Neuropeptide Y (NPY) occurs in adrenergic as well as in non-adrenergic nerves innervating the islets of Langerhans and inhibits glucose-stimulated insulin secretion. Recently we demonstrated that NPY is expressed within islet beta cells of the rat pancreas following treatment with dexamethasone in vivo. In this study we examined the cellular expression of NPY following dexamethasone treatment of the insulin-producing cell line RINm5F, which under control conditions does not express or release NPY. The cells were cultured with or without dexamethasone (100 nM) for 5 days. Over the 5-day culture period, dexamethasone time dependently induced an increased release of NPY with a concomitant decrease in the release of insulin. Northern blot and in situ hybridization revealed a corresponding time-dependent increase in the amount of NPY transcripts and in the number of cells labeled for NPY mRNA, whereas immunocytochemistry for NPY revealed only a few immunoreactive cells, indicating a rapid release of the formed peptide. Following 5 days of culture with dexamethasone, acute stimulation with D-glyceraldehyde (10 mM) or KCl (20 mM) Ca2+ dependently stimulated the release of insulin. In contrast neither stimulation with D-glyceraldehyde or KCl nor removal of extracellular Ca2+ affected the release of NPY. Furthermore the D-glyceraldehyde- and KCl-induced increase in cytosolic Ca2+, evident in control RINm5F cells, was impaired after dexamethasone treatment. We conclude that RINm5F cells show steroid-sensitive plasticity and express NPY after dexamethasone treatment concomitantly with a decreased insulin secretion and impaired increase in cytosolic Ca2+ upon depolarization with KCl or stimulation with D-glyceraldehyde. We also conclude that NPY and insulin secretion are regulated differently and suggest that the inability of the removal of extracellular Ca2+ to inhibit NPY secretion and the failure of D-glyceraldehyde and KCl to stimulate NPY secretion reflect a constitutive release of this peptide from the cells in contrast to the regulated release of insulin.

Neuropeptide Y (NPY) is a widely distributed neurotransmitter in the mammalian central and peripheral nervous systems (1, 2) and belongs to the NPY family of peptides also comprising pancreatic polypeptide (PP) and peptide YY (PYY) (3) (for review, see Ref. 2). In the pancreas of several species, including the rat, NPY has been demonstrated in adrenergic as well as in non-adrenergic nerve fibers around blood vessels, ducts, and acini as well as within the islets (4, 5). In the pancreas NPY inhibits glucose-stimulated insulin secretion as demonstrated both in vitro and in vivo (6, 7). Recent studies have revealed expression of NPY also in islet cells, e.g. in hamster islet somatostatin cells (8), in mouse islet glucagon cells (9), and in rat islet cells following in vivo treatment or treatment of isolated islets with dexamethasone (10–12). The dexamethasone-induced expression of NPY in rat islet cells has been localized predominantly to the insulin-producing beta cells (11, 12). However, the mechanisms of dexamethasone-induced NPY expression and the regulation of NPY secretion from insulin-producing cells after dexamethasone treatment are not yet established.

To study whether insulin-producing cells show steroid plasticity and display induced expression and secretion of NPY after dexamethasone treatment in a defined environment without influence of peptides derived from other islet cells, we examined the RINm5F cells by using Northern blot, in situ hybridization (ISH), and immunocytochemistry. Furthermore, we examined the release of insulin and NPY by radioimmunoassay from the RINm5F cells after dexamethasone treatment during basal conditions and after stimulation with D-glyceraldehyde and KCl in the presence or absence of extracellular Ca2+. The levels of cytosolic Ca2+ in control- and dexamethasone-treated cells during basal and stimulated conditions were also measured.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—Stock cultures of RINm5F cells were grown continuously at 37°C in humidified air equilibrated with 5% CO2 in RPMI 1640 medium supplemented with 2 mM l-glutamine, 10% fetal calf serum, 100 units/ml penicillin G, 100 µg/ml streptomycin, and 2.5 µg/ml amphotericin B. Cells were subcultured once weekly after trypsinization and the medium was changed every 3–4 days. All experimental work was performed with cells in passages number 76–80.

