PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 185, 211–218 (1987)

Down-Regulation of Glucocorticoid and β-Adrenergic Receptors on Lectin-Stimulated Splenocytes[1] (42537)

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Abstract. Activation of porcine splenocytes with the mitogen, concanavalin A, increases the number of glucocorticoid and β-adrenergic receptors with no change in the apparent dissociation constant. Incubation of splenocytes with concanavalin A in the presence of hydrocortisone 21-sodium succinate prevented this mitogen-induced increase in glucocorticoid receptors. Isoproterenol also prevented the concanavalin A-induced increase in β-adrenoceptors at 24 hr and reduced the binding affinity of these receptors at 48 hr. Neither agonist had any significant effect on the receptor number or binding affinity of nonstimulated cells. These data demonstrate that the increase in the number of glucocorticoid and β-adrenergic receptors that occur on lymphoid cells after activation by a T-cell mitogen can be prevented by appropriate hormone agonists. Down-regulation of receptor number by appropriate agonists appears to be a common regulatory system that is shared by both the neuroendocrine and the immune systems.

Lymphoid cells possess specific receptors for a variety of hormones and neurotransmitters, including glucocorticoids (1), estradiol (2), testosterone (2), insulin (3), growth hormone (4, 5), prolactin (6), adrenocorticotropin (7), β-endorphin (8), β-adrenergic agents (9, 10), substance P (11), vasoactive intestinal peptide (12), and acetylcholine (13). Some hormones, such as ACTH (14), the endogenous opiates (15, 16), and oxytocin (17), are even produced by activated lymphoid cells, so specific receptors on lymphocytes may respond to both endogenously and exogenously produced hormones. Several studies have concentrated on identifying hormone receptors on lymphocytes and estimating their number and affinity, but few experiments have provided insights into the regulation of lymphoid hormone receptors. In nonlymphoid tissue, it is well known that many ligands, such as glucocorticoids in the brain, regulate their receptor density and binding affinity *in vivo* (18) and *in vitro* (19). Both glucocorticoid and β-adrenergic receptors on the ACTH-secreting mouse pituitary tumor cell line, AtT-20, can be down-regulated by appropriate ligands (20).

It is now clear that the endocrine and immune systems share common messenger molecules (21). Reciprocal systems of communication also exist between these two physiologic systems (22). If hormone receptors on lymphoid cells could be regulated by specific ligands, this system would represent another common regulatory event that is shared by both the endocrine and the immune systems. Recent evidence with the T-cell-derived hormone interleukin 2 has shown that exogenous interleukin 2 down-regulates the biologically active high-affinity receptor (23, 24), but not the low-affinity receptor (25, 26). Similarly, exogenous vasoactive intestinal peptide down-regulates its receptors on lymphoid cells, and disappearance of this receptor reduces homing of lymphocytes to the intestinal lymphoid tissue (27).

We wondered whether such a negative feedback system for receptors might also exist in lymphoid cells for more classical endocrine hormones. In this report, we investigated the modulation of whole-cell (glucocorticoid) and surface (β-adrenergic) receptors on/in splenocytes by the T-lymphocyte mitogen, concanavalin A (Con A). We then determined whether the density and affinity of these lym-

[1] This research was supported by the U.S. Department of Agriculture (83-CSRS-2-2331), University of Illinois Agriculture Experiment Station, and Moorman Manufacturing Co.

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0037-9727/87 $1.50

phoid cell receptors were affected by their hormone agonists, cortisol and isoproterenol.

Materials and Methods. *Cell preparation.* Spleens were collected from 5-month-old pigs immediately following slaughter. Single-cell suspensions were prepared in RPMI 1640 tissue culture medium, pH 7.4, by gently scraping the spleen with a scalpel. The suspension was poured over a fine-mesh screen to remove connective tissue, and the resulting suspension was centrifuged at 400*g*, 21°C for 30 min. The cell pellet was resuspended in 10 ml Tris-buffered ammonium chloride (0.83% NH_4Cl, 0.1% $KHCO_3$, 0.01 m*M* EDTA, pH 7.4) to lyse erythrocytes. Splenocytes were then washed three times in RPMI 1640, 25 m*M* Hepes. Cell viability was determined by trypan blue exclusion and was >95% for all cell suspensions.

