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Studies on Neuropeptide Y Receptors in a Mouse Adrenocortical Cell Line

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SUMMARY

The mouse adrenocortical Y-1 cell line has been found to express high affinity binding sites for neuropeptide Y (NPY). Pharmacological studies have shown that these NPY binding sites are of the Y_1 type. Reverse transcription-polymerase chain reaction using primers specific for the rat Y_1 receptor revealed that the NPY Y_1 receptor mRNA is present in Y-1 cells. The K_d of the receptor for NPY was found to be 1.75 ± 0.20 nM and the B_{max} was 265 ± 18 fmol/mg. The NPY Y_1 receptors in this adrenocortical cell line were shown to be coupled to pertussis toxin-sensitive G proteins. Stimulation of Y_1 receptors resulted in the inhibition of forskolin- and adrenocorticotropic hormone (ACTH)-stimulated cAMP synthesis. NPY had no effect on basal steroid release from the Y-1 cells. At an ACTH concentration of 0.1 μM, NPY did not affect ACTH-stimulated steroid release, although NPY did inhibit cAMP production under the same hormonal conditions. cAMP profoundly affected the density of the NPY receptors in Y-1 cells. Treatment of the cells with N^6,2′-*O*-dibutyryl-cAMP or ACTH reduced the Y_1 receptor density by >50%. On the other hand the steroid dexamethasone increased the density of Y_1 receptors by 35%. Although additional detailed studies are necessary, these results may have interesting implications for the functions of ACTH, steroids, and NPY in the pituitary-adrenocortical axis.

NPY, a 36-amino acid peptide with a characteristic carboxyl-terminal tyrosine amide, has a widespread distribution in both the central and peripheral nervous systems. High affinity NPY binding sites were found to be widely distributed in the brain and adrenal gland (1–3). Pharmacological studies have identified at least three distinct NPY receptor subtypes (4). The NPY Y_1 receptor binds NPY and PYY with equal affinity and is the only receptor subtype that binds the NPY analogue [Leu^{31},Pro^{34}]NPY. The NPY Y_2 receptor also binds NPY and PYY with similar affinities. However, in contrast to the Y_1 receptor, the Y_2 receptor binds long carboxyl-terminal fragments of either peptide with high affinity but does not bind [Leu^{31},Pro^{34}]NPY. The main characteristic of the Y_3 receptor is that it binds NPY with at least 100-fold higher affinity than it binds PYY.

The possible physiological roles of NPY have been under extensive investigation. Upon intracerebroventricular administration, in ovariectomized rats, NPY elicits consummatory behavior (5) and the release of luteinizing hormone (6). Immunocytochemical studies have localized NPY-containing neuronal terminals in the vicinity of neurons that secrete corticotrophin-releasing factor in the paraventricular nucleus. This site forms a morphological basis for an action of NPY in the hypothalamic control of the pituitary-adrenocortical axis. Microinjection of NPY into the area of the paraventricular nucleus stimulated the release of ACTH and corticosterone (7). NPY was also found to stimulate corticotrophin-releasing factor release in the rat hypothalamus *in vitro* (8, 9). In bovine adrenal cortex, specific NPY binding sites are concentrated in the zona glomerulosa (1). NPY-like immunoreactivity was also observed in the nerve fibers invading the zona glomerulosa of the rat adrenal cortex, as well as the zona fasciculata and the zona reticularis (2).

The function of NPY in the neuroendocrine regulation of adrenal cortex, however, is not very clear. Inconsistencies are found in experimental data. According to Neri *et al.* (10), 1 μM NPY inhibited both basal and ACTH-stimulated aldosterone and 18-hydroxycorticosterone secretion in isolated rat zona glomerulosa cells. In contrast, Lesniewska *et al.* (11) observed that 1 μM NPY enhanced basal steroid secretion in cells cultured from the same area. NPY was also found to increase aldosterone release in isolated rat zona glomerulosa cells, and this stimulation was speculated to be via the NPY Y_3 receptor (12).

The discrepancies observed regarding the effect of NPY

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ABBREVIATIONS: NPY, neuropeptide Y; ACTH, adrenocorticotropic hormone; Bt_2cAMP, N^6,2′-O-dibutyryl-cAMP; PYY, peptide YY; RT, reverse transcription; PCR, polymerase chain reaction; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

may be the result of the variations in tissue preparation. To avoid these variations, we examined the possible NPY binding sites using the adrenal gland-derived Y-1 cell line. These Y-1 cells, derived originally from a mouse adrenocortical tumor, exhibit many properties of adrenocortical cells, including expression of ACTH receptors and maintenance of steroid secretion in response to ACTH. In this study, Y-1 cells were found to express high levels of NPY binding sites. The subtype of these binding sits was verified. The possible influence of NPY on intracellular cAMP concentrations and some of the effects of NPY on steroid secretion were investigated. The influences of ACTH, cAMP, and steroid on the density of NPY receptors were also studied.

