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Regulation of hamster α_{1B}-adrenoceptors expressed in Chinese hamster ovary cells

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Abstract

Chinese hamster ovary (CHO) cells were stably transfected to express the hamster α_{1B}-adrenoeceptor, and the function and agonist-induced regulation of the binding properties of these receptors were characterized. The cells expressed approximately 230000 receptors per cell, with a K_D for [^{3}H]prazosin of 140 pM. In assays of competition by epinephrine for [^{3}H]prazosin binding to receptors on intact cells, 88% of the receptors were in a low affinity form. The protein kinase C activator phorbol 12-myristate, 13-acetate (PMA) did not further increase the fraction in the low affinity form, but the protein kinase C inhibitor staurosporine reduced the low affinity fraction to 51%. In sucrose density gradient centrifugation assays of receptor internalization, the percentage of receptors in the light vesicle fraction was 25% for control cells, 53% for epinephrine-pretreated cells, 44% for PMA-pretreated cells, and 53% for cells pretreated with epinephrine plus PMA. Staurosporine completely blocked PMA-induced internalization, but only partially inhibited epinephrine-induced internalization. These results suggest a relationship between low affinity binding and internalization for α_{1B}-adrenoceptors and the involvement of protein kinase C in both processes. Longer-term (24 h) exposure of cells to epinephrine induced an unexpected up-regulation of receptor density of approximately 2-fold that was accompanied by an increase in maximal agonist-stimulated phosphoinositide turnover. These studies document several regulatory differences between α_{1B}-adrenoceptors expressed in transfected CHO cells and those natively expressed in DDT$_1$ MF-2 hamster smooth muscle cells, and they provide additional information on the molecular mechanisms involved in agonist-induced regulation of α_{1B}-adrenoceptors.

Keywords: Adrenoceptor; Desensitization; Internalization; Up-regulation; Intact cell binding

1. Introduction

Exposure of cells to agonists leads to multiple adaptive changes in the binding properties of β- and α_1-adrenoceptors. These include desensitization of receptor coupling to GTP-binding proteins and/or second messenger generation, receptor sequestration and/or internalization, and receptor down-regulation. These phenomena have been extensively characterized at the molecular level in the case of β-adrenoceptors, but much less is known for α_1-adrenoceptors (Perkins et al., 1991; Toews et al., 1991; Collins et al., 1992; Hadcock and Malbon, 1993). Studies to date indicate that α_{1B}-adrenoceptor desensitization shares some common features with β_2-adrenoceptor desensitization (Leeb-Lundberg et al., 1987; Hoffman, 1987) but is different from β_2-adrenoceptor desensitization in other features (Morris et al., 1991; Toews, 1987; Zhu et al., 1992; Zhu and Toews, 1993).

In previous studies we have documented several differences in the adaptive regulation of the β_2-adrenoceptors and the α_{1B}-adrenoceptors in DDT$_1$ MF-2 hamster smooth muscle cells. Agonist alone appeared to induce only a sequestration of α_1-adrenoceptors within the plasma membrane, whereas agonist plus a protein kinase C activator led to receptor internalization within endocytotic vesicles (Toews, 1987; Cowlen and Toews, 1988). In contrast, agonist alone led to internalization of β-adrenoceptors, and protein kinase C activators had no further effect (Toews, 1987; Cowlen and Toews, 1987). Whereas β_2-adrenoceptors were down-regulated by nearly 80% following 24-h agonist exposure, only minimal (10–20%) down-regulation

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occurred for α_{1B}-adrenoceptors (Toews, 1987). In addition, a guanine nucleotide-induced shift in agonist competition binding curves in membrane preparations has been observed for β_2-adrenoceptors but not for α_{1B}-adrenoceptors in DDT_1 MF-2 cells (Cornett et al., 1982; Toews, 1987).

Agonist exposure also leads to a rapid change in the binding properties of both β- and α_1-adrenoceptors measured on intact cells (reviewed in Toews et al., 1991). During the course of equilibrium competition binding assays, agonists induce conversion of receptors from a native high affinity form to a form exhibiting markedly lower apparent binding affinity. This change is not observed in assays with antagonists in intact cells or in assays with agonists in isolated membrane preparations. Similar phenomena have also been shown to occur for α_2-adrenoceptors (McKernan et al., 1987) and muscarinic acetylcholine receptors (Fisher, 1988; Hoover and Toews, 1989). The low apparent affinity exhibited by these receptors has been postulated to be related to agonist-induced receptor internalization and the consequent relative inaccessibility of hydrophilic agonists to the internalized receptors because of the plasma membrane barrier (Toews et al., 1991). Previous studies from this laboratory have supported this hypothesis for β_2-adrenoceptors (Zhu et al., 1992; Zhu and Toews, 1993, 1994) but suggested that different mechanisms might be involved in the case of α_{1B}-adrenoceptors (Toews, 1987; Zhu et al., 1992; Zhu and Toews, 1993).

