Rat Neuropeptide Y Precursor Gene Expression

mRNA STRUCTURE, TISSUE DISTRIBUTION, AND REGULATION BY GLUCOCORTICOIDS, CYCLIC AMP, AND PHORBOL ESTER

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Rat brain neuropeptide Y precursor (prepro-NPY) cDNA clones were isolated and sequenced in order to study regulation of the prepro-NPY gene. Rat prepro-NPY (98 amino acid residues) contains a 36-residue NPY sequence, followed by a proteolysis/amidation site Gly-Lys-Arg, followed by a 30-residue COOH-terminal sequence. The strong evolutionary conservation of rat and human sequences of NPY (100%) and COOH-terminal peptide (93%) suggests that both peptides have important biological functions.

In the rat central nervous system, prepro-NPY mRNA (800 bases) is most abundant in the striatum and cortex and moderately abundant in the hippocampus, hypothalamus, and spinal cord. The rat adrenal, spleen, heart, and lung have significant levels of prepro-NPY mRNA.

Regulation of the prepro-NPY mRNA abundance was studied in several rodent neural cell lines. PC12 rat pheochromocytoma and N18TG-2 mouse neuroblastoma cells possess low basal levels of prepro-NPY mRNA, while NG108-15 hybrid cells possess high levels. Treatment of PC12 cells with a glucocorticoid such as dexamethasone or elevation of cAMP by forskolin increased the prepro-NPY mRNA level 2–3-fold or 3–10-fold, respectively. In N18TG-2 cells dexamethasone and forskolin synergistically increased prepro-NPY mRNA 7-fold. Treatment of PC12 cells with the protein kinase C activator phorbol 12-myristate 13-acetate alone elevated prepro-NPY mRNA marginally, but the phorbol ester plus forskolin elicited 20–70-fold increases, which were further enhanced to over 200-fold by dexamethasone and the calcium ionophore A23187. These results indicate that NPY gene expression can be positively regulated by synergistic actions of glucocorticoids, cAMP elevation, and protein kinase C activation.

Neuropeptide Y (NPY), a 36-amino acid COOH-terminally amidated peptide, was originally isolated from porcine brain by Tatamoto et al. (1, 2) and found to have a structural similarity to peptide YY and pancreatic polypeptide, which are found in the gastrointestinal tract and pancreas. One of the most abundant neuropeptides, NPY is widely distributed in central and peripheral neurons (3–5) as well as cells of neural crest origin such as adrenal chromaffin cells (6–8). NPY is localized with catecholamines, y-aminobutyric acid, or other neuropeptides in many neurons (9–12). Evidence is mounting that NPY is involved in the regulation of peripheral and cardiac artery blood pressure (4, 10, 13–15), circadian rhythms (16), release of hypothalamic hormones (17), and feeding behavior (18). NPY also inhibits catecholamine release from the rat vas deferens (19) and cultured bovine chromaffin cells (20). Brief reviews on NPY neuronal systems have appeared recently (21, 22).

The nucleotide sequence of human pheochromocytoma prepro-NPY mRNA (NPY mRNA) and the organization of the human NPY gene have been determined (23, 24). Human prepro-NPY (97 amino acid residues) contains a signal peptide sequence, the 36-amino acid NPY sequence, and a 30-residue COOH-terminal peptide sequence. The COOH-terminal peptide ("CPON") is reported to be colocalized with NPY in many tissues but as yet has no known biological or pharmacological activity (25, 26).

Information about the regulation of the NPY gene by hormones, neurotransmitters, and second-messenger systems is currently lacking. In order to study this regulation in experimental animals and in rodent NPY-containing cell lines (27, 28), we cloned and sequenced rat prepro-NPY cDNA. Using this probe we have examined the tissue-specific expression of the NPY gene in the rat and have delineated regulatory influences of glucocorticoids, cAMP, and phorbol esters on the NPY mRNA abundance in neural cell lines.