Mitogen activation of splenocytes. Splenocytes were prepared as described above, adjusted to a concentration of 6×10^6 viable cells/ml in RPMI 1640 supplemented with 5% fetal calf serum and then incubated in 75-cm^2 flasks (Lux, Miles Scientific, Naperville, IL) at 39°C, 7% CO_2. As in other species, Con A stimulates porcine T lymphocytes to undergo DNA synthesis (28). Splenocytes were incubated with Con A (Sigma Chemical Co., St. Louis, MO) at a final concentration of 2.5 μg/ml for 24 or 48 hr in the absence or presence of a water-soluble form of cortisol (hydrocortisone 21–sodium succinate) at a final concentration of 7.4 μ*M* (Sigma) or isoproterenol (final concentration, 1 μ*M;* Sigma) dissolved in RPMI 1640. Con A was not added to control cultures. In preliminary experiments, we determined the optimal concentrations of Con A, cortisol, and isoproterenol which were used in these experiments. After a 24- or 48-hr incubation, nonadherent cells were harvested and washed three times in RPMI 1640, 25 m*M* Hepes, and then used in the whole-cell binding assays. Viability of cells after incubation with mitogen was determined by trypan blue exclusion.

Glucocorticoid and β-adrenergic receptor assays. A whole-cell binding technique was used to quantify receptor density and binding affinity for both β-adrenergic and glucocorticoid receptors. The whole-cell binding assay for β-adrenoceptors was modified after Davies and Lefkowitz (29). In brief, 300 μl of cells (containing $3–10 \times 10^6$ viable cells) was incubated in 12 × 75-mm polypropylene culture tubes with 75 μl of 0.1 to 15 n*M* [^{3}H]CGP 12177 (4-(3-t-butylamino-2-hydroxypropoxy)-[5,7-^{3}H]benzimidazol-2-one) (sp act 38 Ci/mmole; Amersham Corp., Arlington Heights, IL). CGP 12177 is a hydrophilic β-antagonist that binds predominantly to surface receptors (30). Total binding was determined in the presence of 75 μl of 6×10^{-3} *M* ascorbate in saline and nonspecific binding was estimated by adding 75 μl of 6×10^{-6} *M* (−)-propranolol–HCl (gift from Ayerst Laboratories, New York, NY) that was also dissolved in ascorbate–saline. Tubes were incubated in a 37°C shaking water bath for 20 min. Incubation was terminated by addition of 3.0 ml ice-cold wash buffer (75 m*M* Tris–HCl, 25 m*M* $MgCl_2$, pH 7.6). Cells were pelleted and the supernatant was aspirated and washed three times by centrifugation at 800*g* for 5 min at 4°C. After the final wash, cells were resuspended in 1.0 ml HP/b scintillation cocktail (Beckman Instruments, Fullerton, CA), vortexed, and transferred to counting vials. Culture tubes were rinsed three more times with 1.0 ml HP/b and transferred to the same counting vial.

Glucocorticoid binding was measured by a modification of the whole-cell binding technique of Distelhorst *et al.* (31). Single-cell suspensions containing $2–5 \times 10^6$ viable cells in 300 μl of RPMI 1640, 25 m*M* Hepes, were incubated in 12 × 75-mm polypropylene culture tubes. The [6,7-^{3}H(N)]dexamethasone (sp act 35 Ci/mmole dissolved in benzene:ethanol (9:1); New England Nuclear Corp., Boston, MA) was diluted greater than 20,000 times in RPMI 1640, 25 m*M* Hepes, and 300 μl was added to tubes containing the cell suspensions to yield final concentrations ranging from 0.01 to 50 n*M*. Total binding was determined from tubes containing both cells and [^{3}H]dexamethasone by adding 100 μl of RPMI 1640, 25 m*M* Hepes, whereas nonspecific binding was estimated by adding 3 μ*M* unlabeled dexamethasone (Sigma). Culture tubes were incubated in a 25°C shaking water bath for 2 hr. This incubation was terminated by addition of 3.0 ml ice-cold phosphate-buffered saline (PBS), pH 7.4. Cells were pelleted by centrifugation at 800*g*, 4°C, for 5 min. Supernatants were aspirated, and the cell pellet was resuspended in 1.0 ml RPMI 1640, 25 m*M* Hepes, and then incubated for an additional