To better understand the cellular and molecular mechanisms involved in agonist-induced regulation of α_{1B}-adrenoceptors and the differences in regulation between β_2-adrenoceptors and α_{1B}-adrenoceptors, we have transfected hamster α_{1B}-adrenoceptors into Chinese hamster ovary (CHO) cells. The function and agonist-mediated regulation of these receptors was then characterized, including changes in intact cell receptor binding properties, receptor internalization, and regulation of receptor number.

2. Materials and methods

2.1. Materials

Growth medium, serum and transfection reagents were purchased from Gibco BRL Life Technologies (Grand Island, NY, USA). Phentolamine was a gift from Ciba-Geigy (Summit, NJ, USA). [^{3}H]Prazosin was purchased from NEN (Boston, MA, USA) and [^{3}H]inositol from either Amersham (Arlington Heights, IL, USA) or American Radiolabeled Chemicals (St. Louis, MO, USA). Hamster α_{1B}-adrenoceptor cDNA was kindly provided by Dr. Robert Lefkowitz (Duke University, Durham, NC, USA). Restriction enzymes were from Promega (Madison, WI, USA). Other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sources of molecular biology reagents are indicated below.

2.2. α_{1B}-Adrenoceptor expression in CHO cells

The cDNA coding for hamster α_{1B}-adrenoceptor (Cotecchia et al., 1988) was subcloned into the *Eco*RI site of pBluescript (Stratagene, La Jolla, CA, USA). A clone with correct orientation was subcloned into the *Hin*dIII and *Xba*I sites of the pRC/CMV expression vector (Invitrogen, San Diego, CA, USA). Stable transfection into CHO-K1 cells was achieved by calcium phosphate precipitation (Chen and Okayama, 1988). G-418-resistant CHO cell clones were screened for intact cell [^{3}H]prazosin binding, and positive clones covering a range of α_{1B}-adrenoceptor expression levels were further expanded to establish cell lines. Cells were maintained in Ham's F12 medium supplemented with 10% fetal bovine serum, 500 μg/ml G-418, and 5 μg/ml gentamicin at 37°C in a 5% CO_2 atmosphere.

2.3. Intact cell binding assays

For competition binding assays, cells grown on 35-mm culture dishes were washed and incubated at 37°C in Ham's F12 medium buffered to pH 7.5 with 20 mM Hepes (Ham's-Hepes) containing 0.5 nM [^{3}H]prazosin in the absence or presence of various concentrations of epinephrine for 60 min. Nonspecific binding was defined as the binding occurring in the presence of 10 μM phentolamine. Cells were then washed 2 or 3 times with warm Ham's-Hepes and dissolved in 1 ml of 0.2 N NaOH. Radioactivity was quantitated by liquid scintillation counting after neutralization of the samples with 0.2 ml of 1 N HCl. A similar protocol was used for assays of the time course and epinephrine concentration dependence of up-regulation, except that only binding in the absence and presence of 10 μM phentolamine was measured.

For saturation binding assays, the binding was essentially identical to that above, except that the concentration of [^{3}H]prazosin was varied. Before washing at the end of the binding reaction, an aliquot of the supernatant medium was taken for scintillation counting to determine the concentration of free radioligand. Bound radioligand associated with the cells was determined as described above.

2.4. Receptor internalization assays

Internalization assays were conducted as previously described (Cowlen and Toews, 1988). Cells incubated for 60 min at 37°C in the absence or presence of 100 μM epinephrine, 1 μM PMA, or both epinephrine and PMA were lysed with lysis buffer at 4°C. In some experiments, 1 μM staurosporine was added 10 min prior to epinephrine or PMA treatment. Cell lysates were then homogenized and a 3.5-ml portion of the lysate was layered on top of a sucrose density gradient consisting of 3 ml of 55% (w/v), 3 ml of 32%, and 1.5 ml of 5% sucrose in 12-ml tubes. Centrifugation was at 4°C for 60 min at 35000 rpm in a

