYCLASE RESPONSES FOLLOWING EXPOSURE TO IP PROSTANOID RECEPTOR AGONISTS

HOMOLOGOUS AND HETEROLOGOUS DESENSITIZATION EXHIBIT THE SAME TIME COURSE

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Abstract—Pretreatment of NG108-15 cells with 0.03–25 μM prostaglandin E_1 (PGE_1) produced decreases in the maximal stimulation of adenylate cyclase activity produced by iloprost, *N*-ethylcarboxamidoadenosine and sodium fluoride. The rate of desensitization to all three agents was dependent on the concentration of PGE_1 used, but at each concentration of PGE_1 the rate of loss of responsiveness to each agent was the same, suggesting that the decreases in responsiveness may be mediated by a single process. Functional desensitization was accompanied by a decrease in the specific binding of [^{3}H]iloprost, consistent with a 75–80% decrease in IP receptor number, with no change in the coupling of the remaining IP receptors to G protein. At each concentration of PGE_1 used, the times taken for half maximal decreases in receptor number and functional responsiveness were similar, suggesting that IP receptor down-regulation is a relatively early event in desensitization. IP receptor down-regulation could be inhibited partially by 100 μM chloroquine, suggesting that lysosomal breakdown of receptors may be occurring.

Key words: iloprost; prostaglandin E_1; G_s; down-regulation

Prolonged exposure of NG108-15 neuroblastoma × glioma cells to IP prostanoid receptor (prostacyclin receptor) agonists such as iloprost and PGE_1‡ produces a pronounced loss of responsiveness to IP receptor agonists which is accompanied by a loss of IP receptors [1, 2]. Pretreatment with IP receptor agonists also produces heterologous desensitization, in which the responses to other stimulators of adenylate cyclase, such as the adenosine A_2 receptor agonist NECA and sodium fluoride, which activates adenylate cyclase via a direct activation of guanine nucleotide binding proteins [3], are also reduced [2, 4]. This heterologous desensitization is accompanied by loss of the stimulatory guanine nucleotide binding protein, G_s [2, 5].

Heterologous desensitization and down-regulation of $G_{s\alpha}$ do not inevitably accompany receptor desensitization and appear to be dependent both on the desensitizing agonist used and on cell type. Pretreatment of NG108-15 cells with adenosine A_2 receptor agonists produces a homologous loss of A_2 receptor responses, but responses to IP receptor agonists and NaF are unaffected [4, 6]. Similarly, pretreatment of NG108-15 cells with forskolin, or pretreatment of the closely related NCB20 cell line with IP receptor agonists, produces desensitization at receptor level, with no effect on G_s [2, 7].

In this study we have attempted to elucidate further the mechanisms which underlie these desensitization phenomena by examining the time course of homologous and heterologous desensitization following pretreatment of NG108-15 cells with IP receptor agonists, and investigating whether internalization of IP receptors and/or G_s accompany these processes.

MATERIALS AND METHODS

Materials. Iloprost was a generous gift from Schering AG, Berlin. Cell culture media were obtained from Gibco (Uxbridge, U.K.) and, unless otherwise indicated, all other drugs and biochemicals were obtained from the Sigma Chemical Co. or BDH Chemicals (both Poole, U.K.).

Cell culture. NG108-15 is a neuroblastoma × glioma somatic hybrid cell line derived from a fusion of the 6-thioguanine-resistant N18 TG2 mouse neuroblastoma with 5-bromodeoxyuridine-resistant rat glioma cells, C6 BU-1 (see Ref. 8). Cells (passage 16–25) were cultured in DMEM containing 10% v:v) foetal calf serum and supplemented with 1 μM aminopterin, 100 μM hypoxanthine and 16 μM thymidine as a hybrid selection medium. Culture flasks (80 cm^2) were maintained at 37° in a humidified atmosphere of 10% CO_2 and 90% air.

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‡ Abbreviations: DMEM, Dulbecco's modified Eagle's medium; GppNHp, 5′-guanylylimidodiphosphate; G_s, the stimulatory guanine nucleotide binding protein; $G_{s\alpha}$, the α-subunit of G_s; NECA, *N*-ethylcarboxamidoadenosine; PGE_1, prostaglandin E_1.