Materials and Methods

Adrenocortical Y-1 cell line. Y-1 cells were obtained from the American Type Culture Collection and were cultured in Ham's F-10 medium (Gibco) supplemented with 15% horse serum, 2.5% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. Cells were maintained at 37°, in 5% CO_2 with 96% humidity.

Binding assays. Y-1 cells cultured in 24-well plates were washed once with Krebs-Ringer buffer (135 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 5 mM $NaHCO_3$, 1.2 mM $CaCl_2$, 20 mM HEPES, pH 7.4) and incubated in the same buffer containing 0.5% bovine serum albumin (Sigma), 0.1% bacitracin (Sigma), and the radioactive ligand *N*-[*propionyl*-^{3}H]NPY (Amersham) or iodinated PYY (NEN), with or without unlabeled displacer NPY or PYY at appropriate concentrations. The mixtures were incubated at 37° for 1 hr, with slow shaking, and then washed three times with ice-cold Tris buffer (50 mM Tris·HCl, pH 7.7). The cells were lysed with 0.1% Triton X-100, and the radioactivity was counted with a scintillation counter or a γ counter. The protein concentrations were determined with the Bio-Rad protein assay. NPY, [Leu^{31},Pro^{34}]NPY, and ACTH were from Bachem and Bt_2cAMP was from Sigma. NPY_{13-36} was a kind gift from Dr. Alain Fournier (Dept. of Psychology, McGill University, Montreal, Quebec, Canada).

RT-PCR. Y-1 cells were cultured in 75-cm^2 flasks, total RNA was isolated from the culture according to the method of Dahle and Macfarlane (13), and first-strand cDNAs were synthesized by using reverse transcriptase (Gibco). The forward primer (TTCCAAAATG-TATCACTTGCGGC) and reverse primer (GCAGCTTCAGATTTTT-TCATTGTCATT) specific for the rat Y_1 receptor were used in the PCR (13). Both primer sequences were different from the corresponding mouse Y_1 receptor sequence at only one base (14). The following PCR conditions were used: 5 min at 95° for one cycle and then 1 min at 93°, 2 min at 55°, and 2 min at 72° for 35 cycles.

cAMP measurement. Y-1 cells were cultured in 24-well plates. After treatment with forskolin or different concentrations of peptide, cAMP was extracted from cells with ice-cold ethanol containing 0.1 M HCl and was chilled immediately (for details, see Ref. 15). The samples were stored at −20° overnight, the cells were then scraped from the plates, and cAMP in the ethanol extracts was quantitated with immuno-radioactivity assay kits (PerSeptive Diagnostics).

Steroidogenesis. Cells were cultured in 24-well plates. After 24 hr, the culture medium was changed to low-serum medium (F-10 medium containing 1.5% fetal bovine serum and antibiotics), and cells were incubated for another 24 hr. Peptides were added to each well in 0.5 ml of low-serum medium. After specific time periods, the medium was collected into tubes, 50 μl of 0.25 N NaOH were added to each tube, and the steroids were extracted with 1 ml of methylene chloride, according to the method of Reyland *et al.* (16). After vortex-mixing and centrifugation, the medium in the upper layer was removed by aspiration. One milliliter of 65% H_2SO_4 in ethanol was then added to the methylene chloride extract. After the mixture was maintained at room temperature for 90 min, the samples were centrifuged and the upper layer of methylene chloride was removed by aspiration. Steroids in the H_2SO_4/ethanol solution were quantitated by measuring fluorescence excited at 470 nm and emitted at 526 nm, using 4-pregenen-20α-ol-3-one as the standard.

Results

Y-1 cells express high affinity binding sites for NPY. The saturation binding study revealed that the adrenocortical Y-1 cells displayed high affinity binding sites for [^{3}H]NPY. The Scatchard analysis showed that the NPY binding had a K_d of 1.75 ± 0.20 nM and a B_{max} of 263 ± 18 fmol/mg (Fig. 1). These NPY binding sites showed equal affinity for NPY, PYY, and the NPY Y_1 receptor-specific agonist [Leu^{31},Pro^{34}]NPY. However, the carboxyl-terminal fragment NPY_{13-36}, which is a NPY Y_2 receptor-specific agonist, had very low potency in displacing bound [^{3}H]NPY (Fig. 2).

The binding of both [^{3}H]NPY and ^{125}I-PYY to the endogenous NPY binding sites of Y-1 cells could be displaced by unlabeled NPY and PYY, to the same extent. The competition binding studies carried out with [^{3}H]NPY and ^{125}I-PYY showed the same level of nonspecific binding using a 1000-fold excess of nonradiolabeled NPY (0.1 μM), PYY (0.1 μM), or [Leu^{31},Pro^{34}]NPY (0.1 μM). These data suggest that this adrenocortical cell line expresses a single subtype of NPY receptors, which we have shown to be of the Y_1 type.