Beckman SW41 rotor. The membranes at the 5/32% interface were collected as the light vesicle fraction and those at the 32/55% interface were collected as the plasma membrane fraction. Aliquots of each fraction were assayed for α_{1B}-adrenoceptors by incubation in binding buffer (20 mM Tris, pH 7.5, 10 mM $MgCl_2$, 1 mM EDTA) containing 0.5 nM [^{3}H]prazosin for 60 min at 37°C. Nonspecific binding was defined as the binding occurring in the presence of 10 μM phentolamine. Assays were terminated by rapid filtration over S&S No. 30 filters and washing the filters 3 times with wash buffer (10 mM Tris, pH 7.5, 140 mM NaCl) using a Brandel (Gaithersburg, MD, USA) cell harvester. Radioactivity associated with the filters was quantitated by liquid scintillation counting.

2.5. Inositol phosphates assays

Cells grown on 35-mm culture dishes were prelabeled overnight with 2 μCi [^{3}H]inositol in 1 ml of inositol-free high glucose Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. After labeling, cells were washed and then stimulated with various concentrations of epinephrine in Ham's-Hepes containing 10 mM LiCl for 10 min. Inositol phosphates were extracted as described by Nakahata et al. (1986) and separated on Dowex 1-X8 (formate form) columns. Total inositol phosphates were eluted with 8 ml of 1 M ammonium formate and 0.1 M formic acid. A 3-ml portion of the eluate ('a') and a 0.375-ml portion of the organic phase ('b') were counted. The percentage of conversion of inositol phospholipids to inositol phosphates was then calculated by the formula $a/(a+b) \times 100\%$.

2.6. Data analysis

Data (means ± S.D.) from competition binding experiments were fit to one- and two-site binding models using the computerized nonlinear regression curve-fitting capacity of InPlot (GraphPAD, San Diego, CA, USA). The two-site fit was used where it was significantly ($P < 0.05$) better than the one-site fit. Dose-response curves for inositol phosphates assays were fit with the one-site model. Nontransformed saturation binding data were fit by nonlinear regression to a one-site saturation binding equation for bound vs. free radioligand; however, data in the figures are presented in the Rosenthal (1967) transformation format of bound vs. bound/free radioligand.

3. Results

3.1. Receptor expression

Hamster α_{1B}-adrenoceptors were stably expressed in CHO cells as described in the Materials and methods section. Among 144 colonies picked, 51 were positive in [^{3}H]prazosin binding assays with intact cells. Six clones covering a wide range of expression levels were chosen for further assessment of α_{1B}-adrenoceptor expression, and a single clone, referred to as CHO-α_{1B}, was then used for the regulation studies below. Rosenthal (1967) analysis of [^{3}H]prazosin saturation binding assays with intact CHO-α_{1B} cells revealed a K_D of 139 ± 37 pM and a B_{max} of 230000 ± 30000 receptor binding sites/cell. This K_D value is similar to that reported for DDT$_1$ MF-2 cells in our previous study, but the level of receptor expression is about 20-fold higher. Binding was inhibited by the antagonists prazosin, phentolamine and yohimbine with the expected potencies (data not shown).

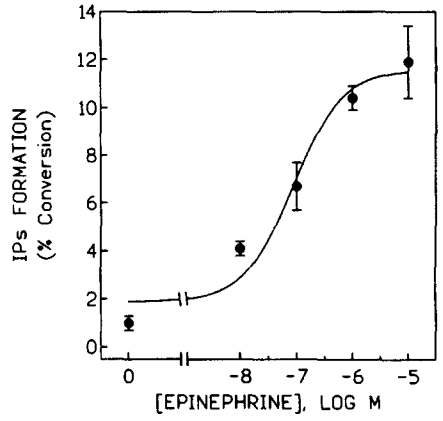

Fig. 1. Agonist-stimulated phosphoinositide turnover in CHO-α_{1B} cells. Cells were incubated with the indicated concentrations of epinephrine for 10 min in the presence of 10 mM LiCl. Data are expressed as percent conversion of inositol phospholipids to inositol phosphates and are means ± S.D. from 3 experiments, each performed in triplicate.