Some of these data have been presented in abstract form (29).

EXPERIMENTAL PROCEDURES AND RESULTS

Structure of Rat Brain NPY mRNA—Rat brain NPY cDNA clones were isolated (Fig. 1, Miniprint). The sequences of rat NPY mRNA and its gene product are compared in Fig. 2 with the previously determined human sequences (23). An uncommon AUGAUG sequence begins the 294-nucleotide open reading frame in rat NPY mRNA, in contrast to human NPY mRNA, in which a single AUG opens the reading frame (29).

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* The abbreviations used are: NPY, neuropeptide Y; NPY mRNA, prepro-neuropeptide Y messenger RNA; DME medium, Dulbecco's modified Eagle's medium; poly(A) RNA, polyadenylated RNA; HPLC, high-performance liquid chromatography; bp, base pairs; PMMA, phorbol 12-myristate 13-acetate or 12-O-tetradecanoylphorbol 13-acetate.
The overall similarity of rat and human NPY mRNA sequences is high (81 and 94% for nucleotides and amino acids, respectively), with extensive conservation in the coding region and near the polyadenylation signal. The amounts of amino acid identity in the three coding domains are 86, 100, and 93% for the signal, NPY, and COOH-terminal peptides, respectively. The amounts of nucleotide identity are 82, 94, and 90%, respectively.

**Tissue Distribution of NPY mRNA**—NPY mRNA from various rat brain regions electrophoresed as a broad band of mean length 800 bases (Fig. 3A). A similar estimate of length was obtained with purified poly(A)+ RNA from rat striatum, cortex, hypothalamus, and spinal cord (not shown). NPY mRNA from peripheral tissues electrophoresed in narrower bands of smaller average size (720 bases) (Fig. 3A). The levels of NPY immunoreactivity in rat brain regions are found in striatum, with cortex slightly lower. The spinal cord, hypothalamus, and hippocampus have intermediate levels. The highest abundance in brain was found in striatum, with cortex slightly lower. The spinal cord, hypothalamus, and hippocampus have intermediate levels. The levels of NPY immunoreactivity in rat brain regions are
in good agreement with NPY mRNA levels except in the hypothalamus, which has much more NPY peptide relative to its NPY mRNA content. NPY mRNA was found to be abundant also in the adrenal gland, spleen, heart, and lung.

**Regulation of NPY Gene Expression by Glucocorticoids and cAMP in Cultured Cells—**PC12 rat pheochromocytoma cells, which are studied as a model of neural crest cell differentiation (33, 34), possess a low level of NPY mRNA (0.16 ± 0.02 pg/µg total RNA (n = 39)) that is consistent with its low NPY content, previously reported (28). Treatment of cells with the potent glucocorticoid dexamethasone (1 µM) elicited a gradual elevation to 2.1–2.6 times the control between 24 and 96 h (Fig. 5). The half-maximal and maximal effects were achieved with 1.7 × 10⁻⁴ M and 10⁻³ M dexamethasone, respectively (not shown). As shown in Fig. 5, treatment of cells with 10 µM forskolin, a diterpene activator of adenylate cyclase, elicited a rapid but transient increase in the NPY mRNA level, which was maximally 2.7 times the control at 8 h. The response to forskolin was apparently desensitized by 72 h. The response to dexamethasone + forskolin at early times equaled that by forskolin alone, but at 48 and 72 h was greater than the effects of each drug alone. The results suggest that the rat NPY mRNA abundance can be positively regulated by cAMP and glucocorticoids, acting rapidly (≤ 4 h) and slowly (> 12 h), respectively.

Untreated NIH3T3 mouse neuroblastoma cells (35) also possess a low level of NPY mRNA (0.13 ± 0.02 pg/µg RNA (n = 5)). As shown in Fig. 6, this level was only slightly increased by dexamethasone or forskolin alone during 6–48 h of treatment, but the combination of both drugs produced marked elevations of up to 6.7 times the control. These results demonstrate that NPY mRNA in mouse cells is positively regulated by both glucocorticoids and cAMP elevation, with greater synergism than with PC12 cells under the conditions employed.