RT-PCR using rat NPY Y_1 receptor primers confirmed that this Y-1 cell line expresses NPY Y_1 receptor mRNA. The two primers used in PCR were specific for the rat Y_1 gene and were different from the mouse Y_1 gene sequence at only one base (13, 14). The PCR result revealed a product of 608 base pairs, which is the same size as the control product from total RNA isolated from 293 cells stably transfected with the rat

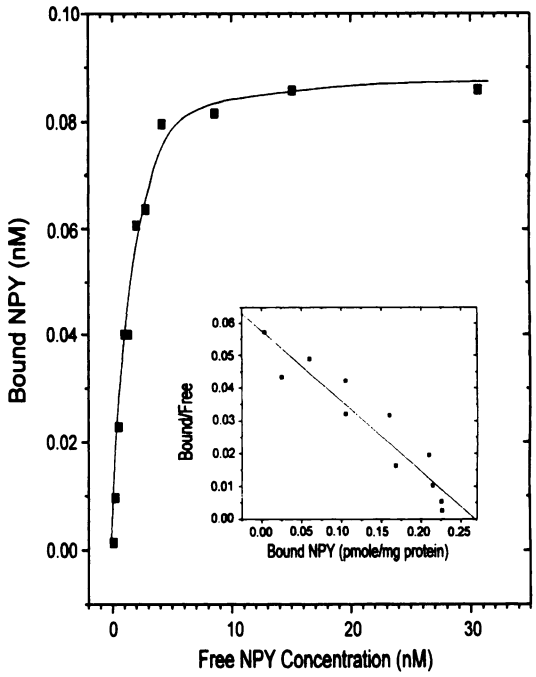

Fig. 1. Specific [^{3}H]NPY binding to adrenocortical Y-1 cells. The dissociation constant (K_d) and the number of binding sites (B_{max}) were obtained by Scatchard transformation (*inset*) of the saturation data (K_d = 1.75 ± 0.20 nM, B_{max} = 263 ± 18 fmol/mg of protein).

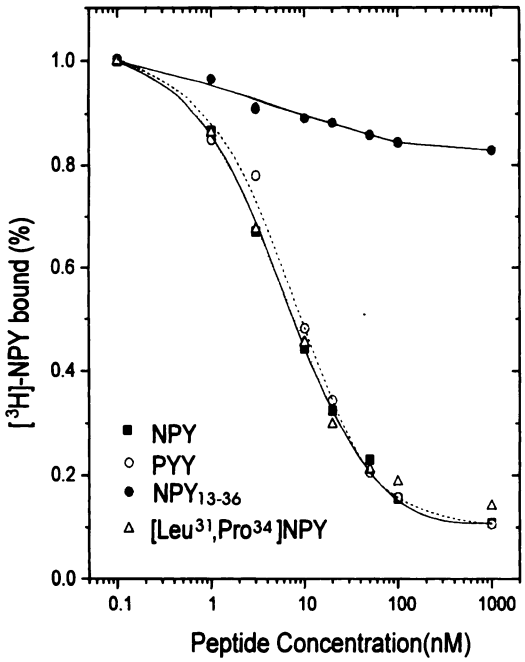

Fig. 2. Ligand competition for [^{3}H]NPY binding in Y-1 cells. Competition data are presented as the percentage of total binding in the absence of competitor. Each data point is the mean of two triplicate experiments. The concentration of [^{3}H]NPY was 0.1 nM.

Y_1 receptor cDNA (Fig. 3). Total RNA isolated from COS-1 cells was used as a negative control.

The Y_1 receptors of adrenocortical Y-1 cells are coupled to a G protein that inhibits adenyl cyclase. In cells that express the NPY Y_1 receptor, such as neuroblastoma SK-N-MC cells, the receptor is coupled to a heterotrimeric G protein that inhibits adenyl cyclase (17). In the adrenocortical Y-1 cells, NPY receptor activation also inhibited the production of cAMP. Fig. 4A shows that the application of NPY to the culture medium inhibited forskolin-stimulated cAMP production, in a concentration-dependent manner. This inhibition was abolished by pretreatment of the cells with pertussis toxin, which suggests that the NPY receptor in Y-1 cells is coupled to a pertussis toxin-sensitive G protein.

The mouse adrenocortical Y-1 cells are widely used as a model to study steroid secretion. This cell line expresses ACTH receptors, which, upon activation, stimulate steroid release. This action of ACTH is mediated by activation of

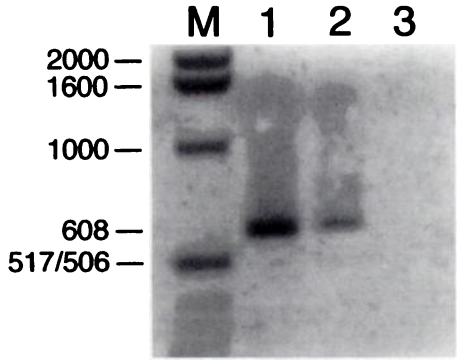

Fig. 3. RT-PCR results obtained using first-strand cDNAs synthesized from total RNA from the Y-1 cell line (*lane 2*), a 293 cell line transfected with rat Y_1 receptor cDNA (*lane 1*), or COS-1 cells (*lane 3*), under the same reaction conditions. *Lane M*, molecular mass standards.