3.2. Receptor coupling to phosphoinositide turnover

α_{1B}-Adrenoceptors in these cells were functionally coupled to the phosphoinositide turnover second messenger system as expected. The agonist epinephrine stimulated phosphoinositide turnover in a dose-dependent manner (Fig. 1). The basal and maximally stimulated levels of inositol phosphates formation were 1.9% and 11.6% conversion, respectively. The EC_{50} (88 ± 40 nM) was somewhat lower than that reported previously for DDT$_1$ MF-2 cells (about 500 nM; Hoyer, 1984; Schachter and Wolfe, 1992), but this is not unexpected given the higher α_{1B}-adrenoceptor expression level in these cells than in DDT$_1$ MF-2 cells. Although this stimulation of phosphoinositide turnover is presumably mediated by activation of a GTP-binding protein, agonist competition for [^{3}H]prazosin binding to isolated membrane preparations was insensitive to added Gpp(NH)p (data not shown), which is in agreement with previous findings for α_{1B}-adrenoceptors in DDT$_1$ MF-2 cells from our laboratory and others (Cornett et al., 1982; Toews, 1987).

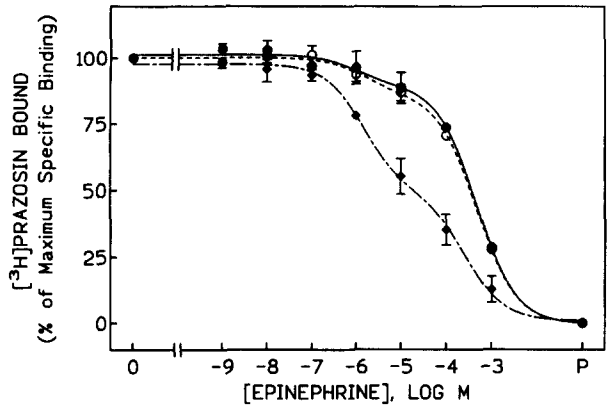

Fig. 2. Equilibrium binding of the agonist epinephrine to intact CHO-α_{1B} cells. Binding of [^{3}H]prazosin to α_{1B}-adrenoceptors in control (●), PMA-treated (○), or staurosporine-treated (♦) cells was measured in 60 min assays in the absence (0) or presence of the indicated concentrations of the agonist epinephrine or 10 μM phentolamine (P). Data are expressed as percent of maximal specific binding and are means ± S.D. from three experiments, each performed in duplicate.

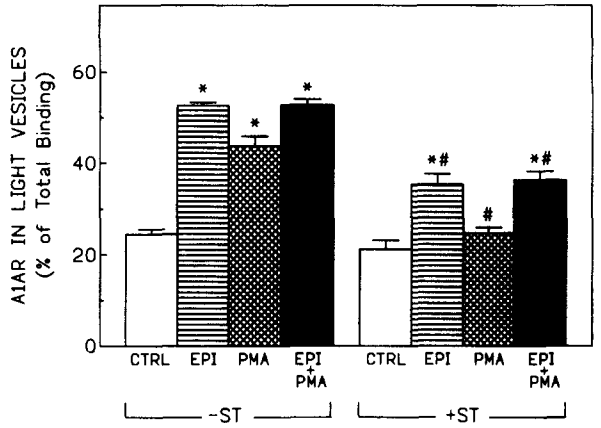

Fig. 3. Internalization of α_{1B}-adrenoceptors in CHO-α_{1B} cells. CHO-α_{1B} cells treated as described in Materials and methods were incubated for 60 min in the absence (CTRL) or presence of 100 μM epinephrine (EPI), 1 μM PMA, or epinephrine plus PMA, each in the absence (−ST) or presence (+ST) of 1 μM staurosporine. Cell lysates were then subjected to sucrose density gradient centrifugation assays of receptor internalization. Data are expressed as the percentage of total α_{1B}-adrenoceptors present in the light vesicle fraction and are the means ± S.D. from 3 experiments. * Treated value significantly different from its corresponding control value, $P < 0.05$; # +ST value significantly different from its corresponding −ST value, $P < 0.05$.

3.3. Intact cell binding properties

The intact cell binding properties of the transfected α_{1B}-adrenoceptors were investigated. Assays of competition by the agonist epinephrine for [^{3}H]prazosin binding to α_{1B}-adrenoceptors were performed on intact CHO-α_{1B} cells for 60 min at 37°C (Fig. 2, Table 1). Binding to control cells was better fit by the two-site model, with 88 ± 3% of the receptors in the low affinity form. A similar level of the low affinity form was also observed in another cell clone expressing about one-fourth as many α_{1B}-adrenoceptors (data not shown).