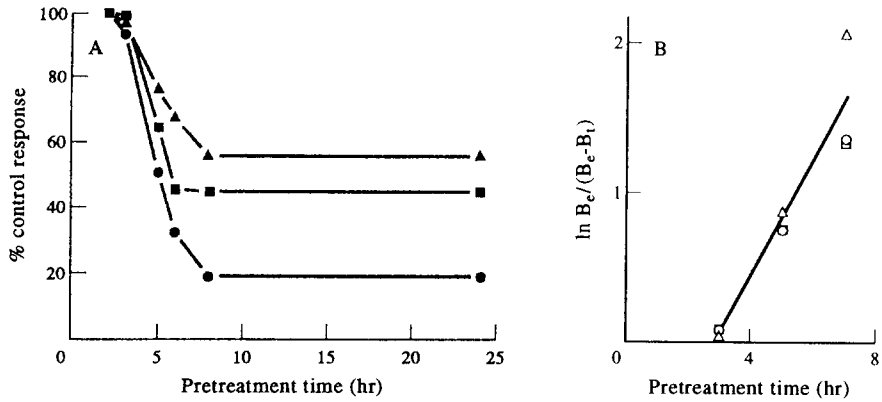

Fig. 1. Time courses of desensitization following pretreatment of NG108-15 cells with 25 μM PGE_1. NG108-15 cells were pretreated with 25 μM PGE_1 or vehicle for the times indicated and the cells were then harvested and washed as described in Materials and Methods. (A) Stimulation of adenylate cyclase activity produced by 1 μM iloprost (circles), 100 μM NECA (squares) and 10 mM NaF (triangles) in membranes from PGE_1-treated cells expressed as a percentage of the response obtained in control cell membranes. Each point is the mean of three determinations from a single experiment, representative of three. (B) Rate plot of the same data where B_t is the percentage decrease in response at time t and B_e is the maximal decrease in response, achieved after pretreatment for 24 hr.

In pretreatment studies, the growth medium was removed from confluent cells and replaced with DMEM containing the stated concentration of IP receptor agonist iloprost or PGE_1 or the appropriate vehicle. The cells were returned to the incubator for between 1 and 24 hr as stated and then washed in Dulbecco's PBS to remove the desensitizing agent. The cells were pelleted by centrifugation at 200 g for 2 min and frozen at −70° until required. Different washing procedures were employed for the different desensitizing agents. In order to remove all traces of iloprost, cells were first harvested by agitation in PBS and then centrifuged and resuspended three times in warm PBS before being pelleted and frozen. PGE_1 was removed by rapidly rinsing the monolayer of cells twice with cold PBS. The cells were harvested, pelleted and frozen, or, in order to investigate recovery from desensitization, fresh DMEM was added and the cells returned to the incubator. As PGE_1 could be removed from the cells much more quickly than iloprost, PGE_1 was used in the time course experiments.

In order to investigate the effects of 100 μM chloroquine on desensitization, cells were incubated in DMEM in the presence or absence of chloroquine for 30 min prior to the addition of 10 μM iloprost or vehicle.

Adenylate cyclase. Adenylate cyclase activity was measured as described previously [2]. Cell pellets were thawed and homogenized immediately before use and the conversion of [α-^{32}P]ATP (Amersham International, Amersham, U.K.) to [^{32}P]cAMP was measured after a 15 min incubation at 37°. The [^{32}P]cAMP was separated from [α-^{32}P]ATP by a two-step chromatographic procedure [9] and [^{3}H]-cAMP was included as an internal recovery standard. Protein was measured using the Lowry method [10] with BSA as standard.