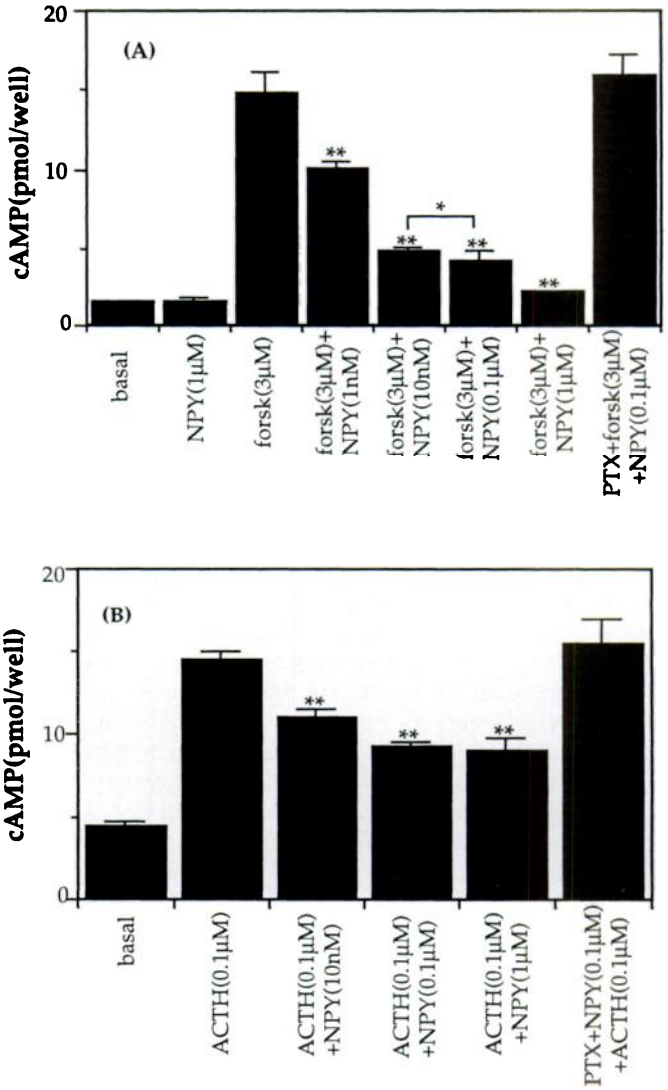

Fig. 4. Effects of NPY on cAMP formation. A, Concentration-response curve for NPY effects on forskolin (*forsk*) (3 μM)-stimulated cAMP accumulation. Cells were incubated with different concentrations of NPY for 1 min at 37° and then stimulated with 3 μM forskolin for another 15 min. B, Concentration-response curve for NPY effects on ACTH (0.1 μM)-stimulated cAMP accumulation. ACTH and different concentrations of NPY were added simultaneously. The incubation was for 15 min. Results for cells treated overnight with 100 ng/ml pertussis toxin (*PTX*) are also shown. The values indicated are means ± standard errors of six independent determinations. *, $p < 0.01$; **, $p < 0.001$, compared with control experiments.

adenyl cyclase, which increases cellular cAMP concentrations. We examined the interactions of the NPY Y_1 receptor and the ACTH receptor at the second messenger level. ACTH stimulated cAMP production in Y-1 cells. The half-maximal and saturating concentrations of ACTH for this stimulation were 50 nM and 1 μM, respectively (data not shown). NPY inhibited the ACTH-stimulated cAMP increase in a concentration-dependent manner, similarly to the inhibition of the forskolin stimulation of cAMP (Fig. 4B). However, the NPY inhibition of ACTH stimulation was not as effective as its inhibition of forskolin-mediated cAMP accumulation. NPY at 1 μM inhibited only 50% of the cAMP increased by ACTH, in contrast to forskolin stimulation, in which case NPY reduced the cAMP concentration almost to basal levels.

The stimulatory ACTH and inhibitory NPY effects on cAMP production were long lasting effects. These effects could be observed even after 6 hr of incubation, as long as the peptides were present in the medium.

Some effects of NPY on steroidogenesis were investigated. Fig. 5 shows the concentration-response relationship for NPY effects on basal and ACTH-stimulated steroid secretion. NPY did not affect the basal level of steroid secretion. Although ACTH stimulation of steroid production is mediated by elevation of intracellular cAMP concentrations (18) and NPY inhibits ACTH-stimulated cAMP production, up to 1 μM NPY did not inhibit steroid secretion significantly, with ACTH concentrations of 10 nM to 1 μM. The result obtained with 0.1 μM ACTH is shown in Fig. 5. These phenomena were not time dependent, as shown by the time course of steroid secretion (Fig. 6).

Increased intracellular levels of cAMP reduced NPY receptor density. In many systems, second messengers such as cAMP not only produce acute responses but also regulate long term gene expression. We examined the effect of cAMP on the NPY binding sites in Y-1 cells. Treatment of the Y-1 cells with Bt_2cAMP in the micromolar range reduced the density of NPY binding sites by >70% (Fig. 7A).