Since one of the consequences of α_{1B}-adrenoceptor activation is stimulation of protein kinase C, the possible role of protein kinase C in generation of the low affinity form of these receptors was investigated. Epinephrine competition binding assays were performed as above, but in the presence of 1 μM PMA to activate protein kinase C or 1 μM staurosporine to inhibit protein kinase C (Fig. 2, Table 1). PMA did not further increase the fraction of α_{1B}-adrenoceptors in the low affinity form (86 ± 2%), but staurosporine was effective in decreasing the fraction of the receptors in the low affinity form (51 ± 4%).

Table 1
Intact cell binding properties of CHO-α_{1B} cells for the agonist epinephrine in control, PMA- or staurosporine-treated cells

	I_H (μM)	R_H (%)	I_L (μM)	R_L (%)
Control	1.0 ± 0.7	12 ± 3	470 ± 70	88 ± 3
PMA	1.6 ± 0.7	14 ± 2	470 ± 50	86 ± 2
Staurosporine	1.5 ± 0.3	49 ± 4	260 ± 70	51 ± 4

IC_{50} values and % of receptors in each fraction were obtained from the displacement of 0.5 nM [^{3}H]prazosin as described in Materials and methods. Each value represents the mean ± S.D. of three experiments preformed in duplicate. I_H: high affinity IC_{50}. R_H: % of receptors in the high affinity form. I_L: low affinity IC_{50}. R_L: % of receptors in the low affinity form.

3.4. Receptor internalization

To test the possible role of receptor internalization in generating the low affinity form, the agonist-induced internalization properties of α_{1B}-adrenoceptors transfected in these cells were investigated using sucrose density gradient centrifugation assays (Fig. 3). Exposure of CHO-α_{1B} cells to the agonist epinephrine induced a shift of α_{1B}-adrenoceptors on sucrose density gradients from the plasma membrane fraction to the light vesicle fraction that is thought to represent receptors in endocytotic vesicles (Toews, 1987; Cowlen and Toews, 1987). The basal level of receptors in the light vesicle fraction in cells not exposed to epinephrine was 25 ± 1%, whereas 53 ± 1% were present in the light vesicle fraction in cells exposed to 100 μM epinephrine for 60 min.

The possible role of protein kinase C in α_{1B}-adrenoceptor internalization also was investigated in CHO-α_{1B} cells, using the protein kinase C activator PMA and the protein kinase C inhibitor staurosporine (Fig. 3). Exposure of cells to 1 μM PMA alone for 60 min induced receptor internalization, with 44 ± 2% of receptors in the light vesicle fraction. PMA did not further increase the epinephrine-induced α_{1B}-adrenoceptor internalization, since epinephrine plus PMA resulted in the same extent of receptors in the light vesicle fraction (53 ± 1%) as with epinephrine alone. Treatment of cells with 1 μM staurosporine alone did not alter the basal level of receptors in the light vesicle frac-

tion (21 ± 2%). Although staurosporine only partially blocked epinephrine-induced internalization (35 ± 2% of the receptors in the light vesicle fraction), it almost completely prevented PMA-induced internalization (25 ± 1% in light vesicle fraction). Inclusion of PMA during the epinephrine treatment did not antagonize the effect of staurosporine, since cells exposed to epinephrine and PMA plus staurosporine still showed 36 ± 2% of the receptors in the light vesicle fraction, essentially the same as that with epinephrine and staurosporine without PMA.

3.5. Receptor up-regulation

The effects of long-term exposure to agonist on receptor levels were investigated by assessment of [^{3}H]prazosin binding to intact CHO-α_{1B} cells following various treatments. Surprisingly, treatment of these cells with epinephrine resulted in an up-regulation of receptor density that was both time- and agonist concentration-dependent (Fig. 4). Up-regulation was apparent by 4 h of epinephrine exposure and continued to increase during the 24-h exposure. Half-maximal up-regulation with 24-h exposure occurred with approximately 1 nM epinephrine. Saturation binding analyses following 24 h exposure of cells to 10 nM epinephrine revealed that both the K_D and the B_{max} values were increased (Fig. 5). Based on results from three experiments, the B_{max} values calculated as number of receptor binding sites per dish increased by 2.1 ± 0.1-fold. However, epinephrine pretreatment also decreased the number of cells per dish by approximately 25%, so that the B_{max} values expressed as receptors per cell increased by nearly 3-fold, from 179 000 ± 26 000 receptors per cell in control conditions to 514 000 ± 32 000 receptors per cell following epinephrine pretreatment. The K_D for [^{3}H]prazosin increased by 2.1 ± 0.2-fold, from 149 ± 7 pM in control cells to 306 ± 23 pM following epinephrine pretreatment. The up-regulation was not an artefact of the intact cell binding assay, since similar results were obtained in saturation binding assays with membrane preparations from up-regulated cells (data not shown). A corresponding increase (1.75-fold) in epinephrine-stimulated maximal inositol phosphates formation occurred following exposure of cells to 1 μM epinephrine for 24 h. The percentage of conversion value in control cells was 11.2 ± 1.5% and in cells treated with epinephrine prior to assay was 19.5 ± 3.1% (n = 3). The phenomenon of epinephrine-induced up-regulation also was observed in the cells expressing lower levels of α_{1B}-adrenoceptors (data not shown).