5 min in a 25°C shaking water bath. The incubation was again terminated with ice-cold PBS and the cells were pelleted by centrifugation and washed again with PBS. After the final wash, cell pellets were resuspended in 0.5 ml ethanol and incubated at room temperature for 1 hr. After this incubation, tubes were vortexed vigorously and their contents were transferred to counting vials which contained 3.5 ml HP/b scintillation cocktail.

Radioactivity for both assays was determined in a Beckman LS 5801 scintillation counter. Binding at each concentration of [^{3}H]CGP 12177 and [^{3}H]dexamethasone was measured in duplicate. Specific binding was calculated by subtracting disintegrations per minute in nonspecific binding tubes from those tubes to which no competitor was added. Data were analyzed using the computer program LIGAND (32). All results were expressed as receptor density or affinity per viable cell at the end of the 24- or 48-hr incubation period.

Statistics. Data were analyzed by analysis of variance as a randomized complete block design with repeated measures. Post hoc comparisons between individual treatment means were by the Newman–Keuls' test (33).

Results. *Glucocorticoid and β-adrenergic receptors on splenocytes.* We first confirmed that porcine splenocytes possess receptors for glucocorticoids and β-adrenergic agents. Freshly isolated porcine splenocytes displayed an average of 2300 glucocorticoid receptors per cell with a K_D of 3.4 nM as determined by Scatchard analysis (Fig. 1A). Surface membrane β-adrenoceptors were then identified on porcine splenocytes. These cells showed 1100 β-adrenoceptors with a K_D of 0.45 nM (Fig. 1B). When larger numbers of animals were tested, similar numbers and affinities of both glucocorticoid and β-adrenergic receptors were measured (Table I; see footnote). Receptor density and binding affinity for both of these hormones were similar to those found in human (34) and murine (35, 36) lymphoid cells. Although glucocorticoid receptors had previously been identified in porcine lymphoid cells (37), this is the first report to demonstrate the presence of β-adrenoceptors on the surface membrane of porcine splenocytes.

Con A increases the number of hormone receptors. In Table I and subsequent tables, cells incubated in the absence or presence of Con A displayed similar viabilities at both 24 and 48 hr. Activation of porcine splenocytes with Con A resulted in a significant increase in the number of glucocorticoid and β-adrenergic receptors per cell (Table I). In control cultures that contained no mitogen, there was no significant difference between number of receptors at time zero or after 24 and 48 hr of incubation. However, there was a 1.5-fold increase ($P < 0.01$) in the number of glucocorticoid receptors in Con A-activated cells at 24 hr. By 48 hr of incubation with Con A, there was a 2.5-fold increase ($P < 0.01$) in the number of splenic glucocorticoid receptors when compared to control cultures. Incubation of splenocytes with Con A also resulted in an increase ($P < 0.01$) in β-adrenoceptors at 24 hr. However, at 48 hr, there was no significant difference in the number of β-adrenoceptors on Con A-activated splenocytes when compared to nonstimulated cells. Activation of porcine splenocytes with Con A did not affect the apparent dissociation constant of either glucocorticoid or β-adrenergic receptors at any time period.

