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Chronic exposure of NG108-15 cells to inhibitory acting drugs reduces stimulatory prostaglandin E_1 receptor number

Hermann Ammer *, Rüdiger Schulz

Institute of Pharmacology, Toxicology and Pharmacy, University of Munich, Königinstraße 16, 80539 München, Germany

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Abstract

Prolonged exposure of neuroblastoma × glioma (NG108-15) hybrid cells to inhibitory acting drugs results in sensitization of adenylate cyclase. We now report that chronic activation (3 days) of either inhibitory δ-opioid receptors, α_{2B}-adrenoceptors, or muscarinic M_4 receptors significantly decreases the number of stimulatory, adenylate cyclase-coupled prostaglandin E_1 receptors. Pharmacological characterization further revealed that the loss of [^{3}H]prostaglandin E_1- binding sites most likely corresponds to a reduction of the number of high-affinity, G protein-coupled prostaglandin E_1 receptors. The decline in functionally active prostaglandin E_1 receptors developed in a time- and dose-dependent manner and could be prevented by pretreatment of the cells with pertussis toxin. Heterologous prostaglandin E_1 receptor regulation was blocked by concomitant exposure of the cells to antagonists for inhibitory receptors and was rapidly reversed ($t_{1/2} < 30$ min) upon termination of chronic inhibitory drug treatment. The decrease in high-affinity prostaglandin E_1 receptors developed regardless of whether full or partial agonists were used for pretreatment. In addition, the concentrations of inhibitory drugs required to maximally affect prostaglandin E_1 receptor number closely resembled those mediating maximal adenylate cyclase inhibition. The data demonstrate that chronic inhibitory drug treatment of NG108-15 hybrid cells reduces the number of functionally active, excitatory prostaglandin E_1 receptors. Thus, it is proposed that adaptations at the level of stimulatory receptor systems contribute to the regulatory mechanisms associated with drug dependence.

Keywords: Adenylate cyclase; α_{2B}-Adrenoceptor; Muscarinic M_4 receptor; δ-Opioid receptor; Prostaglandin E_1 receptor; Receptor cross-talk

1. Introduction

Neuroblastoma × glioma (NG108-15) hybrid cells are frequently used to study acute and chronic aspects of transmembrane signalling processes, since they express a wide variety of different receptors, G proteins and effectors (for review, see Milligan et al., 1990). Among these, the δ-opioid receptors, α_{2B}-adrenoceptors, and muscarinic M_4 receptor systems are coupled to the inhibition of adenylate cyclase (Graeser and Neubig, 1993) via pertussis toxin-sensitive G proteins (Kurose et al., 1983), which, in the case of δ-opioid receptors and α_{2B}-adrenoceptors, has been demonstrated to be of the G_i2 type (McClue and Milligan, 1990; McKenzie and Milligan, 1990). Besides the acute inhibitory effect of these receptors on adenylate cyclase activity, long-term activation of either one results in sensitization of adenylate cyclase as characterized by the production of a cAMP overshoot phenomenon upon withdrawal of the drug (Nathanson et al., 1978; Sabol and Nirenberg, 1979; Sharma et al., 1975). This transient increase in intracellular cAMP levels is generally accepted to represent a cellular correlate for drug dependence (Thomas and Hoffman, 1987).

The adenylate cyclase effector system is under dual control of both inhibitory and stimulatory receptor systems (Gilman, 1987). Due to this functional interdependence, cross-regulation between adenylate cyclase-coupled signal transduction pathways has been reported for several cell systems in response to chronic treatment with agonists for either inhibitory or stimulatory receptors (Hadcock et al., 1991; Morris et al., 1991; Reithmann et al., 1992; Paraschos and Karliner, 1994). In this context, we could recently demonstrate chronic opioid induced adaptations within the stimulatory prostaglandin E_1 receptor system in NG108-15 hybrid cells (Ammer and Schulz, 1993). The individual

* Corresponding author. Tel.: (49) (89) 21 80 – 26 66; fax: (49) (89) 34 23 16.