**Regulation of NPY Gene Expression by Combinations of Phorbol Ester, Calcium Ionophore, Glucocorticoid, and cAMP—**To elucidate the possible effects of calcium-depend-

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**TABLE I**

| Tissue            | A, abundance of NPY mRNA | B, abundance of immunoreactive NPY peptide |
|-------------------|--------------------------|----------------------------------------|
|                   | pg/µg RNA | % of striatum | pmol/g tissue | % of striatum |
| Central nervous system |   |    |   |   |
| Striatum          | 6.1 ± 0.9 | 100 | 54 ± 4 (8) | 100 |
| Frontal cortex    | 5.0 ± 0.8 | 82  | 55 ± 5 (8) | 102 |
| Hippocampus       | 2.1       | 34  | 23 ± 1 (8) | 43  |
| Hypothalamus      | 2.0 ± 0.1 | 33  | 120 ± 10 (8) | 222 |
| Medulla oblongata | 1.1       | 18  | 8 ± 1 (10) | 15  |
| Midbrain          | 0.6       | 10  | 3 ± 1 (8) | 6   |
| Cerebellum        | 0.4       | 7   | <0.3 | <0.6 |
| Spinal cord       | 1.8       | 30  | 7 ± 1 (6) | 13  |
| Other tissues     |   |    |   |   |
| Adrenal           | 2.4       | 39  | 210 ± 70° | 390 |
| Spleen            | 1.3       | 21  | 1 ± 0.3 (4) | 2 |
| Heart             | 0.7       | 11  | 27 ± 7° (4) | 50 |
| Lung              | 0.4       | 7   | 2 ± 0.9 (4) | 4   |
| Skeletal muscle   | 0.4       | 7   | NT       | NT   |
| Stomach           | 0.3       | 5   | NT       | NT   |
| Thyroid           | 0.2       | 3   | NT       | NT   |
| Liver             | 0.1       | 2   | <0.3 (4) | <0.6 |
| Kidney            | 0.1       | 2   | 0.8 ± 0.4 (4) | 1 |
| Small intestine   | 0.06      | 1   | 55 ± 26° (4) | 102 |
| Testis            | <0.06     | <1  | <0.3 (4) | <0.6 |
| Pancreas          | <0.06     | <1  | NT       | NT   |

* Ref. 8.

* Peptide values are for atrium only, while mRNA values are for whole heart.

* Peptide values are for duodenum only. High values are probably due to cross-reacting peptide YY.
Regulation of Rat Neuropeptide Y Precursor Gene Expression

The effects of combinations of cyclic nucleotide elevation, PMA, A23187, and dexamethasone on the NPY mRNA abundance of PC12 cells

PC12 cells (50-60% confluent) were treated for 24 h with 1 μM dexamethasone (Dex), 20 μM forskolin, 0.4 μM PMA, 0.4 μM 4a-phorbol 12,13-didecanoate, 0.4 μM A23187, 1 mM 8-bromo-cyclic nucleotide, and/or 1 mM N6,2' O-dibutyryl cAMP, as indicated. In experiment 1 the culture medium contained fetal bovine and horse serum that were both steroid-depleted by charcoal treatment, while in experiments 2 and 3, neither serum was charcoal-treated. The abundances of NPY mRNA are expressed as ratios to the control levels (without drugs), which were 0.05, 0.1, and 0.08 pg/μg total RNA in experiments 1-3, respectively.