**Immunocytochemistry and in Situ Hybridization**—RINm5F cells were cultured on glass slides for 1 to 5 days in control medium and in medium supplemented with dexamethasone (100 nM), respectively. Each day of the culture period, slides were processed for immunocytochemistry or ISH. For immunocytochemistry, each slide was washed twice with phosphate-buffered saline (PBS), pH 7.2, and then fixed in Stefanini’s fixative (2% formaldehyde and 0.2% picric acid in phosphate...
buffer, pH 7.2) for 15 min at room temperature, then rinsed repeatedly with PBS, and immediately processed for the immunocytochemical demonstration of NPY, insulin, glucagon, somatostatin, PP, and PYY. The slides were incubated overnight with primary antibodies against pro-NPY, F5–97 (C-PO) (1:1280, code CA 300, Cambridge Research Biochemicals, Cambridge, United Kingdom). Incubation was performed with primary antibodies against preproinsulin (1:1280, code 9003), glucagon (1:2560, code 7811), PYY (1:2560, code 8415), PP (1:1280, code 7823) (EuroDiagnostica, Malmö, Sweden), and somatostatin (1:1600, code N-SOM, Incstar, Stillwater, MN). The site of the antigen-antibody reaction was visualized by fluorescein isothiocyanate-conjugated antibody (Jackson, West Grove, PA) or biotin IgG raised in pig (Dakopatts, Copenhagen, Denmark).

For ISH, slides were rinsed twice with PBS and then fixed for 10 min in buffered 4% paraformaldehyde (pH 7.2), permeabilized in 0.25% Triton X-100, rinsed in PBS for 5 min, and dehydrated. For detection of NPY mRNA, a 36-mer probe complementary to the nucleotide sequence 266–301 of rat NPY cDNA was used. The probe has been checked for complementarity to other genes present in the GenBank25 data base, using the NCBI BLAST E-mail server, but no such complementarity was found (11, 12). For detection of insulin mRNA, a probe mixture consisting of six different 30-mer oligodeoxynucleotides was used (BPR 236; R & D Systems, Abingdon, Oxfordshire, UK). ISH was also performed for glucagon mRNA using a probe complementary to nucleotides 153–182 of rat glucagon cDNA, for somatostatin mRNA using a probe complementary to nucleotides 310–339 (code NEP-503, DuPont NEN, Solna, Sweden) of rat somatostatin cDNA, for PYY and PP mRNAs using probes complementary to nucleotides 266–295 of rat PYY cDNA, and nucleotides 265–294 of rat PP cDNA. The probes were 3'-end-labeled with 32P-dATP using a terminal transferase (both supplied by DuPont NEN, Stockholm, Sweden) yielding a specific activity of approximately 2 x 10⁶ cpm/μg. Hybridization was carried out overnight at 37 °C in sealed moisturizing chambers using a probe concentration of 1.3 pmol/ml. After hybridization, the slides were washed first in 1 x SSC (0.15 M NaCl, 0.015 M sodium citrate) at room temperature, then in 0.5 x SSC (four times for 15 min at 55 °C), and finally in 1 x SSC (once for 30 min at room temperature), followed by dehydration. The slides were coated with Ifford K-5 emulsion and stored in light-sealed boxes at 4 °C for 3–4 days. They were then developed and counterstained with hematoxylin. For control purposes, hybridization was also performed after incubation in RNase A (Sigma) (45 μg/ml for 30 min at 37 °C) or in the presence of a 100-fold molar excess of unlabeled probe. In the control experiments no autoradiographic labeling was obtained.

The percentage of NPY mRNA-labeled cells of the total number of cells counted was calculated for each day during the 5-day culture period, with and without dexamethasone. For determination of NPY mRNA the slides were cross-linked to the filter by exposure to UV light (120,000 μW/cm²). Prehybridization was performed at 63 °C for 60 min. Hybridization with the 32P-labeled actin probe relative to the autoradiographic signal after hybridization with the NPY probe was performed, and cells (1000 cells/slide, n = 6) were examined in randomly selected visual fields in the microscope for labeling of NPY mRNA. The values are the percent of cells (mean ± S.E) labeled for NPY mRNA.

NPY (50 μl) were then frozen and stored at −20 °C until assayed. NPY and Insulin Assays—For the radioimmunoassay of NPY, a rabbit antiserum to synthetic porcine NPY was used (gift from Dr. P. C. Emmson, Cambridge, UK). Porcine125I-NPY was used as a tracer. The antiserum used has been characterized previously (13). For the radioimmunoassay of insulin, we used a guinea pig anti-rat insulin antibody, 125I-labeled human insulin, as tracer and rat insulin as standard (Linco Research, St. Louis, MO). The separation of free and bound radioactivity was performed by the double antibody technique.