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regulatory changes were found to comprise a paradoxical loss of [^{3}H]prostaglandin E_1 binding sites, which is compensated by a concomitant increase in functional coupling efficiency between prostaglandin E_1 receptors and their associated stimulatory G proteins, leading to an overall enhanced stimulatory adenylate cyclase activity as observed during the state of opioid dependence (Ammer and Schulz, 1995)

The aim of the present study was to investigate heterologous prostaglandin E_1 receptor regulation more closely by chronic inhibitory drug treatment of NG108-15 hybrid cells. Our results indicate that the decrease in [^{3}H]prostaglandin E_1 binding sites following chronic opioid treatment most likely corresponds to a reduction in the number of high-affinity, G protein-coupled prostaglandin E_1 receptors. The rapid recovery of prostaglandin E_1 receptor number upon termination of chronic opioid treatment further suggests sequestration rather than true down-regulation of the receptors as the underlying regulatory mechanism. In addition, we further characterized regulation of stimulatory receptors as a general adaptive phenomenon associated with chronic inhibitory drug action in NG108-15 hybrid cells, since chronic treatment with agonists for a variety of inhibitory receptor types, such as δ-opioid, α_{2B}-adrenergic, and muscarinic M_4 receptors, produced a similar effect at stimulatory prostaglandin E_1 receptors.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM) and hypoxanthine/aminopterin/thymidine (HAT) supplement were from GIBCO/BRL, fetal calf serum was from PAN Systems (Aidenbach, Germany). [5,6(n)-^{3}H]Prostaglandin E_1 (47 Ci/mmol) was obtained from Amersham Buchler. The following ligands were used: [D-Ala^2,D-Leu^5]enkephalin (DADLE), clonidine HCl, naloxone HCl (Research Biochemicals International), and oxotremorine sesquifumarate (Sigma). Guanosine 5′-(γ-thio)triphosphate (GTPγS) was purchased from Boehringer (Mannheim, Germany), whereas pertussis toxin, and indomethacin were from Sigma. All other chemicals were from local sources and of reagent grade or better.

2.2. Cell culture

NG108-15 hybrid cells were grown as monolayers in DMEM, supplemented with 5% fetal calf serum, 100 μM hypoxanthine, 1 μM aminopterin, and 16 μM thymidine, at 37°C in a humified atmosphere of 5% CO_2 and 95% air. Stock cultures were maintained in 150-cm^2 tissue culture flasks in the presence of 40 ml medium. At 80% confluency, the cells were split in the ratio 1 : 10, incubated overnight in growth medium, and were then chronically treated for a further 3 days with either the δ-opioid receptor agonist, DADLE (1 μM), the low-potency opiate, morphine (10 μM), the muscarinic acetylcholine receptor agonist, oxotremorine (10 μM), or the α_2-adrenoceptor ligand, clonidine (10 μM). In some experiments cells were exposed to the opioid antagonist, naloxone (10 μM, 3 days), alone or together with DADLE (1 μM). To investigate the effect of pertussis toxin pretreatment on prostaglandin E_1 receptor down-regulation, cells were incubated in serum-free medium for 16 h followed by a further 3-day exposure to either pertussis toxin (16 ng/ml) alone or to pertussis toxin together with DADLE (1 μM). In each experiment untreated cells of the same passage served as control. At the end of pretreatment, the cells were harvested in ice-cold phosphate-buffered saline (PBS; 154 mM NaCl, 0.61 mM Na_2HPO_4, 0.38 mM KH_2PO_4, pH 7.4), washed twice (10 min, 300 × g), and stored at −70°C until use.