[^{3}H]*Iloprost binding.* The binding of [^{3}H]iloprost (Amersham) to NG108-15 cell homogenates was assayed using a modification of the method described previously [2]. Crude cell homogenates (300–800 μg protein/sample) were routinely incubated for 30 min at room temperature in Tris–HCl, pH 7.4, containing 5 mM $MgCl_2$ in the presence of 1 or 10 nM [^{3}H]-iloprost. Each incubation was performed in the absence or presence of 100 μM GppNHp and 10 μM unlabelled iloprost was used to define non-specific binding. In self-competition experiments, cell homogenates were incubated with 5–10 nM [^{3}H]-iloprost in the absence or presence of 0.1 nM–10 μM unlabelled iloprost, as indicated. At the end of the incubation the samples were filtered through Whatman GF/B glass fibre filters and washed three times with 3.5 mL ice-cold buffer using a Brandel cell harvester. Protein was measured using the Lowry method [10].

Statistics. Data are presented as means ± SEM. Differences were compared using paired or unpaired t-tests, as appropriate, and were considered significant if P was less than 0.05.

RESULTS

Effect of PGE_1 pretreatment on adenylate cyclase responses

Pretreatment of NG108-15 cells with 30 nM–25 μM PGE_1 led to a time-dependent decrease in the responses to maximal concentrations of iloprost (1 μM), NECA (100 μM) and NaF (10 mM). With the concentration range used in these experiments, a maximal loss of response had occurred after 24 hr, and the maximal level of desensitization did not appear to depend on the concentration of the desensitizing agonist used. Following pretreatment with PGE_1, the mean reduction in the response to 1 μM iloprost was 81.1 ± 6.7%, which produced an increase over basal activity of 66.3 ± 21.4 pmol

cAMP/min·mg protein in membranes from control cells and 19.8 ± 12.2 pmol cAMP/min·mg protein in membranes from treated cells; the mean reduction in the response to 10 mM NaF was 44.6 ± 23.1%, from 35.1 ± 16.1 pmol cAMP/min·mg protein in membranes from control cells to 19.7 ± 12.3 pmol cAMP/min·mg protein in membrane from treated cells; the mean reduction in the response to 100 μM NECA was 40.0 ± 17.1%, from 25.5 ± 12.9 pmol cAMP/min·mg protein in membranes from control cells to 14.8 ± 5.7 pmol cAMP/min·mg protein in membranes from treated cells (all values means ± SEM, N = 11).

Pretreatment of NG108-15 cells for 17 hr with 10 μM iloprost produced the same pattern of desensitization as PGE_1. The mean reduction in the response to 1 μM iloprost was 75.7 ± 6.3%, which produced an increase above basal activity of 78.7 ± 16.4 pmol cAMP/min·mg protein in membranes from control cells and 19.1 ± 3.6 pmol cAMP/min·mg protein in membranes from treated cells; the mean reduction in the response to 10 mM NaF was 36.4 ± 8.7%, from 39.8 ± 9.5 pmol cAMP/min·mg protein in membranes from control cells to 25.4 ± 9.4 pmol cAMP/min·mg protein in membranes from treated cells; the mean reduction in the response to 100 μM NECA was 39.4 ± 7.2%, from 34.6 ± 10.5 pmol cAMP/min·mg protein in membranes from control cells to 19.7 ± 3.7 pmol cAMP/min·mg protein in membranes from treated cells (all values means ± SEM, N = 7).

Pretreatment of NG108-15 cells with either PGE_1 or iloprost did not produce any statistically significant effect on basal adenylate cyclase activity. Following pretreatment with PGE_1, basal activity was 13.7 ± 7.4 pmol cAMP/min·mg protein, compared to 15.7 ± 7.6 pmol cAMP/min·mg protein in membranes from control cells (mean ± SEM, N = 11). Following pretreatment with iloprost, basal activity was 19.6 ± 10.4 pmol cAMP/min·mg protein, compared to 22.1 ± 9.0 pmol cAMP/min·mg protein in membranes from control cells (mean ± SEM, N = 7).