Table II

| Addition to the culture medium (other than Dex) | NPY mRNA abundance relative to control |
|-----------------------------------------------|----------------------------------------|
| None                                          |                                      |
| Forskolin                                     | (1.0)                                  |
| PMA                                           | 3.7                                    |
| A23187                                        | 1.2                                    |
| Forskolin + PMA                               | 1.5                                    |
| Forskolin + A23187                            | 21.4                                   |
| PMA + A23187                                  | 2.1                                    |
| Forskolin + PMA + A23187                      | 45.0                                   |
| None                                          | (1.0)                                  |
| Forskolin                                     | 9.6                                    |
| PMA                                           | 1.8                                    |
| 4a-Phorbol didecanoate                        | 0.4                                    |
| Forskolin + PMA                               | 67.0                                   |
| Forskolin + 4a-phorbol didecanoate            | 9.8                                    |
| None                                          | (1.0)                                  |
| 8-Bromo-cAMP                                  | 3.0                                    |
| Dibutyryl cAMP                                | 14.0                                   |
| PMA                                           | 2.8                                    |
| A23187                                        | 1.1                                    |
| PMA + A23187                                  | 7.5                                    |
| 8-Bromo-cAMP + PMA                            | 34.0                                   |
| Dibutyryl cAMP + PMA                          | 60.0                                   |
| 8-Bromo-cAMP + PMA + A23187                   | 49.0                                   |
| Dibutyryl cAMP + PMA + A23187                 | 67.0                                   |
| 8-Bromo-cGMP                                  | 1.6                                    |
| 8-Bromo-cGMP + PMA                            | 3.7                                    |

As shown in Table II (experiment 3), 1 mM 8-bromo-cAMP or N6,2'-O-dibutyryl cAMP, which activates cAMP-dependent protein kinase directly, elevated NPY mRNA moderately. (The former derivative was less active than the latter or forskolin in both this effect and the production of short processes extended from the cell bodies.) The combination of either cAMP derivative with PMA elicited much higher elevations (20-70-fold) that were further potentiated (up to 200-fold) by A23187 and/or dexamethasone. The half-maximally and maximally effective concentrations of PMA were 6 and 40 nM, respectively, in the presence of forskolin (not shown). 4a-Phorbol 12,13-didecanoate, which is inactive in protein kinase C activation in contrast to 4β-phorbol esters such as PMA, was inactive in increasing the NPY mRNA abundance in the presence of forskolin (Table II, experiment 2).
The high conservation of NPY sequences in human and rat species was expected, it is especially interesting that the 5' flanking region of the recently reported sequence of the rat NPY gene (38) for the presence of these motifs, we noted a 6292 strongly synergistic positive regulation of NPY gene expression by A23187, forskolin, and PMA, and/or 0.4 μM A23187 for the durations indicated. The serum in the culture medium was not charcoal/Dextran-treated. The abundances of NPY mRNA were measured as ratios to the control level at zero time, which was 0.24 pg/μg total RNA. Symbols: ○, no drug; □, forskolin (F); △, PMA; ○, forskolin + PMA; ■, forskolin + A23187; ▲, PMA + A23187; ▼, forskolin + PMA + A23187.

The results of Table II and Fig. 7 indicate a strong and synergistic positive regulation of NPY gene expression by concomitant activations of protein kinase C and cAMP-dependent protein kinase. Glucocorticoid appears to exert a weaker, probably permissive, action. The calcium ionophore A23187 also exerts a modest potentiating action, probably by facilitating the action of PMA. Qualitatively similar results were also obtained with NPY mRNA levels in SK-N-MC human neuroblastoma cells, suggesting that these conclusions are applicable to human as well as rat NPY gene expression.

**DISCUSSION**

In this study we have attempted to lay the groundwork for investigations of the regulation of NPY gene expression in a variety of experimental systems. Furthermore, we show that NPY gene regulation appears to be controlled more strongly by the synergistic actions of multiple regulators (cAMP, protein kinase C activation, and glucocorticoids) than by single regulators.