2.3. Determination of prostaglandin E_1 receptor levels

Radioligand binding studies were performed in a heavy membrane preparation obtained as follows with all steps performed at 4°C. Cell pellets (~ 1.5×10^7 cells) were thawed, resuspended in 10 ml ice-cold homogenization buffer (50 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$, 1 mM benzamidine), and lysed for 20 s using a Polytron (Brinkman Instruments, setting 6). Unbroken cells and nuclei were removed by centrifugation at 500 × g for 10 min. The supernatant fraction was taken and membranes were recovered at 22 000 × g for 30 min. The membranes were washed once in 10 ml of the above buffer (22 000 × g, 30 min) and finally resuspended in assay buffer (10 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$, 100 mM NaCl, 100 μM indomethacin, and 10 mM benzamidine) at a concentration of ~ 2 mg/ml. Homologous displacement studies were performed essentially as described by McGee and Kenimer (1982). Membranes (~ 200 μg protein/tube) were incubated with 8 nM [^{3}H]prostaglandin E_1 tracer and increasing amounts of unlabelled competing prostaglandin E_1 in a total volume of 200 μl for 45 min at 25°C. Nonspecific binding was determined in the presence of 10 μM prostaglandin E_1. Under these assay conditions, only high-affinity binding sites are detected by the agonistic radioligand, [^{3}H]prostaglandin E_1. In some experiments, the stable GTP analogue, guanosine 5′-[γ-thio]triphosphate (100 μM), was included in the reaction mixtures in order to promote uncoupling of prostaglandin E_1 receptors from their associated G proteins (Adie et al., 1992). Binding reactions were terminated by rapid filtration over Whatman GF/B glass fibre filters followed by 3 × 5-ml washes of ice-cold assay buffer lacking benzamidine and indomethacin. The filters were soaked overnight in scintillation fluid and counted at 66% efficiency in a Beckman LS 1801 scintillation system. Protein content was determined by the method of Peterson (1983) using bovine serum albumin as standard.

2.4. Data analysis

The values for prostaglandin E_1 receptor binding parameters were calculated according to the method of DeBlasi et al. (1989) where $B_{max} = B_0 \cdot IC_{50}/L$ and $K_D = IC_{50} - L$ (B_0 = specific binding of the radioligand; L = concentration of radioligand used; IC_{50} = median effective dose of prostaglandin E_1 to inhibit specific [^{3}H]prostaglandin E_1 tracer binding for 50%). IC_{50} values were obtained by nonlinear regression analysis of self-competition curves using 9 different concentrations of the cold ligand. Each data point was determined in duplicate, and the results are presented as mean ± S.E.M. values unless otherwise indicated. Statistical differences were compared using Student's two-tailed t-test for paired data.

3. Results

NG108-15 hybrid cells express large amounts of prostaglandin E_1 receptors (McGee and Kenimer, 1982). The binding of the agonistic radioligand, [^{3}H]prostaglandin E_1 (8 nM tracer concentration), to a heavy membrane preparation of NG108-15 hybrid cells was of high affinity and could be dose-dependently displaced by increasing amounts of unlabelled prostaglandin E_1 (0.1 nM–1 μM). Calculation of the values for binding parameters from self-competition curves by the method of DeBlasi et al. (1989) revealed a maximum binding capacity (B_{max}) of 231.4 ± 32.1 fmol/mg membrane protein and an equilibrium binding constant (K_D) of 2.9 ± 1.1 nM (n = 17) in membranes prepared from naive NG108-15 hybrid cells. Chronic exposure of the cells to DADLE (1 μM, 3 days) largely reduced specific [^{3}H]prostaglandin E_1 binding by some 39% (139.8 ± 22.1 fmol/mg membrane protein) as compared to untreated control cells, without any change in drug affinity (K_D = 2.7 ± 0.9 nM, n = 12) (Fig. 1). To more closely characterize the reduction in specific [^{3}H]prostaglandin E_1 binding to membranes from chronically DADLE-treated NG108-15 hybrid cells, additional binding studies were performed in the presence of the stable guanine-nucleotide analogue GTPγS. Uncoupling of prostaglandin E_1 receptors from their associated G proteins by increasing amounts of GTPγS dose-dependently reduced specific [^{3}H]prostaglandin E_1 binding, indicating that the assay system employed only detects the high-affinity, G protein-coupled fraction of prostaglandin E_1 receptors (Fig. 2). When the number of GTPγS (100 μM)-resistant prostaglandin E_1 binding sites was compared, no difference between naive and chronically DADLE-exposed cells could be observed (45.3 ± 4.8 vs. 54.9 ± 8.9 fmol/mg membrane protein, n = 6). Thus, the decrease in specific [^{3}H]prostaglandin E_1 binding to membranes derived from chronically DADLE-treated NG108-15 hybrid cells most likely corresponds to a loss of high-affinity, G protein-coupled prostaglandin E_1 receptors.