First order rate plots of the time courses for desensitization were linear (Fig. 1) and a single exponential with the addition of an initial lag phase was fitted to the data. The rate of desensitization was dependent on the concentration of PGE_1 used when desensitization rate was assessed either as the rate constant for the exponential phase or as the time taken for half maximal desensitization to be achieved (i.e. $T_{1/2}$ + lag phase). The length of the lag phase did not appear to be dependent on the concentration of PGE_1 used (Table 1). Although the maximal losses of responsiveness to iloprost, NaF and NECA were not the same, the rate of loss of responsiveness to all three agents appeared to be the same at all concentrations of PGE_1 used, and this was true whether the rate was assessed as the rate constant for the exponential phase of desensitization or as the time taken for half maximal desensitization to be achieved (Fig. 1, Table 1, Fig. 2).

Effect of PGE_1 pretreatment on [^{3}H]iloprost binding

Pretreatment of NG108-15 cells with 30 nM–25 μM

Table 1. Comparison of the half time ($T_{1/2}$) and lag time for the decrease in responsiveness to iloprost (1 μM), NaF (10 mM) and NECA (100 μM) and IP receptor number (IP-R no.) following pretreatment of NG108-15 cells with 0.03–25 μM PGE_1

PGE_1 (μM)	Response	$T_{1/2}$ (hr)	Lag time (hr)
25	Iloprost	1.7 ± 0.2	1.3 ± 0.8
	NaF	1.9 ± 1.0	1.1 ± 0.9
	NECA	2.3 ± 0.7	1.0 ± 0.9
	IP-R no.	4.1 ± 0.8	—
5	Iloprost	2.9 ± 1.7	2.1 ± 0.5
	NaF	3.5 ± 0.5	1.9 ± 0.4
	NECA	3.8 ± 0.4	1.9 ± 0.8
	IP-R no.	5.4 ± 1.4	—
1	Iloprost	5.2 ± 0.8	2.9 ± 0.2
	NaF	5.2 ± 0.8	2.9 ± 0.3
	NECA	5.2 ± 0.7	3.8 ± 1.2
	IP-R no.	7.3 ± 3.3	—
0.1	Iloprost	6.7 ± 1.1	2.1 ± 0.5
	NaF	5.9 ± 1.2	2.9 ± 0.3
	NECA	6.3 ± 0.3	2.6 ± 0.9
	IP-R no.	9.1 ± 0.7	—
0.03	Iloprost	12.0 ± 2.7	1.5 ± 0.6
	NaF	9.1 ± 2.4	1.7 ± 0.3
	NECA	12.6 ± 7.9	1.8 ± 0.3
	IP-R no.	ND	ND

Experiments were carried out as described in Materials and Methods and the $T_{1/2}$ and lag time values were obtained as explained in the legend to Fig. 1.

All values are means ± SEM of three independent determinations.

ND, not determined.

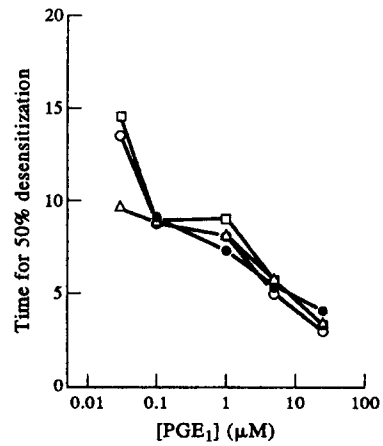

Fig. 2. Comparison of the time taken for a half maximal decrease in responsiveness to iloprost (1 μM; ○), NaF (10 mM; △) and NECA (100 μM; □) and IP receptor number (●) following pretreatment of NG108-15 cells with 0.03–25 μM PGE_1. Experiments were carried out as described in Materials and Methods. Data points represent the total time taken for a half maximal decrease to be achieved, i.e. $T_{1/2}$ + lag time, which were obtained as explained in the legend to Fig. 1. Each point represents the mean of three independent determinations.