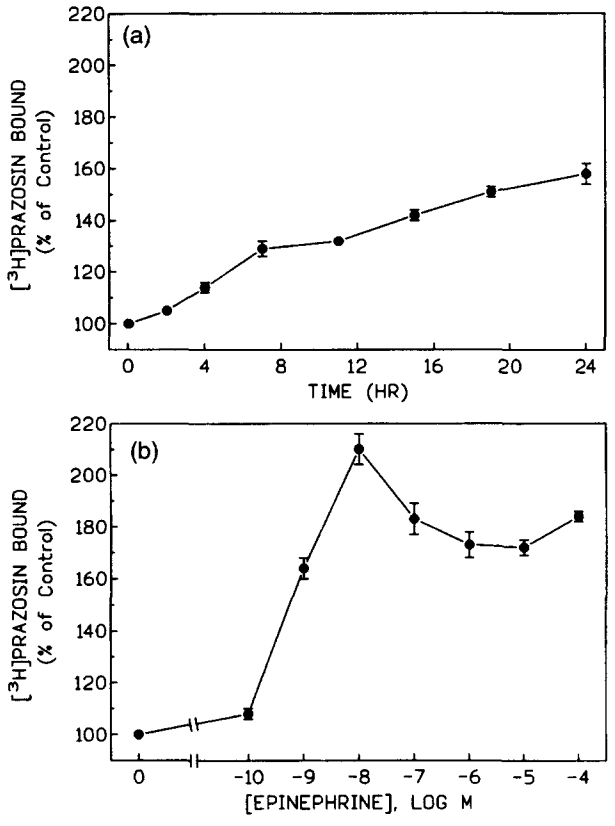

Fig. 4. Time- and concentration-dependent agonist-induced up-regulation of α_{1B}-adrenoceptors in CHO-α_{1B} cells. Cells were pretreated with 10 nM epinephrine for the indicated time period (top panel) or with the indicated concentrations of epinephrine for 24 h (bottom panel), washed, and then subjected to intact cell [^{3}H]prazosin binding. Data are expressed as percent of binding relative to that in cells not exposed to epinephrine treatment and are means ± S.E.M. from 3 (top panel) or 4 (bottom panel) experiments.

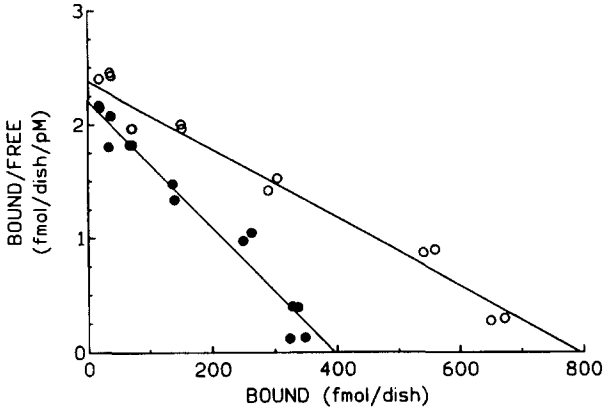

Fig. 5. Saturation analysis of [^{3}H]prazosin binding to control and up-regulated cells. Cells pretreated in the absence (●) or presence (○) of 10 nM epinephrine for 24 h were washed and then subjected to intact cell binding assays with various concentrations of [^{3}H]prazosin. Data are expressed in the form of a Rosenthal transformation and represent triplicate determinations from a single experiment representative of three. For the data shown, the B_{max} was 396 fmol/dish for control cells and the K_D was 179 pM; for epinephrine-treated cells, the B_{max} was 794 fmol/dish and the K_D was 337 pM.