The loss of prostaglandin E_1 binding sites upon chronic DADLE pretreatment turned out to be selectively δ-opioid receptor-mediated, since coincubation of the cells for 3 days with DADLE (1 μM) together with the opioid antagonist naloxone (100 μM) abolished this effect (B_{max} = 251.1 ± 10.3 vs. 245.7 ± 12.4 fmol/mg membrane protein, mean ± S.D. values of two separate experiments).

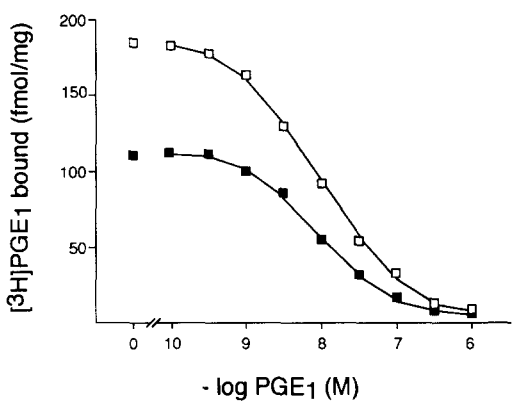

Fig. 1. Specific [^{3}H]prostaglandin E_1 binding to membranes of NG 108-15 hybrid cells. Membranes from control (□) or chronically DADLE (1 μM; 3 days)-treated NG 108-15 hybrid cells (■) were prepared and homologous displacement of a single [^{3}H]prostaglandin E_1 tracer concentration (8.1 nM) with increasing concentrations of unlabelled prostaglandin E_1 was performed as described under Materials and methods. Nonspecific binding was determined in the presence of 10 μM of cold prostaglandin E_1. IC_{50} values were calculated by nonlinear regression analysis and the values for binding parameters were determined by the method of DeBlasi et al. (1989). In the experiment displayed, calculated B_{max} values were 233.7 and 138.5 fmol/mg membrane protein for control and chronically DADLE-exposed cells, respectively, whereas no change in K_D was observed (2.3 vs. 2.1 nM). Each data point represents the mean value for duplicate determination in a representative experiment.

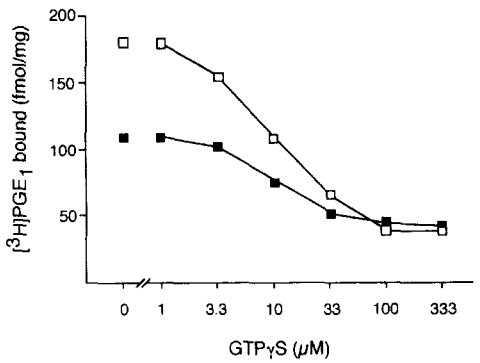

Fig. 2. Loss of specific [^{3}H]prostaglandin E_1 binding by uncoupling of receptor/G protein interaction. Binding of a single concentration of [^{3}H]prostaglandin E_1 tracer to membranes from control (□) or chronically DADLE (1 μM, 3 days)-treated NG 108-15 hybrid cells (■) was measured either in the absence (○) or in the presence of various concentrations of the stable guanine-nucleotide analogue, GTPγS, as indicated. Nonspecific binding was determined in the presence of 10 μM of cold prostaglandin E_1 and was unaffected by co-incubation with GTPγS. Each data point represents the mean value of a determination from a representative experiment done in duplicate.

Table 1
Regulation of prostaglandin E_1 receptors in NG108-15 hybrid cells by chronic inhibitory treatment

Pretreatment	n	B_{max}		K_D (nM)
		fmol/mg protein	Reduction (%)	
None	17	231.4 ± 32.1		2.9 ± 1.1
DADLE (1 μM, 3 days)	12	139.8 ± 22.1 [a]	(39)	2.6 ± 0.9
Morphine (10 μM, 3 days)	3	147.3 ± 10.8 [a]	(36)	2.2 ± 1.2
Oxotremorine M (10 μM, 3 days)	9	158.1 ± 21.2 [a]	(32)	2.3 ± 0.6
Clonidine (10 μM, 3 days)	9	156.2 ± 28.3 [a]	(32)	2.4 ± 0.7

[a] Significantly different from control ($P < 0.05$) NG108-15 hybrid cells were cultured for 3 days either in the absence (no pretreatment) or in the presence of maximal effective concentrations of various inhibitory agonists as indicated. Prostaglandin E_1 binding parameters were calculated according to the method of DeBlasi et al. (1989) from self-competition curves obtained by the displacement of a fixed concentration of [^{3}H]prostaglandin E_1 and increasing concentrations of unlabelled prostaglandin E_1. The data given represent mean ± S.E.M. values for the number of experiments indicated. The values in parentheses indicate % reduction in the maximal binding capacity of prostaglandin E_1 receptors as compared to untreated controls.