In the adrenal cortex, ACTH controls a wide spectrum of cell activities, such as regulation of steroidogenesis, gene expression, and cell division, through a single signal transduction pathway involving cAMP. The effect on NPY receptor expression seen after treatment of cells with the cAMP analogue was also observed after treatment of cells with ACTH. The binding assays were performed on cells treated with ACTH. The down-regulation of NPY receptors occurred after 24 hr of ACTH treatment. A >50% reduction of NPY receptors was observed after 48 hr (Fig. 7B). As a control, treatment of Y-1 cells with different concentrations of NPY for the same period of time did not affect the number of NPY binding sites.

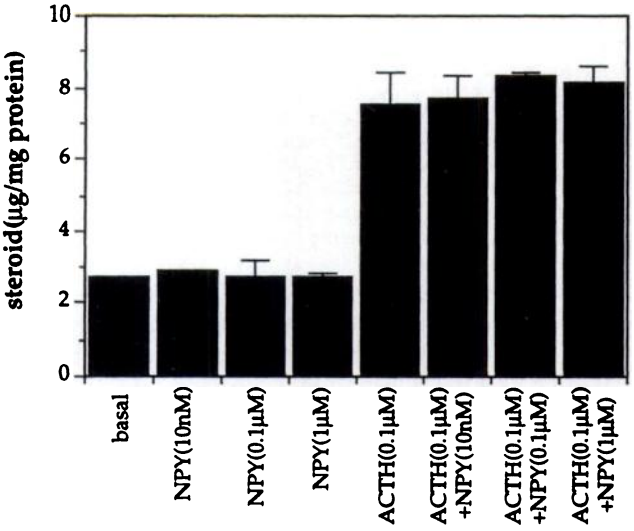

Fig. 5. Effect of different concentrations of NPY on basal and ACTH-stimulated steroid production in Y-1 cells in a 24-hr period. The values indicated are means ± standard errors of triplicate determinations. There was no effect of NPY on basal or ACTH (0.1 μM)-stimulated steroid secretion. For all values with NPY treatment, $p > 0.05$, compared with control measurements.

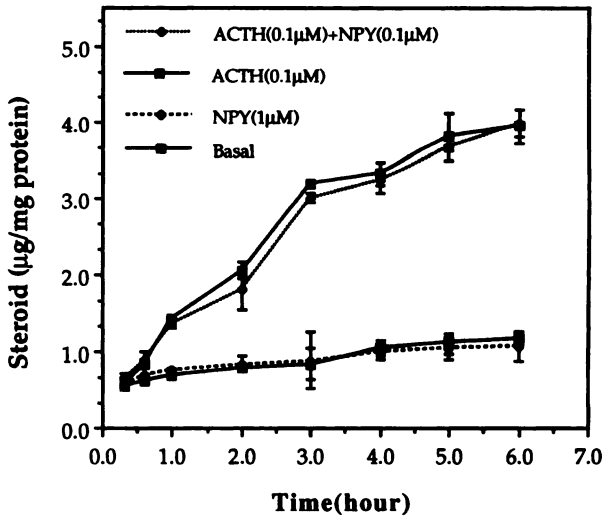

Fig. 6. Time course of NPY effects on ACTH-stimulated steroid production in mouse adrenocortical Y-1 cells. Cells were cultured in 24-well plates. At the indicated times, medium from identical wells was removed and total fluorogenic steroids in the medium were measured. The steroid production is expressed as fluorescence intensity. The data are the means ± standard errors of triplicate experiments. This is a typical result from six repeated experiments.

Steroid treatment increased NPY receptor density in Y-1 cells. Steroids are important regulators of many physiological processes. Steroid action is often mediated by binding to steroid receptors. The steroid-receptor complex may bind to steroid response elements in a gene promoter region, thus controlling gene transcription. In the mouse NPY Y_1 gene, there are several steroid response elements (14). We were interested in how steroids may affect NPY receptor gene expression. Treatment of Y-1 cells with dexamethasone for 48 hr increased receptor density. The increase in the receptor density was dependent on the steroid concentration (Fig. 8). Dexamethasone at 10 μM increased the receptor density by about 35%. Although the increase was small, it was statistically significant ($p < 0.001$).

Discussion

In previous studies, the subtype of NPY binding sites in bovine and rat adrenal cortex was not fully determined (1, 19). In this study, we have found that the adrenocortical Y-1 cell line expresses a single class of NPY/PYY receptors, which is of the Y_1 type. The K_d value of the receptor is in the nanomolar range, which is comparable to the affinity of the Y_1 receptor measured in other cell lines. In the CHP-212 neuroblastoma cell line the K_d of the endogenous Y_1 receptor was found to be 4.8 nM (20), in SK-N-MC cells the K_d of the Y_1 receptor was determined to be 0.27 nM, and in COS-1 cells that transiently express the cloned Y_1 receptor the K_d value was 0.68 nM (4, 13). The level of receptor expression in Y-1 cells is similar to that in SK-N-MC cells (4).