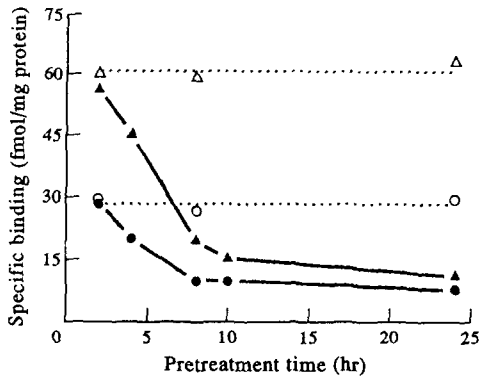

Fig. 3. Time course of the decrease in specific binding of [^{3}H]iloprost binding following pretreatment of NG108-15 cells with 25 μM PGE_1. The specific binding of 10 nM [^{3}H]-iloprost to membranes from NG108-15 cells pretreated for various times with 25 μM PGE_1 (closed symbols) or vehicle (open symbols). Binding was measured in the absence (triangles) or the presence (circles) of 100 μM GppNHp. Each point is the mean of duplicate determinations from a single experiment, representative of three.

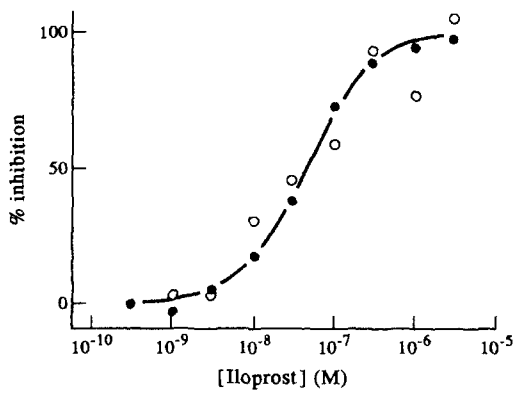

Fig. 4. Effect of PGE_1 pretreatment on iloprost binding curves in NG108-15 cell homogenates. NG108-15 cells were pretreated for 6 hr with vehicle (●) or 5 μM PGE_1 (○) and binding assays were carried out in the absence of GppNHp, as described in Materials and Methods. Each data point represents the % inhibition of the specific binding of 7.5 nM [^{3}H]iloprost, which was 48 fmol/mg protein in control cell homogenates and 21 fmol/mg protein in treated cell homogenates. Each point is the mean of duplicate determinations from a single experiment, representative of three.

PGE_1 led to a time-dependent decrease in the specific binding of [^{3}H]iloprost measured both in the absence and presence of 100 μM GppNHp (Fig. 3). However, self-competition curves obtained for the inhibition of the specific binding of [^{3}H]iloprost by unlabelled iloprost in homogenates from control and PGE_1-treated cells suggest that pretreatment with PGE_1 did not affect the affinity of iloprost for its binding sites (Fig. 4).

The binding of [^{3}H]iloprost to NG108-15 cell membranes is characteristic of agonist binding to a G protein-coupled receptor in that it is sensitive to guanine nucleotides: GTP or its non-hydrolysable analogues produce a shift to the right and a steepening of the iloprost binding curve, consistent with conversion of high affinity receptor–G protein complexes to low affinity, uncoupled receptors [11]. In this study we have measured the specific binding of two concentrations of [^{3}H]iloprost, 1 and 10 nM, in the absence or presence of 100 μM GppNHp in an attempt to distinguish changes in IP receptor number from changes in receptor–G protein coupling or gross changes in [^{3}H]iloprost binding affinity. Specific binding measured in the presence of GppNHp should reflect predominantly binding to uncoupled receptors. Pretreatment with PGE_1 led to a decrease in the specific binding of [^{3}H]iloprost measured in the presence of GppNHp and the percentage decrease was the same at both concentrations of [^{3}H]iloprost (data not shown), suggesting that the decrease was due to a loss of IP receptors with no change in the binding affinity of [^{3}H]iloprost. Thus, pretreatment with PGE_1 appeared to produce IP receptor down-regulation.