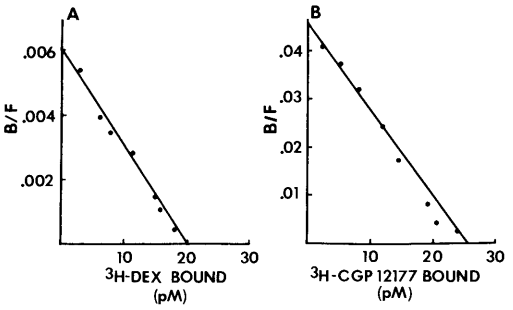

FIG. 1. Scatchard plots of [^{3}H]dexamethasone (A) and [^{3}H]CGP 12177 (B) binding curves to freshly isolated porcine splenocytes. The ratio of the amount of bound ligand to free ligand is plotted against the amount of specifically bound radiolabeled ligand. The dissociation constant (K_D) is the negative reciprocal of the slope of the line and r is the correlation coefficient ($r = -0.91$ and $r = -0.98$ for glucocorticoid and β-adrenergic receptors, respectively).

Cortisol prevents the Con A-induced increase in glucocorticoid receptors. It was then determined whether cortisol affected the number of glucocorticoid receptors in Con A-activated splenocytes. Cortisol did not affect cell viability at either 24 or 48 hr (see footnote, Table II). Con A again increased the number of glucocorticoid receptors at both 24 and 48 hr, but

TABLE I. INCREASE IN RECEPTOR DENSITY OF CONCANAVALIN A-ACTIVATED PORCINE SPLENOCYTES[a]

Type of receptor	24 hr		48 hr	
	No./cell	K_D (nM)	No./cell	K_D (nM)
Glucocorticoid				
−Con A	3031 ± 331	1.70 ± 0.16	2905 ± 331	2.10 ± 0.56
+Con A	4587 ± 696[b]	1.80 ± 0.15	7189 ± 1040[b]	2.20 ± 0.25
β-Adrenergic				
−Con A	1098 ± 169	0.30 ± 0.02	943 ± 196	0.32 ± 0.04
+Con A	3436 ± 317[b]	0.48 ± 0.10	1103 ± 207	0.42 ± 0.04

Note. Values are means ± SEM (n = 7–8/treatment). Viabilities of control cells at 24 and 48 hr were 86 ± 1 and 81 ± 1%, and for Con A-incubated cells the respective viabilities were 85 ± 2 and 82 ± 2%.

[a] Glucocorticoid receptor density at time zero averaged 2787 with a K_D of 3.7 nM, and neither of these values differed significantly from those glucocorticoid receptors at 24 or 48 hr. Similarly, β-adrenergic receptor density at time zero averaged 1186 with a K_D of 0.31 nM, and these values did not differ significantly at any time from β-adrenergic receptors on cells that were not incubated with Con A.

[b] Different from control cultures without Con A, $P < 0.01$.

this increase was only significant at 48 hr (Table II). However, cortisol prevented ($P < 0.05$) the Con A-induced increase in glucocorticoid receptors at 48 hr. This failure of Con A to increase the number of glucocorticoid receptors in cells treated with cortisol was specific for activated cells because exogenous cortisol did not significantly affect the number of receptors in control cells (those in which cortisol was added in the absence of Con A). There was no effect of Con A or cortisol on the apparent dissociation constant, which averaged 1.6 nM at 24 hr and 1.7 nM at 48 hr.

Isoproterenol prevents the Con A-induced increase in β-adrenoceptors. We then determined whether a β-agonist, isoproterenol, could modulate the number of β-adrenoceptors on Con A-activated splenocytes. Viabilities of cells incubated in the presence or absence of isoproterenol were similar (see footnote, Table III). At 24 hr, Con A again caused a 2.5-fold increase in the number of β-adrenoceptors (Table III). The β-agonist, isoproterenol, inhibited ($P < 0.01$) this Con A-induced increase in the number of β-adrenergic receptors at 24 hr. There was no effect of Con A or isoproterenol on the K_D (average 0.48 nM) at 24 hr. After 48 hr of incubation, there was neither a Con A-induced increase nor an isoproterenol-induced decrease in β-adrenoceptors. However, there was a significant reduction in the binding affinity (or increased

TABLE II. MODULATION OF GLUCOCORTICOID RECEPTORS IN PORCINE SPLENOCYTES BY CONCANAVALIN A AND CORTISOL[a]

Treatment	24 hr		48 hr	
	No cortisol	Cortisol	No cortisol	Cortisol
Control	3672 ± 881	4341 ± 552	4229 ± 1179	5171 ± 1428
Con A	4700 ± 1812	3236 ± 653	7020 ± 1614[b]	3200 ± 828

Note. Values are means ± SEM (n = 5/treatment); cortisol was used at 7.4 μM. Viabilities of control and Con A-treated cells in the absence of cortisol did not differ and averaged 94 ± 2% at 24 hr and 87 ± 2% at 48 hr. Viabilities of cells incubated with cortisol (averaged over control and Con A treatments) were similar, averaging 91 ± 2% at 24 hr and 88 ± 2% at 48 hr.