4. Discussion

In our previous studies of regulation of the hamster α_{1B}-adrenoceptor endogenously expressed in DDT$_1$ MF-2

cells, we observed several differences between α_{1B}-adrenoceptor regulation and that of the β_2-adrenoceptor also endogenously expressed in these cells (Toews, 1987; Cowlen and Toews, 1988; Zhu et al., 1992; Zhu and Toews, 1993). A major goal of the current studies was to compare the regulatory properties of these α_{1B}-adrenoceptors expressed in a different cell background with those of α_{1B}-adrenoceptors natively expressed in DDT_1 MF-2 cells. Our results allow us to address the generality of the differences in regulation between α_{1B}-adrenoceptors and β_2-adrenoceptors observed previously in DDT_1 MF-2 cells. They also provide new mechanistic information regarding α_{1B}-adrenoceptor regulation.

Previous studies in our laboratory presented evidence that 'sequestration' and 'internalization' are separable steps for α_{1B}-adrenoceptors in DDT_1 MF-2 cells (Cowlen and Toews, 1988). 'Sequestration' is defined as a loss of receptor binding sites accessible at the cell surface; 'internalization' is defined as a shift of receptors from the plasma membrane to a light vesicle fraction, presumably endocytotic vesicles, in sucrose density gradient centrifugation assays. In DDT_1 MF-2 cells, agonist alone could induce α_{1B}-adrenoceptor sequestration, but the receptors remained associated with the plasma membrane fraction in sucrose density gradient assays (Toews, 1987). Only when cells were treated with agonist plus PMA did α_{1B}-adrenoceptor internalization into the light vesicle fraction occur (Cowlen and Toews, 1988). In contrast, agonist alone was able to induce both sequestration and internalization of β_2-adrenoceptors in these cells (Toews, 1987) and addition of PMA had no further effect (Cowlen and Toews, 1987).

The studies presented here suggest that differences in α_{1B}-adrenoceptor sequestration and internalization exist between DDT_1 MF-2 and CHO-α_{1B} cells. In contrast to results for α_{1B}-adrenoceptors in DDT_1 MF-2 cells but similar to results for β_2-adrenoceptors in DDT_1 MF-2 cells, agonist alone was sufficient to mediate α_{1B}-adrenoceptor internalization in CHO-α_{1B} cells. Furthermore, adding PMA plus epinephrine did not induce further internalization than that with epinephrine alone as it did in DDT_1 MF-2 cells.

Our studies of α_{1B}-adrenoceptor internalization in CHO-α_{1B} cells also provide further evidence for the involvement of protein kinase C in this process. Treatment of CHO-α_{1B} cells with the protein kinase C activator PMA induced α_{1B}-adrenoceptor internalization. The extent of internalization was smaller than that with epinephrine in these cells, but much larger than that with PMA alone in DDT_1 MF-2 cells. The protein kinase C inhibitor staurosporine completely blocked PMA-induced internalization, suggesting complete inhibition of protein kinase C. Staurosporine only partially inhibited epinephrine-induced internalization under the same conditions, suggesting that other elements in addition to protein kinase C activation may be involved in agonist-induced α_{1B}-adrenoceptor internalization. However, it is also possible that agonist-induced internalization involves an isozyme of protein kinase C that is not activated by PMA and not fully inhibited by staurosporine.

Our results are in general agreement with a recent study of internalization of α_{1B}-adrenoceptors transfected into HEK293 cells by Fonseca et al. (1995), who also suggested a role for protein kinase C in α_{1B}-adrenoceptor internalization. In their studies, both agonist and PMA induced receptor internalization as assessed by confocal immunofluorescence microscopy, similar to our results in CHO-α_{1B} cells. However, in their studies staurosporine was able to completely inhibit both agonist- and PMA-induced internalization.

Our studies of the agonist binding properties of α_{1B}-adrenoceptors on intact cells revealed differences between DDT_1 MF-2 and CHO-α_{1B} cells corresponding to the differences in internalization between these two cell lines. Although CHO-α_{1B} cells exhibited low affinity binding of agonists to intact cells as did DDT_1 MF-2 cells, the fraction of the receptors in this low affinity form was greater in CHO-α_{1B} cells than in DDT_1 MF-2 cells (Toews, 1987; Cowlen and Toews, 1988). In fact, the fraction of α_{1B}-adrenoceptors in the low affinity form in CHO-α_{1B} cells was closer to that observed for β_2-adrenoceptors in DDT_1 MF-2 cells. This correlates with the greater similarity of α_{1B}-adrenoceptor internalization in CHO-α_{1B} cells to that for β_2-adrenoceptors in DDT_1 MF-2 cells than to that for α_{1B}-adrenoceptors in DDT_1 MF-2 cells. In DDT_1 MF-2 cells, PMA increased the fraction of receptors in the low affinity form, presumably due to its enhancement of receptor sequestration and internalization in these cells (Cowlen and Toews, 1988). In contrast, PMA did not further increase the proportion of α_{1B}-adrenoceptors in the low affinity form in CHO-α_{1B} cells, correlating with the inability of PMA to enhance epinephrine-induced receptor internalization in CHO-α_{1B} cells. Thus agonist alone appears sufficient to induce both maximal internalization and maximal conversion to the low affinity form in CHO-α_{1B} cells, whereas agonist plus PMA is required in DDT_1 MF-2 cells.