The time courses of IP receptor down-regulation could be well described by a single exponential and no lag phase was apparent (Fig. 3, Table 1). The rate of receptor down-regulation was dependent on the concentration of PGE_1 used (Table 1, Fig. 2), but the maximal decrease in IP receptor number produced by pretreatment with PGE_1 or iloprost appeared to be the same. Following pretreatment with 1 μM PGE_1 for 24 hr the specific binding of 10 nM [^{3}H]iloprost measured in the presence of GppNHp was reduced by 79.2 ± 7.0% (mean ± SEM, N = 4) and following pretreatment for the same period with 10 μM iloprost the specific binding of 10 nM [^{3}H]iloprost measured in the presence of GppNHp was reduced by 75.9 ± 14.3% (mean ± SEM, N = 3).

Throughout the time course of desensitization, the specific binding of 1 and 10 nM [^{3}H]iloprost measured in the absence of GppNHp was always greater than the specific binding measured in the presence of GppNHp (Fig. 2). This difference in specific binding presumably reflects the coupling of IP receptors to G_s. In the absence of GppNHp, a proportion of the IP receptors were coupled to G_s and thus had a higher affinity for [^{3}H]iloprost than the uncoupled receptors. In the presence of GppNHp these complexes were disrupted, which reduced the amount of [^{3}H]iloprost bound. Thus it seems that IP receptors in NG108-15 cell membranes were capable of an interaction with G_s following pretreatment with PGE_1. Furthermore, the degree of receptor–G protein coupling, expressed as the percentage increase in specific binding of [^{3}H]iloprost in the absence of GppNHp compared to the specific binding in its presence, was the same in membranes from cells which had been pretreated for 24 hr with PGE_1 as in membranes from control cells. Using 10 nM [^{3}H]iloprost, the increase in specific binding in the absence compared with the presence of GppNHp was 106.7 ± 15.6% in control membranes and 115.4 ± 17.4% in membranes from cells which had

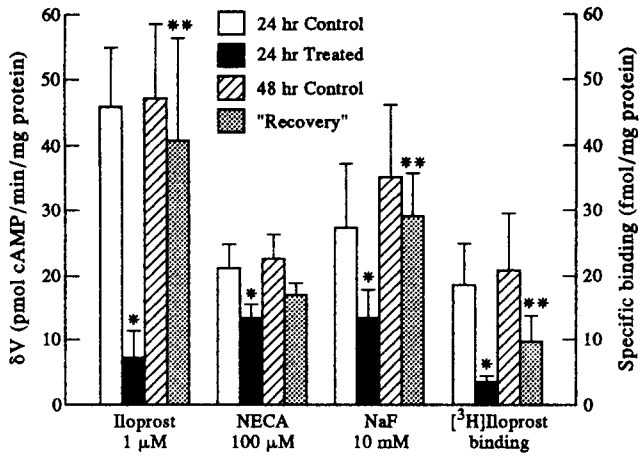

Fig. 5. Recovery of NG108-15 cells from the effects of pretreatment with PGE_1. Cells were pretreated for 24 hr in the absence (24 hr control and 48 hr control) or presence (24 hr treated and "recovery") of 1 μM PGE_1. After 24 hr, the PGE_1 was removed as described in Materials and Methods and the cells were either harvested immediately (24 hr control and 24 hr treated) or cultured for a further 24 hr in DMEM alone (48 hr control and "recovery"). The bars represent the increase in adenylate cyclase activity produced by 1 μM iloprost, 100 μM NECA and 10 mM NaF and the specific binding of 10 nM [^{3}H]iloprost measured in the presence of 100 μM GppNHp; all values means ± SEM from three separate experiments. *Indicates that the value obtained in membranes obtained from PGE_1 was significantly different ($P < 0.05$, in a paired t-test) from the relevant control value and ** indicates that the value obtained in membranes from cells which had undergone "recovery" was significantly different ($P < 0.05$, in a paired t-test) from the value obtained in cells harvested immediately after exposure to PGE_1.

been pretreated for 24 hr with PGE_1; using 1 nM [^{3}H]iloprost, the increase in specific binding in the absence compared with the presence of GppNHp was 271 ± 72% in control and 246 ± 71% in PGE_1-treated cell membranes (all values means ± SEM, N = 16).