[a] At time zero, glucocorticoid receptor density averaged 3310 (K_D = 3.5 nM) and did not differ from nonstimulated cultures without exogenous cortisol at either 24 or 48 hr.

[b] Different from all other means at 48 hr $P < 0.05$.

TABLE III. REGULATION OF β-ADRENOCEPTORS ON PORCINE SPLENOCYTES BY CONCANAVALIN A AND ISOPROTERENOL[a]

	24 hr			48 hr		
Treatment	No isoproterenol	Isoproterenol	Cortisol	No isoproterenol	Isoproterenol	Cortisol
Control	1577 ± 173	1872 ± 383	2771 ± 871[b]	830 ± 26	1394 ± 261	1184 ± 164
Con A	3970 ± 423[c]	1926 ± 346	2694 ± 417	1298 ± 65	1117 ± 1213	1708 ± 83

Note. Values are means ± SEM (n = 6/treatment); isoproterenol was used at 1 μM and cortisol at 7.4 μM. Viabilities of control and Con A-treated cells in the absence of isoproterenol or cortisol were similar and averaged 94 ± 2% at 24 hr and 87 ± 2% at 48 hr. Viabilities of isoproterenol-treated cells (averaged over control and Con A treatments) were 93 ± 2% at 24 hr, and 86 ± 4% at 48 hr, and respective values for cortisol-treated cells were 91 ± 2 and 88 ± 2%.

[a] The number of β-adrenoceptors on splenocytes at time zero averaged 1242 (K_D = 0.54 nM) and these values did not significantly differ from the values at 24 and 48 hr.

[b] Differs from 1577, 1872, and 1926, $P < 0.05$.

[c] Differs from 1577, 1872, and 1926, $P < 0.01$.

K_D) of β-adrenoceptors on splenocytes which were incubated with Con A and isoproterenol for 48 hr (Table IV).

Cortisol affects lymphoid cell β-adrenoceptors. Cortisol caused a significant increase ($P < 0.05$) in the number of β-adrenoceptors on nonstimulated splenocytes at 24 hr (2771 receptors/cell) when compared to nonstimulated splenocytes incubated without cortisol (1577 receptors/cell). This effect was not apparent at 48 hr. Furthermore, incubation of splenocytes with cortisol did not affect the number of β-adrenoceptors on Con A-stimulated splenocytes at either 24 or 48 hr. However, cortisol, like isoproterenol, significantly increased ($P < 0.01$) the K_D of β-adrenoceptors at 48 hr on Con A-activated splenocytes (Table IV).

Discussion. In this report, we demonstrate that receptors for both glucocorticoids (38, 39) and β-adrenergic agents increase during T-cell activation. More importantly, appropriate hormone agonists do not affect either glucocorticoid or β-adrenergic receptors on resting lymphoid cells, but they do prevent the augmentation in receptor number that is caused during cellular activation by Con A. Both T and B cells possess receptors for glucocorticoids and catecholamines, so we chose to use unseparated cells to measure receptor number on splenocytes that were incubated with a lectin that stimulates T lymphocytes (28). These results therefore suggest that the increase in the number of receptors occurs on T cells.