The rat prepro-NPY cDNA sequence reported here is in agreement with reports of Allen et al. (37) and Larhammar et al. (38) published after the initial submission of this paper. While the high conservation of NPY sequences in human and rat species was expected, it is especially interesting that the sequences of the 30-residue COOH-terminal peptide, which has no reported biological or pharmacological activity, are highly conserved (85%, two divergent residues at positions 87 and 96). This degree of conservation between human and rat sequences is typical of that for polypeptide hormones such as the A and B chains of insulin (90–95%) or corticotropin (95%) and is higher than that of such sequences as insulin C-peptide (71–74%) and signal peptides (70–85%), which are apparently important mainly for precursor biosynthesis and processing (Ref. 39 and references therein). Therefore, we propose that the prepro-NPY COOH-terminal sequence or a fragment thereof has an important biological function.

The abundances of NPY mRNA in brain regions (Table I) are generally parallel to those of NPY peptide, even though some NPY neurons possess cell bodies (containing mRNA) and terminals (containing stored NPY) in different brain regions. As expected, NPY mRNA is highly abundant in the striatum and cerebral cortex, where numerous intrinsic NPY-containing neurons are found (12, 21, 22). The hypothalamus is the only region having a major discrepancy between relative NPY mRNA and NPY peptide levels, suggesting that much of the stored NPY in hypothalamus is from extrinsic rather than intrinsic neurons and/or that NPY turnover is relatively low in hypothalamus. The significant amounts of NPY mRNA in rat heart and lung may reflect the innervation of these organs by intrinsic neurons containing NPY (40, 41).

Glucocorticoids positively regulate several, perhaps many, genes governing neurotransmitter and neuropeptide biosynthesis such as tyrosine hydroxylase (42) and proenkephalin genes (43, 44). During rat development glucocorticoids can direct the differentiation of bipotential neural crest cells toward the chromaffin cell phenotype (45). In the present study we demonstrate positive regulation of NPY mRNA levels by glucocorticoids in three rodent cell lines. The steroid specificity of this response reflects a glucocorticoid receptor-mediated response (Fig. 8). These results are consistent with a previous report showing a 2-fold increase in NPY peptide content in NG108-15 cells treated with dexamethasone (27).

Cyclic AMP elevation stimulates the transcription of several neuropeptide genes (46–49) and tyrosine hydroxylase (42). The actions of cAMP and glucocorticoids are sometimes synergistic, as in the case of proenkephalin gene transcription (49). In the present study we demonstrate positive synergism between cAMP and glucocorticoids in PC12 and N18TG-2 cells (Figs. 5 and 6). However, we found a much more powerful synergism between cAMP and PMA (Table II, Fig. 7). A similar synergism between these two compounds, although smaller in magnitude, was previously reported for the regulation of vasoactive intestinal polypeptide precursor mRNA levels in human neuroblastoma cells (50). Another possible example of such synergism is a cAMP regulatory element of the human proenkephalin gene that responds to phorbol ester only in the presence of elevated cyclic nucleotide levels (48). The elevations of the NPY mRNA abundance elicited by forskolin + PMA are of sufficient magnitude to involve, most likely, transcriptional activation.

Regulatory elements required for either cAMP- or phorbol ester-stimulated transcription have been identified for several genes, and consensus sequences for upstream regulatory element cores have recently been proposed for cAMP (46–48, 51, 52) and phorbol ester (53, 54) regulation. In examining the 5'-flanking region of the recently reported sequence of the rat NPY gene (38) for the presence of these motifs, we noted a 28-base imperfect palindrome (bases −88 to −65, GGGAGT-CACCCGGGCGTGACTGCC), which is well conserved in the

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2 S. L. Sabol, preliminary results.
human NPY gene (24), that contains two sequences (−97 to −80 in the upper strand and −66 to −73 in the lower strand) that resemble the phorbol ester motif (TG/T)ACGTCAG(G/C) and to a lesser extent the cAMP motif (TG/A)CGTCTAG. Phorbol ester regulatory elements can bind the transcription factor AP-1 (53, 54), while the cAMP regulatory element binds an apparently similar or identical factor (52).