Pretreatment of the cells with naloxone alone (100 μM, 3 days) had no effect on overall prostaglandin E_1 receptor levels. Additional experiments demonstrated that the decrease in high-affinity [^{3}H]prostaglandin E_1 binding is independent of the nature of the opioid used for chronic pretreatment. Table 1 shows that there was no difference in the extent of prostaglandin E_1 receptor regulation regardless of whether the highly potent enkephalin derivative, DADLE, or the partial δ-opioid receptor agonist, morphine (Vachon et al., 1987), was used for chronic pretreatment.

Since NG108-15 hybrid cells also express several other receptor types which are coupled to the inhibition of adenylate cyclase, like α_{2B}-adrenoceptors and muscarinic M_4 receptors, we also tested the ability of agonists for these receptors to decrease high-affinity [^{3}H]prostaglandin E_1 binding. As with chronic opioid treatment, exposure of the cells for 3 days to maximal effective concentrations of oxotremorine (10 μM) and clonidine (10 μM) significantly reduced the capacity of [^{3}H]prostaglandin E_1 binding sites by some 32%. B_{max} values were 158.1 ± 21.1 ($n = 9$) and 156.2 ± 28.3 ($n = 9$) fmol/mg membrane protein for oxotremorine- and clonidine-pretreated cells, respectively (Table 1).

The pharmacokinetics of prostaglandin E_1 receptor cross-regulation was investigated using the δ-opioid receptor agonist, DADLE, for chronic inhibitory treatment. The reduction in the number of high-affinity prostaglandin E_1 receptors developed in a time-dependent manner with a half-life of ~ 8 h, and was complete after 2 days of DADLE (1 μM) exposure (Fig. 3a). In addition, the maximal extent of prostaglandin E_1 receptor regulation was also dependent on the concentration of DADLE used for chronic pretreatment. While exposure of the cells to 1 nM DADLE for 3 days had no effect on prostaglandin E_1 receptor capacity, increasing concentrations of the opioid produced a clear reduction in maximal [^{3}H]prostaglandin E_1 binding, displaying a median effective concentration of ~ 30 nM and a maximal effective concentration of 1 μM DADLE (Fig. 3b).

To determine whether the decrease in prostaglandin E_1 receptor number is reversible on termination of chronic inhibitory treatment, NG108-15 hybrid cells were kept for 3 days in the presence of 1 μM DADLE. The cells were then washed free of ligand and reincubated in DADLE-free