The NPY receptor in the Y-1 cell line is linked to a pertussis toxin-sensitive G protein that inhibits adenyl cyclase. In our experiments, NPY inhibited forskolin-stimulated cAMP accumulation and ACTH-stimulated cAMP accumulation. However, the inhibitions were to different extents. NPY inhibition of ACTH stimulation is not as effective as its inhibition of forskolin-stimulated cAMP accumulation. This phe-

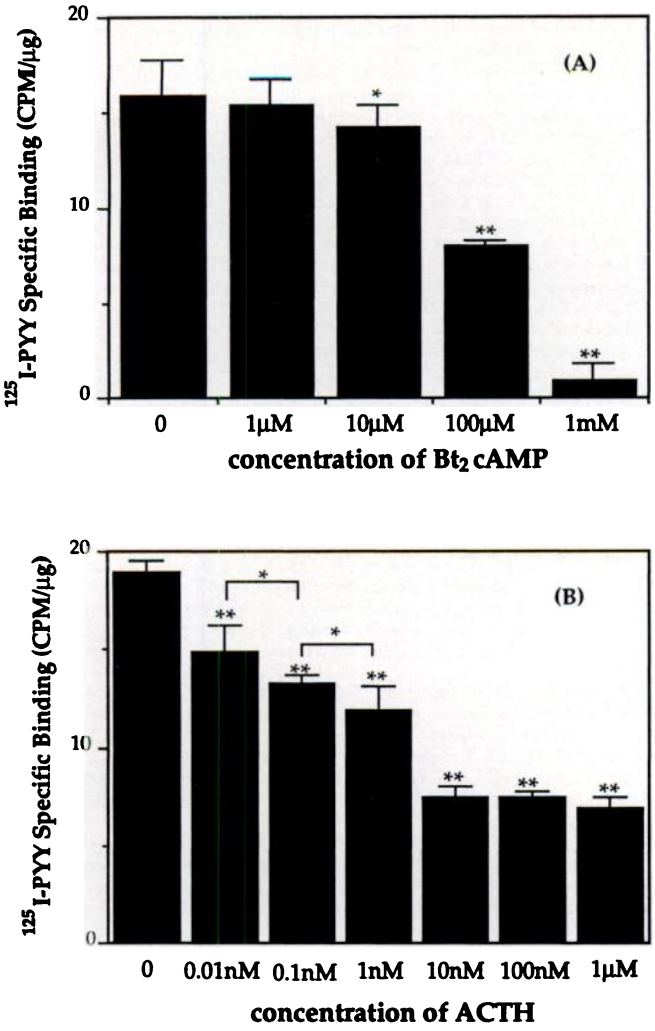

Fig. 7. Effects of increasing intracellular cAMP levels on NPY Y_1 receptor density in the Y-1 cell line. Cells were incubated for 48 hr with low-serum medium containing different concentrations of Bt_2cAMP (A) or ACTH (B). Binding assays were carried out under the same conditions for all samples. The results were normalized to protein content and expressed as specific ^{125}I-PYY binding per microgram of protein. Data are means ± standard errors of nine independent measurements. *, $p < 0.01$; **, $p < 0.001$, compared with control measurements.

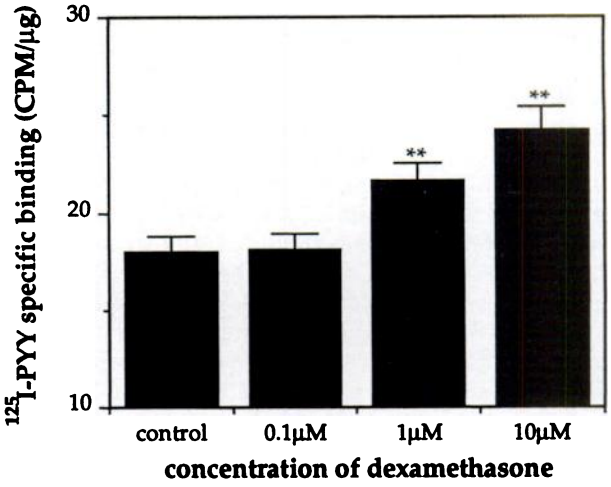

Fig. 8. Effects of steroid treatment on NPY Y_1 receptor density in the Y-1 cell line. Cells were incubated for 48 hr with low-serum medium containing different concentrations of dexamethasone. Binding assays were carried out under the same conditions as indicated for Fig. 7. Data are means ± standard errors of nine independent measurements. **, $p < 0.001$, compared with control.

nomenon may be an indication of the fact that forskolin and the stimulatory G protein G_s stimulate adenyl cyclase through different mechanisms. It has been proposed that forskolin and $G_{s\alpha}$ stimulate adenyl cyclase by regulating the interaction between its cytoplasmic loops. Forskolin might do so by altering the fluidity of the microenvironment around a portion of the transmembrane regions, whereas GTP-liganded $G_{s\alpha}$ interacts with adenyl cyclase in the cytosolic region of the plasma membrane (21). Binding of NPY to its cell surface receptor causes stimulation of the inhibitory G protein G_i. The GTP-bound G_i α subunit may compete with the two stimulating processes induced by forskolin and $G_{s\alpha}$ with different rates, which may result in different levels of enzyme activity. Moreover, in the presence of ACTH, G protein $\beta\gamma$ subunits released from activated $G_{s\alpha}$ could compete with adenyl cyclase for activated $G_{i\alpha}$ binding, reducing the inhibition of the enzyme.