The interaction of the cAMP and protein kinase C regulatory systems reported here is an example of "monodirectional control" discussed by Nishizuka (36). However, the exact point(s) of interaction of the systems with respect to NPY gene expression are unknown. Several mechanisms of cross-talk between the two systems are known (reviewed in Refs. 36 and 55) and may be relevant to this study. However, a more interesting possible mechanism would be activation of one or more nuclear transcription factor(s), such as AP-1 or factor(s) interacting with AP-1, by phosphorylation by both types of protein kinases at different sites on the same factor or on different factors. Cooperation of this nature among multiple regulators may be a feature of many genes.

Our results indicate that the regulation of the NPY gene is complex and interesting. Unraveling the mechanisms involved should shed light on general aspects of gene regulation.

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and phorbol ester were from Sigma. Forskolin and A23187 were from Calbiochem. Horse and fetal calf sera were from Gibco and Armour, respectively. Sources of other materials are described below or elsewhere (4,44).

Cell Culture. PC12 cells, obtained from Dr. Terry Rogers (Univ. of Maryland School of Medicine, Baltimore, MD) and Dr. Gordon Green (National Institutes of Health, Bethesda, MD), were cultured at 37°C in 15 cm2 flasks in 89% MEM medium-9% horse/maintained horse serum-1% charcoal/Dextran-treated fetal calf serum. Cell density was maintained by passage at 30% confluence. Similar results on the regulation of NPY mRNA levels with cells of different passage were obtained with PC12 cells obtained from passage 16-23. NPY mRNA levels were similar in 90% confluent cells and those inactivated by charcoal/dextran treatment. Myelin was removed from passages 10 001 ng/ml to reduce the endogenous steroid content. PC12 cells did not grow well in the presence of charcoal/dextran-treated horse and fetal calf serum, so unless otherwise stated, only fetal calf serum was used.

DNA sequencing. Inserts were subcloned into M13mp8 or M13mp18 phage DNA, and nucleotide sequences were determined by the dideoxyribonuclease chain termination method, as improved (57).

Preparation of purified NPY transcripts. The 511-bp insert of rNPY2 was ligated into the EcoRI site of plasmid pSP65 (Stratagene) containing SP6 promoter sequences. The transcripts were synthesized with SP6 RNA polymerase and purified by phenol extraction and repeated ethanol precipitation in 2.0 M ammonium acetate. Analysis by formamide-agarose gel electrophoresis and ethidium bromide staining revealed that 80% of the largest material migrated according to the expected size (194 bp). The RNA concentration was determined by absorbance at 260 nm. The yield was 500 ng per plate.

Preparation of cellular RNA. Total cellular RNA was extracted with 4M guanidinium thiocyanate, purified by precipitation with 1.5 M NaCl (58), and concentrated by vacuum drying at 260 nm. Adult male Fischer 344 and Sprague-Dawley rats (200-250 g) were used as sources of peripheral tissue and brain, respectively. Brains were removed and dissected according to a method described previously (59). For cultured cells, a single 75 cm2 flask was used for each RNA preparation.

Northern blot analysis. RNA (3-100 ng) was denatured in 0.4 M formaldehyde for 25 min at 58°C and hybridized with 20% dextran sulfate containing 8% formaldehyde at 60°C, and 0.1 M phosphate buffer (pH 6.6). Each gel was prehybridized with 500 ng/ml of NPY cDNA and hybridized overnight with 20 ng/ml of NPY cDNA. After extensive washing, the blotted membranes were stained with 0.01% amido black in 7% acetic acid to assure hybridization. The membranes were then exposed to XAR film for 3 days for the NPY message, 1 week for other regions of rat brain, and 1-3 weeks for PC12 cells. The bands were determined by a densitometry of a Kodak model GS 860 and integration of peak areas. Exposure times were chosen to give the best contrast in our range of approximately linear variation with amount of RNA applied.