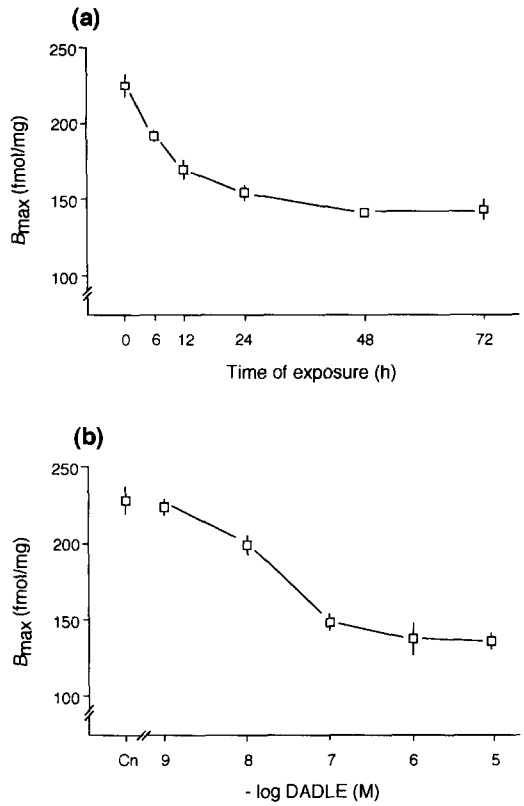

Fig. 3. Pharmacokinetics of prostaglandin E_1 receptor regulation. (a) Time course of DADLE-induced loss of high-affinity prostaglandin E_1 binding sites. NG 108-15 hybrid cells were exposed to DADLE (1 μM) for the times indicated before membranes were prepared and [^{3}H]prostaglandin E_1 binding data were obtained. The calculated value for half-maximal reduction of prostaglandin E_1 receptor capacity was 8.25 h. (b) Concentration-response relationship for heterologous prostaglandin E_1 receptor down-regulation. NG 108-15 hybrid cells were treated chronically for 3 days with increasing concentrations of the δ-opioid agonist, DADLE. The concentration of the inhibitory ligand required to produce a half-maximal reduction in prostaglandin E_1 receptor levels (EC_{50}) was ~ 30 nM. The data shown are mean ± S.D. values from two separate experiments each.

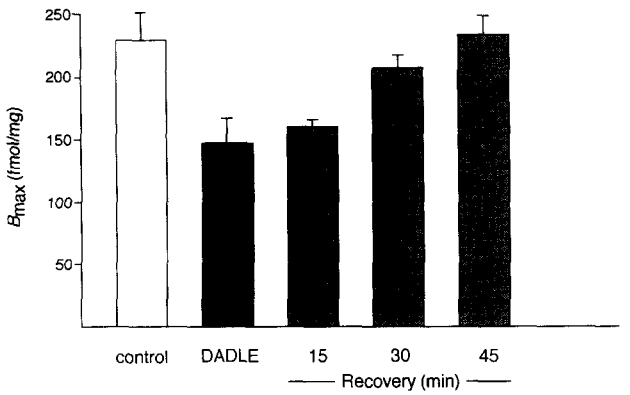

Fig. 4. Recovery of [^{3}H]prostaglandin E_1 binding sites. NG 108-15 hybrid cells were incubated with or without (control) 1 μM of DADLE for 3 days. The cells were then washed 3× and reincubated with growth medium lacking the inhibitory agonist. At the times indicated, the cells were harvested and prostaglandin E_1 receptor levels were measured as described in Materials and methods. Total number of prostaglandin E_1 receptors is expressed as fmol/mg of membrane protein ± S.D. from two experiments.

medium at 37°C for increasing time intervals before the cells were harvested and receptor density was measured in membrane preparations. While after 15 min of agonist removal, only slight recovery of [^{3}H]prostaglandin E_1 binding sites was observed, ~ 76% of the receptors recovered following 30 min of readaptation. The reduction in prostaglandin E_1 receptor number was completely reversed 45 min after termination of chronic DADLE pretreatment (Fig. 4).

Finally, the role of inhibitory G proteins in the mediation of receptor cross-talk was investigated in experiments in which persistent δ-opioid receptor signalling was interrupted by pertussis toxin treatment of the cells. Culture of the cells for 3 days in the presence of pertussis toxin (16 ng/ml medium) moderately reduced the number of high-affinity prostaglandin E_1 receptors by ~ 21% (223.7 ± 5.6 vs. 176.2 ± 4.8 fmol/mg membrane protein for control and pertussis toxin-pretreated cells, respectively; mean ± S.D. values of triplicate determinations). However, as shown in Table 2, no further decrease in the capacity of prostaglandin E_1 binding sites was observed in response to chronic DADLE exposure (1 μM) during the last 2 days of pertussis toxin pretreatment (180.3 ± 2.1 fmol/mg membrane protein; mean ± S.D. value of triplicate determinations).