Steroid hormone production in the adrenal gland is regulated primarily by ACTH via the second messenger cAMP. cAMP activation of cAMP-dependent protein kinase A results in acute responses characterized by changes in cell shape and mobilization, as well as transport of cholesterol from storage sites to the inner mitochondrial membrane, where cholesterol is utilized for steroid hormone biosynthesis (16). In an ACTH concentration range of 10 nM to 1 μM, NPY did not significantly influence steroid hormone secretion, although it partially inhibited cAMP elevation induced by ACTH. This illustrates the fact that the increase in cAMP levels is not the rate-limiting step in the process of steroid secretion. Other studies have shown that it is not cAMP formation but the action of the cytochrome P450-cholesterol side chain cleavage enzyme that constitutes the rate-limiting step in steroidogenesis (22). As a result, steroid production reaches its half-maximal value at a much lower ACTH concentration, i.e., about 40 pM (23), whereas the half-maximal concentration for cAMP stimulation is 50 nM. The saturating concentration of ACTH for steroid secretion is about 0.5 nM. Below this hormone concentration, we expect NPY to inhibit ACTH-stimulated steroid secretion, although this still needs to be substantiated by corresponding experiments. The inhibition of ACTH-stimulated cAMP production by NPY described in this study may offer a possible biochemical basis for the finding that NPY inhibited production of some steroids *in vitro* and *in vivo* (10, 11). The stimulating effect of NPY on steroid secretion observed by others (12, 24) may be mediated through other receptor systems. Recently, Bernet *et al.* (19) found that NPY- and vasoactive intestinal peptide-induced aldosterone secretion by the rat capsule/zona glomerulosa could be mediated by catecholamines via β_1-adrenergic receptors.

In addition to its acute actions on steroidogenesis, ACTH exerts chronic effects on the adrenal gland, including maintenance of steroid hydroxylase gene expression (25). In this study, we found that long term increases in intracellular cAMP concentrations, either produced directly by addition of Bt_2cAMP to the culture or stimulated by treatment of the cells with ACTH, reduced NPY receptor density dramati-

cally. However, we cannot determine from the binding studies alone whether this reduction of receptor density is due to receptor internalization or due to suppression of gene expression, although the time scale of the process suggests that the change may occur at the gene transcription level. The mouse NPY Y_1 receptor gene has an upstream CGTCA sequence that is essential for biological activity of the cAMP-regulating element (14). It is possible that cAMP exerts its effect through a protein that binds to this cAMP response element.

The putative promoter region of the mouse NPY Y_1 receptor gene also contains several reverse complement consensus sequences for steroid receptor binding (14). However, gene structure analysis alone cannot offer information about how steroids may affect gene expression. In this study, we demonstrated that treatment of Y-1 cells with steroids increased NPY receptor density.

We do not know from the current experiments whether these findings would also be observed under physiological conditions. If the same type of process exists *in vivo*, NPY receptors may serve as a sensor of ACTH and steroid levels in the adrenal cortex. The interactions between ACTH, steroids, and NPY receptors may be important in balancing hormonal actions and may have important implications in adrenocortical function and the function of NPY in the pituitary-adrenocortical axis.