Quantitation of NPY mRNA. The amounts of NPY mRNA from rat striatum, frontal cortex, hypothalamus, and NG108-15 cells were determined by densitometric analysis. Total RNA (0.1-0.5 pg) was denatured in formaldehyde-formamide, denatured with 0.3 M NaCl-0.01 M sodium acetate, and filtered slowly into a Gene Screen Plus membrane (New England Nuclear) within a dot-blot manifold. For standardization, at least 25 ng (2.5-100 ng of pSP65-NPY) transcripts were similarly filtered with carrier rat liver RNA (3 pg). The membranes were hybridized with nick-translated 

HPLC analysis of NPY immunoreactivity in rat brain. (Key) analyses of extracts of each rat brain region, as shown for striatum in Fig. 4, demonstrated that NPY immunoreactivity eluted as a single peak having a retention time identical to that of synthetic human NPY but slightly shorter than that of porcine NPY. This result is consistent with the finding that rat NPY is identical to human but not to porcine NPY. The HPLC profiles also showed that the data concerning NPY immunoreactivity in brains (Table I) actually reflects levels of mouse NPY peptide.
Regulation of Rat Neuropeptide Y Precursor Gene Expression

Glucocorticoids resemble that in PC12 cells; however, forskolin treatment rapidly and persistently depressed the NPY mRNA abundance by 60-80% (Fig. 8). The half-maximal reduction due to forskolin occurred at approximately 15 h (not shown). NPY mRNA was also reduced by 1 mM 8-bromo-cAMP or 10 μM prostaglandin E1 (which activates adenylate cyclase), while 1 mM 8-bruno-eGMP had no effect (not shown). Other treatments that differentiate NG108-15 cells without primarily elevating cAMP levels, such as 2% dimethylsulfoxide and serum deprivation, also reduce the NPY mRNA abundance. This suggests that cAMP may be acting negatively in these cells by a mechanism different from that mediating positive regulation in PC12 and N18TG2 cells.

Steroid specificity in elevating NPY mRNA in PC12 and NG108-15 cells. To determine whether the NPY mRNA elevation by dexamethasone is a glucocorticoid response, a variety of steroids were tested at 1 μM. Only the glucocorticoids hydrocortisone, corticosterone, and dexamethasone increased NPY mRNA, while other types of steroids were inactive in either cell line (Fig. 9). The half-maximally effective concentration of dexamethasone was 0.3 ± 1.7 x 10^-8 M in both lines (not shown), which is also consistent with a glucocorticoid-receptor mediated response.

![Graph](image-url)

**FIG. 8.** The effects of dexamethasone and/or forskolin on the NPY mRNA abundance in NG108-15 cells. Confluent cultures of NG108-15 cells were treated for the indicated times with the compounds indicated: no drug (C), 1 μM dexamethasone (D), 10 μM forskolin (F), or 1 μM dexamethasone + 10 μM forskolin (DF). The medium was changed daily. NPY mRNA was quantitated by dot-blot analysis. Results are expressed as percent of the zero-time control value, which was 11 ± 1 pg/μg total RNA.

**FIG. 9.** The effects of various steroids on the NPY mRNA abundance in PC12 and NG108-15 cells. Flasks of PC12 and NG108-15 cells were cultured with various steroids (1 μM) for 48 h. During the two days prior to steroid addition and during steroid exposure, all of the serum supplementing the medium for PC12 cells was charcoal/dextran-treated to remove endogenous steroids. Total RNA was isolated and NPY mRNA abundances were determined. The data are mean percentages (± standard error of duplicate flasks) of the NPY mRNA level of untreated cells. Abbreviations: DEX, dexamethasone; HC, hydrocortisone, CS, corticosterone, DOC, 11-deoxycorticosterone; E, beta-estradiol; P, progesterone; and T, testosterone.