Our results suggest that the properties of α_{1B}-adrenoceptors expressed in transfected CHO-α_{1B} cells are somewhat different from those of the α_{1B}-adrenoceptors natively expressed in DDT_1 MF-2 cells, but more similar to endogenous β_2-adrenoceptors of DDT_1 MF-2 cells and to β_2-adrenoceptors transfected into CHO cells (Zhu et al., 1991), in terms of both receptor internalization and the agonist binding properties on intact cells. They also support our initial hypothesis that the low affinity binding of agonists to intact cells is a reflection of receptor internalization and the subsequent relative inaccessibility of hydrophilic agonists to the internalized receptors (Toews et al., 1991). Agonist alone induced both internalization and conversion to the low affinity form in CHO-α_{1B} cells. PMA did not further increase agonist-induced internalization or increase the conversion to the low affinity form.

Staurosporine showed similar partial inhibition of both processes. Furthermore, the effects of PMA and staurosporine suggest an important role for protein kinase C in regulating both receptor internalization and generation of the low affinity form of α_{1B}-adrenoceptors in CHO-α_{1B} cells.

Long-term exposure of cells to agonists generally causes a decrease in receptor number as detected by radioligand binding assays, a phenomenon referred to as down-regulation. In DDT_1 MF-2 cells, α_{1B}-adrenoceptor down-regulation occurred but to a much smaller extent than for β_2-adrenoceptor down-regulation (Toews, 1987). In the present studies with CHO-α_{1B} cells, epinephrine did not induce any down-regulation, but instead induced an increase in receptor density measured on intact cells or in isolated membrane preparations. This agonist-induced up-regulation of α_{1B}-adrenoceptors was both time- and concentration-dependent, and it was accompanied by an increase in epinephrine-stimulated phosphoinositide turnover induced under the same conditions. In addition to the up-regulation in the number of binding sites, epinephrine pretreatment also increased the K_D of the receptors for the radioligand [^{3}H]prazosin and decreased the number of cells per dish. The molecular mechanisms involved in these novel effects of epinephrine remain to be determined. However, it should be noted that the up-regulation occurs with significantly lower concentrations of epinephrine than those required to stimulate phosphoinositide turnover. Agonist-induced up-regulation of α_{1B}-adrenoceptors and α_{1B}-adrenoceptor mRNA and increased sensitivity of phosphoinositide turnover have been reported to occur in DDT_1 MF-2 cells (Hu et al., 1993), although modest down-regulation was observed in our previous studies with DDT_1 MF-2 cells (Toews, 1987). Agonist-induced up-regulation has also been reported for β_3-adrenoceptors (Thomas et al., 1992), 5-HT (5-hydroxytryptamine, serotonin) receptors (Akiyoshi et al., 1993) and dopamine receptors (Filtz et al., 1994; Zhang et al., 1994).

Our results suggest that the properties of α_{1B}-adrenoceptors expressed in CHO-α_{1B} cells are somewhat different from those of the same receptor endogenously expressed in DDT_1 MF-2 cells. The differences in receptor regulation properties between DDT_1 MF-2 and CHO-α_{1B} cells presented here appear not to be due to the difference in receptor expression level, because these properties were investigated and confirmed in limited experiments with CHO cells expressing a lower level of α_{1B}-adrenoceptors as well. Our results therefore suggest that the determinants for α_{1B}-adrenoceptor regulation are not only at the receptor level, but probably involve cell-specific determinants as well. These results underscore the importance of careful studies of the recipient cell system in studies of transfected receptors. Further studies on wild-type and mutated α_{1B}-adrenoceptors transfected into various recipient cell systems and careful comparisons between endogenously expressed and transfected α_{1B}-adrenoceptors should greatly enhance our understanding of the molecular mechanisms involved in α_{1B}-adrenoceptor regulation.

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