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References

1. Torda, T., R. A. Cruciani, and J. M. Saavedra. Localization of neuropeptide Y binding sites in the zona glomerulosa of the bovine adrenal gland. *Neuroendocrinology* **48:**207–210 (1988).
2. Maubert, E., G. Tramu, and J. P. Dupouy. Co-localization of vasoactive intestinal polypeptide and neuropeptide Y immunoreactivities in the nerve fibers of the rat adrenal gland. *Neurosci. Lett.* **113:**121–126 (1990).
3. Wharton, J., L. Gordon, J. Byrne, H. Herzog, L. A. Selbie, K. Moore, M. H. F. Sullivan, M. G. Elder, G. Moscosco, K. M. Taylor, J. Shine, J. M. Polak. Expression of human neuropeptide tyrosine Y_1 receptor. *Proc. Natl. Acad. Sci. USA* **90:**687–691 (1993).
4. Wahlestedt, C., D. Regunathan, and D. J. Reis. Identification of cultured cells selectively expressing Y_1-, Y_2-, or Y_3-type receptors for neuropeptide Y/peptide YY. *Life Sci.* **50:**7–12 (1991).
5. Levine, A. S., and J. E. Morley. Neuropeptide Y: a potent inducer of consummatory behaviour in rats. *Peptides* **5:**1025–1029 (1984).
6. Kalra, S. P., and W. R. Crowly. Norepinephrine-like effects of neuropeptide Y on LH release in the rat. *Life Sci.* **35:**1173–1176 (1984).
7. Wahlestedt, C., G. Skagerberg, R. Ekman, M. Heilig, F. Sundler, and R. Haökanson. Neuropeptide Y (NPY) in the area of the hypothalamic paraventricular nucleus activates the pituitary-adrenocortical axis in the rat. *Brain Res.* **417:**33–38 (1987).
8. Haas, D. A., and S. R. George. Neuropeptide Y administration acutely increases hypothalamic corticotropin-releasing factor immunoreactivity: lack of effect in other rat brain regions. *Life Sci.* **41:**2725–2731 (1987).
9. Tsagarakis, S., L. H. Rees, G. M. Besser, and A. Grossman. Neuropeptide Y stimulates CRF-41 release from rat hypothalami *in vitro*. *Brain Res.* **502:**167–170 (1989).
10. Neri, G., P. G. Andreis, and G. G. Nussdorfer. Effects of neuropeptide Y and substance P on the secretory activity of dispersed zona glomerulosa cells of rat adrenal gland. *Neuropeptides* **17:**121–125 (1990).
11. Lesniewska, B., M. Nowak, B. Miskowiak, G. G. Nussdorfer, and L. K. Malendowicz. Long-term effects of neuropeptide Y on the rat adrenal cortex. *Neuropeptides* **16:**9–13 (1990).
12. Bernet, F., E. Maubert, J. Bernard, V. Montel, and J. P. Dupouy. *In vitro* steroidogenic effects of neuropeptide Y (NPY_{1-36}), Y_1 and Y_2 receptor agonists (Leu^{31}-Pro^{34}NPY, NPY_{18-36}) and peptide YY (PYY) on rat adrenal capsule/zona glomerulosa. *Regul. Peptides* **52:**187–193 (1994).
13. Larhammar, D., A. G. Blomqvist, F. Yee, E. Jazin, H. Yoo, and C. Wahlestedt. Cloning and functional expression of a human neuropeptide Y/peptide YY receptor of the Y_1 type. *J. Biol. Chem.* **267:**10935–10938 (1992).
14. Eva, C., A. Oberto, R. Sprengel, and E. Genazzani. The murine NPY-1 receptor: gene structure and delineation of tissue-specific expression. *FEBS. Lett.* **314:**285–288 (1992).
15. Farndale, R. W., L. M. Allan, and R. B. Martin. Adenylate cyclase and cAMP, in *Signal Transduction. The Practical Approach* (D. Rickwood and B. D. Hames, eds.). IRL Press, New York, 75–103 (1992).
16. Reyland, M. E., J. T. Gwynne, P. Forgez, M. M. Prack, and D. L. Williams. Expression of the human apolipoprotein E gene suppresses steroidogenesis in mouse Y-1 adrenal cells. *Proc. Natl. Acad. Sci. USA* **88:**2375–2379 (1991).
17. Shigeri, S., and M. Fujimoto. Two different signal transductions of neuropeptide Y_1 receptor in SK-N-MC cells. *Biochem. Biophys. Res. Commun.* **187:**1565–1571 (1992).
18. Clegg, C. H., M. S. Agrahamsen, J. L. Degen, D. R. Morris, and S. G. McKnight. Cyclic AMP-dependent protein kinase controls basal gene activity and steroidogenesis in Y-1 adrenal tumor cells. *Biochemistry* **31:** 3720–3726 (1992).
19. Bernet, F., J. Bernard, C. Laborie, V. Montel, E. Maubert, and J. P. Dupouy. Neuropeptide Y (NPY)- and vasoactive intestinal peptide (VIP)-induced aldosterone secretion by rat capsule/glomerular zona could be mediated by catecholamines via β_1-adrenergic receptors. *Neurosci. Lett.* **166:**109–112 (1994).
20. Sheikh, S. P., M. M. T. O'Hare, O. Tortora, and T. W. Schwartz. Binding of monoiodinated neuropeptide Y to hippocampal membranes and human neuroblastoma cell lines. *J. Biol. Chem.* **264:**6648–6654 (1989).
21. Iyengar, R. Molecular and functional diversity of mammalian G_s-stimulated adenylyl cyclases. *FASEB. J.* **7:**768–775 (1993).
22. Reyland, M. E., and D. L. Williams. Suppression of cAMP-mediated signal transduction in mouse adrenocortical cells which express apolipoprotein E. *J. Biol. Chem.* **266:**21099–21104 (1991).
23. Cortese, F., and J. Wolff. Cytochalasin-stimulated steroidogenesis from high density lipoproteins. *J. Cell Biol.* **77:**507–515 (1978).
24. Kondo, H. Immunohistochemical analysis of the localization of neuropeptide in the adrenal gland. *Arch. Histol. Jpn.* **48:**453–481 (1985).
25. Black, S. M., G. D. Szklarz, J. A. Harikrishna, D. Lin, R. C. Wolf, and W. L. Miller. Regulation of proteins in the cholesterol side-chain cleavage system in JEG-3 and Y-1 cells. *Endocrinology* **132:**539–545 (1993).

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