The effects of pretreatment with PGE_1 appeared to be reversible. The decrease in the specific binding of [^{3}H]iloprost and the loss of responsiveness to iloprost, NECA and NaF produced by pretreatment with 1 μM PGE_1 for 24 hr were substantially restored 24 hr after the removal of the desensitizing agent (Fig. 5).

The loss of [^{3}H]iloprost binding sites induced by iloprost pretreatment could be partially inhibited by 100 μM chloroquine. Following pretreatment for 17 hr with 10 μM iloprost the specific binding of 10 nM [^{3}H]iloprost measured in the presence of 100 μM GppNHp was 1.7 ± 1.3 fmol/mg protein when cells had been pretreated in the absence of chloroquine and 5.9 ± 2.1 fmol/mg protein when cells had been pretreated in its presence. However, chloroquine pretreatment alone reduced the specific binding of [^{3}H]iloprost to NG108-15 cell homogenates from 31.3 ± 13.5 fmol/mg protein in control cell membranes to 18.0 ± 7.2 fmol/mg protein in membranes from chloroquine-treated cells (all values means ± SEM, N = 3).

DISCUSSION

Prolonged pretreatment of NG108-15 cells with either PGE_1 or iloprost produced the same pattern of desensitization: an 80% reduction in the maximal response to iloprost, about 40% reductions in the maximal responses to NECA and NaF and a decrease in the specific binding of [^{3}H]iloprost which was consistent with a 75–80% decrease in IP receptor number with no change in the ability of the remaining receptors to interact with G_s. Desensitization is likely to have resulted as a consequence of activation of IP prostanoid receptors by PGE_1 and iloprost. Both agonists can also produce activation of EP prostanoid receptors, particularly the EP_1 subtype [12], but NG108-15 cells do not seem to express significant levels of these receptors [11].

The loss of functional responses and IP receptors was time dependent and the rate of decrease depended on the concentration of PGE_1 used, although the maximal level of desensitization or receptor loss achieved appeared to be the same under the conditions of these experiments. The time courses for the decrease in responsiveness to iloprost, NaF and NECA following pretreatment with PGE_1 could be well described by a single exponential preceded by an initial lag phase, whereas no lag phase appeared to precede the loss of IP receptors. The significance of the lag phase is unclear, but one possibility is that it reflects a degree of reserve capacity in the system, such that the number of functional IP receptors and/or G_s molecules may be somewhat reduced without reducing the maximal response to iloprost, NaF or NECA.

The amount of [^{3}H]iloprost binding obtained in the present study is consistent with previous estimates of the number of IP receptors in NG108-15 cell membranes of around 100 fmol/mg protein [2, 11]. Nevertheless, some other studies have suggested

that NG108-15 cells express a more than 10-fold higher density of IP receptors [13, 14]. It is possible that these differences can in part be explained by the use of different membrane preparations (washed membranes as opposed to crude homogenates) or genuine differences in receptor density between cells grown under slightly different conditions. However, the main reason for the apparent discrepancy between IP receptor density in different studies probably lies in the way the binding data is analysed. [^{3}H]Iloprost is the best available radioligand for the IP receptor, but it suffers from a major disadvantage: at concentrations above 30 nM, an appreciable component of the displaceable binding is to a very high capacity, low affinity, non-receptor site [2]. Thus if binding curves are analysed according to a model which does not incorporate this non-receptor site, they yield an artefactually high estimate of IP receptor number.

A striking feature of the time courses of desensitization is that at every concentration of PGE_1 the rates of the loss of responsiveness to iloprost, NaF and NECA were the same. This provides strong circumstantial evidence that the homologous decrease in responsiveness to iloprost and the heterologous decrease in responsiveness to NECA and NaF may be mediated by a single process.