Glucocorticoids have been reported to decrease their own number of receptors on resting cultured human T cells and also block the PHA-induced increase in glucocorticoid receptor activity in cultured T cells (40). These effects may be caused by specific binding of the glucocorticoid–receptor complex to nuclear DNA or perhaps they are indirectly caused by metabolic by-products of cortisol catabolism. However, it was not known whether surface receptors (as compared to cytosolic glucocorticoid receptors) on nontransformed porcine lymphoid cells can be augmented by a T-cell lectin and whether they can also be down-regulated. These results show that similar to the effect of cortisol in regulating its receptor, isoproterenol also prevented the Con A-induced increase in β-adrenergic receptors. At 48 hr, this increase in receptor number was no longer apparent, but there was

TABLE IV. DISSOCIATION CONSTANT (K_D) OF β-ADRENOCEPTORS FOLLOWING 48-hr INCUBATION WITH CONCANAVALIN A, ISOPROTERENOL, AND CORTISOL[a]

Treatment	Control (nM)	Isoproterenol (nM)	Cortisol (nM)
Control	0.58 ± 0.07	0.87 ± 0.08	0.58 ± 0.06
Con A	0.68 ± 0.04	1.40 ± 0.19[b]	1.03 ± 0.14[c]

Note. Values are means ± SE (n = 6/treatment).

[a] Receptor numbers are given in Table III. The K_D averaged 0.54 nM at time zero and 0.48 nM at 24 hr.

[b] Differs from all other treatments except Con A-activated cells in the presence of cortisol, $P < 0.01$.

[c] Differs from all other treatments except control cells in the presence of isoproterenol, $P < 0.01$.

a reduction in binding affinity. Incubation of human astrocytoma cells with isoproterenol also results in a rapid decrease in binding affinity of β-adrenoceptors (41). These combined effects of a reduction in the number of β-adrenoceptors and binding affinity could reduce the physiologic effects of β-adrenergic agents on activated lymphoid cells. Chemical destruction of the peripheral sympathetic nervous system by 6-hydroxydopamine results in an increase in β-adrenoceptors on subpopulations of lymphoid cells isolated from the spleen (42). Therefore, these findings suggest that norepinephrine can also down-regulate splenocyte β-adrenoceptors *in vivo* during an immune response.

We previously reported that cortisol greatly reduces proliferative responses of porcine splenocytes that are stimulated with T-cell mitogens (43). Therefore, cortisol might inhibit the increase in glucocorticoid receptors by preventing splenocytes from passing through the cell cycle (34). However, this interpretation is not consistent with other results which showed that the increase in glucocorticoid receptors in Con A-activated splenocytes is not entirely explained by the increase in cell volume and protein which occurs during the cell cycle (31, 44).

Cortisol has a permissive effect on a variety of tissues to enhance the sensitivity of those tissues to catecholamines (45). Glucocorticoids have been reported to augment β-adrenoceptors on human liver, lung, and astrocytoma cells and on human leukocyte membrane preparations (29, 46–48). In the present experiments, there was an increase in the number of β-adrenoceptors on nonstimulated splenocytes that were treated with cortisol. However, there was no effect of cortisol on the number of β-adrenoceptors on Con A-activated splenocytes. The cortisol-induced increase may have been masked by the augmentation in β-adrenoceptors that was caused by Con A. Although the mechanism for these effects of cortisol on β-adrenoceptors is not understood, our data are consistent with those of other studies in which nonlymphoid cells were used. The heterologous regulation of β-adrenoceptors on nonstimulated lymphoid cells by cortisol may act to increase the sensitivity of lymphoid cells to catecholamine-stimulated adenylate cyclase activity, as in other nonlymphoid tissues.

An important general pattern of cell regulation begins to emerge when regulatory events of the immune and endocrine systems are compared. It appears that receptors for classical hormones, such as glucocorticoids and catecholamines, as well as cytokine hormones derived from lymphoid cells (e.g., interleukin 2), are increased by activating agents (antigens and mitogens) and reduced by specific hormone agonists (e.g., interleukin 2, dexamethasone, isoproterenol). These data therefore support the concept of reciprocal systems of communication between the endocrine and immune systems and also describe a common regulatory system for hormone receptors on both lymphoid and endocrine cells.

The authors thank Ayerst Laboratories, New York, for the generous gift of (−)-propranolol-HCl.

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Received September 26, 1986. P.S.E.B.M. 1987, Vol. 185.
Accepted March 9, 1987.