Table 2
Pertussis toxin pretreatment abolishes heterologous prostaglandin E_1 receptor down-regulation

Pretreatment	Prostaglandin E_1 receptors	
	B_{max} (fmol/mg protein)	Reduction (%)
None	223.7 ± 5.6	
Pertussis toxin (16 ng/ml, 3 days)	176.2 ± 4.8	21
Pertussis toxin (16 ng/ml, 3 days) + DADLE (1 μM; 2 days)	180.3 ± 2.1	19

NG108-15 hybrid cells were grown for 3 days either in the absence (no pretreatment) or in the presence or 16 ng/ml pertussis toxin. In the case of pertussis toxin-pretreated cells, some cultures were further exposed to 1 μM DADLE during the last 2 days of pretreatment. Specific [^{3}H]prostaglandin E_1 binding was assessed at a single tracer concentration (8 nM) and the maximal binding capacity (B_{max}) was calculated using the equation $B_{max} = B_0 (L + K_D)/L$, where B_0 is the specific binding in fmol/mg membrane protein, L is the ligand concentration, and K_D is the apparent affinity constant. The data presented represent mean ± S.D. values from triplicate determination. Values in parentheses indicate % reduction in B_{max} as compared to untreated controls.

4. Discussion

Chronic exposure of a variety of different cell types and tissues to inhibitory acting drugs results in adenylate cyclase supersensitivity upon withdrawal of the drug (for review, see Thomas and Hoffman, 1987). Here we describe that chronic treatment of NG108-15 hybrid cells with agonists for either inhibitory δ-opioid receptors, α_{2B}-adrenoceptors, or muscarinic M_4 receptors significantly reduced the number of stimulatory prostaglandin E_1 receptors. Further pharmacological characterization revealed that this common regulatory phenomenon most likely reflects a transient loss of high-affinity, G protein-coupled prostaglandin E_1 binding sites.

NG108-15 hybrid cells express several stimulatory receptor types, i.e. a binding site for prostaglandin E_1, an adenosine A_2 receptor and a secretin receptor (McGee and Kenimer, 1982; Adie and Milligan, 1993). Among these receptors, the binding site for prostaglandin E_1 predominates and, thus, has been frequently used to investigate regulatory changes in stimulatory receptors. The calculated binding parameters for [^{3}H]prostaglandin E_1 in naive NG108-15 hybrid cells described in the present work (B_{max} ~ 230 fmol/mg of membrane protein; K_D ~ 3 nM) are substantially lower than those communicated in our previous report (B_{max} ~ 650 fmol/mg of membrane protein; K_D ~ 15 nM; Ammer and Schulz, 1995) or by others (Adie et al., 1992; Donnelly et al., 1992) who noted the presence of ~ 1.5 pmol of prostaglandin E_1 receptors/mg of membrane protein displaying a K_D of ~ 10–30 nM. This difference is due to the use of different experimental designs for assessing [^{3}H]prostaglandin E_1 binding parameters. The most important modifications of the present binding studies comprised: (1) the use of a heavy membrane preparation; and (2) the inclusion of Na^+ (100 mM) in the reaction mixture. These changes, in combination with the use of the agonistic radioligand, [^{3}H]prostaglandin E_1, as tracer, allowed us to exclusively measure the high-affinity, G protein-coupled component of total prostaglandin E_1 receptor binding. This strategy proved neces-

sary because NG108-15 hybrid cells contain a large number of low-affinity prostaglandin E_1 binding sites (Kelly et al., 1990), which might cover discrete regulatory changes in the portion of functionally active prostaglandin E_1 receptors. Under the experimental conditions employed, however, measurement of prostaglandin E_1 receptors resulted in a very low [^{3}H]prostaglandin E_1 tracer binding, excluding Scatchard (1949) transformation of the binding data. To circumvent this problem, homologous displacement curves were constructed and binding parameters were made as described by DeBlasi et al. (1989). Using this strategy, we were able to obtain reliable information about the number of functionally active, G protein-coupled prostaglandin E_1 receptors. This notion is further supported by the observation that addition of the guanosine nucleotide analogue, GTPγS (100 μM), which is known to promote uncoupling of the receptors from their associated G proteins (Adie et al., 1992), almost completely abolished specific [^{3}H]prostaglandin E_1 tracer binding.