A comparison of the rate constants of IP receptor down-regulation with the rate constants of the exponential phase of the decrease in responses to iloprost, NaF and NECA suggests that the functional desensitization may precede the loss of IP receptors. However, if the lag phase preceding the decrease in responsiveness is taken into account by comparing the time taken for a half maximal decrease in response or IP receptor number, the rates of these processes appear to be very similar. This tends to suggest that receptor down-regulation may be a relatively early event in desensitization of this system. Adie *et al.* [14] have recently demonstrated that down-regulation of IP receptors and $G_{s\alpha}$ occur at the same rate during pretreatment of NG108-15 cells with PGE_1 or iloprost. Thus, down-regulation of $G_{s\alpha}$ is also a relatively early event in desensitization and the close temporal correlation between $G_{s\alpha}$ down-regulation and the loss of responsiveness to NECA and NaF suggests that heterologous desensitization may well be mediated by a loss of functional G_s from the cell membrane.

MacKenzie and Milligan [5] have demonstrated that the loss of $G_{s\alpha}$ that occurs when NG108-15 cells are exposed to PGE_1 is not mediated by a decrease in transcription of $G_{s\alpha}$ mRNA, but reflects increased breakdown of this protein. It therefore seems likely that the decrease in IP receptor number is also mediated by increased breakdown, although decreased receptor synthesis cannot be ruled out at this stage.

In other systems, receptor down-regulation occurs subsequent to the internalization of the receptors. Both IP receptors and $G_{s\alpha}$ are down-regulated following pretreatment of NG108-15 cells with IP receptor agonists [2, 5] and it seems likely that both may undergo internalization. Furthermore, as the homologous desensitization of responses to IP receptor agonists and the heterologous decrease in responsiveness to other activators of G_s appear to be mediated by a single process, one attractive hypothesis is that the initial event in desensitization is the co-internalization of IP receptors and G_s. This possibility tends to be supported by the slowness of desensitization, which is very similar to the rate of δ-opiate receptor internalization in NG108-15 cells [15], and by the fact that IP receptors do not undergo the rapid uncoupling from G_s which is the initial event in β-adrenoceptor desensitization.

Chloroquine has been shown to inhibit down-regulation of receptors in a number of systems, where it acts by inhibiting the breakdown of internalized receptors by raising the pH within lysosomes [15, 16]. Chloroquine tended to inhibit IP receptor down-regulation in the present study and has also been shown to inhibit partially the down-regulation of $G_{s\alpha}$ following pretreatment of NG108-15 cells with iloprost [17]. Thus, these data tend to support the hypothesis that down-regulation of IP receptors and $G_{s\alpha}$ occurs as a result of internalization and lysosomal breakdown. However, as chloroquine also reduced the level of [^{3}H]iloprost binding sites in membranes from control cells, the effects of chloroquine cannot be attributed unequivocally to inhibition of lysosomal breakdown. Therefore, it remains possible that the down-regulation of IP receptors and/or $G_{s\alpha}$ in NG108-15 cells may not involve proteolysis of internalized molecules in lysosomes.

Thus, while the mechanism of desensitization of IP receptors and G_s has not been conclusively demonstrated, these results indicate that homologous and heterologous desensitization are likely to be mediated by a single process and we propose that the most likely event remains the co-internalization of receptors and G_s. Furthermore, these studies have highlighted some important differences between IP receptor desensitization in NG108-15 cells and the best characterized system of desensitization of a G protein-coupled receptor, the β-adrenoreceptor system. The desensitization produced by PGE_1 was much slower than desensitization of the β-adrenoreceptor. The time taken to achieve half maximal desensitization was about 4 hr with 25 μM PGE_1, the highest concentration used, whereas responses to β-adrenoreceptor agonists may be markedly desensitized following a few minutes exposure to β-adrenoreceptor agonists [18, 19]. IP receptors do not appear to be uncoupled from G_s during desensitization, whereas exposure to β-adrenoreceptor agonists produces a rapid uncoupling of the β-adrenoreceptor from G_s which is the initial event in desensitization [18, 20, 21]. Thus, during desensitization of the β-adrenoreceptor, the decrease in responsiveness occurs long before any decrease in the number of receptors [18, 20, 21], whereas in the IP receptor system receptor down-regulation appears to occur very soon after functional desensitization. These differences suggest that IP receptor desensitization in NG108-15 cells may be mediated by mechanisms different from those that have been elucidated for the β-adrenoreceptor.

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