Regulation of prostaglandin E_1 receptor levels is specifically mediated by chronic activation of inhibitory receptors, since coincubation of the cells with DADLE together with naloxone, an opioid receptor antagonist, prevented this effect. This premise is further supported by the observation that pertussis toxin-mediated interruption of the inhibitory signal transduction cascade also blocks cross-regulation of prostaglandin E_1 receptors. The latter observation points to a more critical role of inhibitory G proteins in the regulatory mechanisms underlying sensitization of adenylate cyclase. Due to the fact that both δ-opioid as well as α_2-adrenoceptors utilize a common G protein (G_i2) for mediating adenylate cyclase inhibition (McClue and Milligan, 1990; McKenzie and Milligan, 1990), extracellular information from a variety of inhibitory receptors is suggested to integrate at the level of inhibitory G proteins. The use of a common regulatory pathway downstream of inhibitory receptors would also provide the basis for the phenomenon of cross-dependence between opioids and α_2-adrenoceptor agonists as previously shown to develop in NG108-15 hybrid cells (Lee et al., 1988).

The decrease in the fraction of high-affinity, G protein-coupled prostaglandin E_1 receptors develops in both a dose- and time-dependent manner with respect to chronic inhibitory drug treatment. The concentration of DADLE required to achieve maximal effects on prostaglandin E_1 receptor levels correlates well with that described for mediating maximal adenylate cyclase inhibition (Law et al., 1983; Vachon et al., 1987). In analogy, the time course observed closely resembles that previously reported for the development of adenylate cyclase supersensitivity. In particular, morphine (Sharma et al., 1975), clonidine (Sabol and Nirenberg, 1979), and carbachol (Nathanson et al., 1978) have been shown to maximally potentiate prostaglandin E_1-stimulated adenylate cyclase after 24–30 h of chronic inhibitory drug treatment. Taken together, the pharmacology of heterologous prostaglandin E_1 receptor regulation is in good agreement with the development of other cellular signs of drug addiction.

The rapid recovery of chronic opioid-induced reduction in high-affinity [^{3}H]prostaglandin E_1 binding following termination of opioid receptor activation is indicative of receptor uncoupling rather than true down-regulation, i.e. degradation, of the binding sites. Because of the difficulties involved in the measurement of low-affinity [^{3}H]prostaglandin E_1 binding sites (Kelly et al., 1990), we were not able to investigate directly a sequestered compartment, either in the plasma membrane or in the cytosolic fraction of NG108-15 hybrid cells. Nevertheless, uncoupling of the receptors seems to represent a plausible regulatory mechanism, since chronic exposure of the cells to opioids has been previously shown to impair heterologously the action of a variety of inhibitory and stimulatory receptors (Lee et al., 1988). Thus, the decrease in the number of high-affinity prostaglandin E_1 binding sites might be considered a form of heterologous prostaglandin E_1 receptor desensitization. This is of particular interest in view of our recent finding of a significantly increased functional prostaglandin E_1 receptor/G protein interaction during the state of opioid dependence (Ammer and Schulz, 1993, 1995), whereas the overall functional capacity for stimulatory adenylate cyclase signalling remains unchanged (Traber et al., 1975; Boyd et al., 1992). Therefore, it seems likely that the loss of high-affinity prostaglandin E_1 receptors might represent a secondary regulatory phenomenon counteracting enhanced adenylate cyclase stimulation on postreceptor level. A similar regulatory mechanism has been described for neonatal rat ventricular myocytes, in which chronic carbachol-induced sensitization of adenylate cyclase is also associated with considerable sequestration of stimulatory β_1-adrenoceptors (Paraschos and Karliner, 1994).

The present findings demonstrate that chronic exposure of NG108-15 hybrid cells to inhibitory acting drugs transiently reduces the number of functionally active, G protein-coupled prostaglandin E_1 receptors. The striking correlation between the development of prostaglandin E_1 receptor cross-regulation and the expression of adenylate cyclase supersensitivity further suggests a causal relationship between these two chronic inhibitory drug effects. Moreover, regulatory changes in stimulatory adenylate cyclase-coupled receptor systems seem not to be exclusively mediated by drugs whose primary site of action are members of the family of inhibitory G protein-coupled receptors. The finding that both chronic in vivo as well as in vitro treatment with tricyclic antidepressants also decreases the capacity of β_1-adrenoceptors in the rat frontal cortex (Hosoda and Duman, 1993) and in C6 glioma cells (Fishman and Finberg, 1987), respectively, further raises the possibility that down-regulation of stimulatory adenylate cyclase-coupled receptors represents a more general type of cellular adaptation in response to chronic inhibitory drug exposure.

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