RNA Extraction and Northern Blot Analysis—Total RNA was isolated from cells cultured without or with dexamethasone (100 nm) for 1–5 days by extraction in guanidium thiocyanate using the single step method described by Chomczynski and Sacchi (14). RNA was quantified spectrophotometrically. In each lane, 20 μg of total cellular RNA were separated electrophoretically on a denaturing 1.2% agarose-formaldehyde gel and transferred to a nylon membrane (Hybond-N, Amersham, Buckinghamshire, UK) by capillary transfer in 20 x SSC. RNA was cross-linked to the filter by exposure to UV light (120,000 μW/cm²). Prehybridization was performed at 63 °C for 60 min. Hybridization with the [γ-32P]ATP end-labeled (Amersham International plc) NPY oligo probe (same sequence as used for ISH) (1.6 x 10⁶ cpm/ml hybridization solution) was carried out at 63 °C overnight. The filter was then washed as follows: twice for 15 min, 2 x SSC, 0.1% SDS, at room temperature; once for 15 min, 0.1 x SSC, 0.1% SDS, at room temperature; and once for 15 min, 0.1 x SSC, 0.1% SDS at 60 °C. As control for the amount of total RNA application, the filter was hybridized with a randomly [5-32P]dCTP-labeled actin probe (15). Autoradiography was performed at −70 °C for 5 days for both actin and NPY by exposing the filter to imaging film (CEA AB, Strängnas, Sweden) and developed according to standard procedures. Densitometric laser scanning of each autoradiograph was performed, and quantitation of NPY mRNA was obtained by expressing the autoradiographic signal after hybridization with the NPY probe relative to the autoradiographic signal after hybridization with the actin probe.

Measurement of Intracellular Ca2+—The free cytoplasmic calcium concentration [Ca2+], was analyzed with the fluorescent probe FURA-2AM (Sigma) as described elsewhere (16). Cells cultured in control medium or dexamethasone-supplemented medium cultured for 1, 3, and 5 days were trypsinized and thereafter allowed to recover at a concentration of 0.5 x 10⁵ cells/ml for 2 h in 10 ml of RPMI 1640 medium supplemented with 10% fetal calf serum at 37 °C. During this period the cells were continuously shaken to avoid attachment. The cells were then loaded with 1 μM indicator for 45 min, rinsed three times in HEPES buffer, and allowed to equilibrate for 20 min in the HEPES medium at room temperature. The cells were then rinsed, counted in a Bürker chamber, resuspended in the HEPES medium at a concentration of 0.5 x 10⁵ cells/ml, and transferred to a cuvette for measurement of [Ca2+]. [Ca2+]i was measured by dual wavelength spectrophotofluorimetry in a Perkin-Elmer LS-50 spectrophotofluorometer. The excitation wavelength was continuously altered between 340 and 380 nm, and the emission wavelength was 510 nm. The cell suspension was continuously

**FIG. 1.** Percentage of RINm5F cells labeled for NPY mRNA during a 5-day culture period. Cells were cultured without or with dexamethasone (100 nm) for 5 days on glass slides. On each day, in situ hybridization for NPY mRNA was performed, and cells (1000 cells/slide, n = 6) were examined in randomly selected visual fields in the microscope for labeling of NPY mRNA. The values are the percent of cells (mean ± S.E.) labeled for NPY mRNA.
stirred with a stir bar mounted at the side of the cuvette. D-Glyceraldehyde (10 mM) and KCl (20 mM) were added to the cuvette in microliter volumes from stock solutions of high concentrations at specific time points as indicated in the figures. When experimental and control series were compared, the substances under study were always added at identical time points from the start of the experiment: at 200 s for D-glyceraldehyde and at 900 s for KCl. At the end of each experiment, fluorescence maximum was obtained by adding 0.03% Triton X-100, and fluorescence minimum was obtained by adding EGTA in excess to the cell suspensions. The $[Ca^{2+}]_i$ was calculated according to the previously described formula (17). The $K_d$ was assumed to be 224 nM.

**Statistical Analysis**—The data are presented as the mean ± S.E. Statistical comparisons of differences between mean values were performed by the use of the Student’s $t$ test or with the Mann-Whitney $U$ test. Bonferroni correction was used when multiple comparisons were performed.

**RESULTS**

**Dexamethasone Induces NPY Expression in RINm5F Cells**—ISH performed on control RINm5F cells displayed no labeling for NPY mRNA at any time (Fig. 1), and no signal for NPY mRNA could be detected with Northern blot (Fig. 2). In contrast, after dexamethasone treatment of RINm5F cells, a time-dependent increase in the amount of NPY mRNA transcripts (Fig. 2) and in the number of NPY mRNA-labeled cells (Fig. 1) was observed. Thus, after 1 day of dexamethasone treatment, Northern blot displayed a weak signal (Fig. 2), and only $5 \pm 1\%$ of the cells displayed labeling (Fig. 1 and Fig. 3). However, after 5 days of culture with dexamethasone, there was a 13-fold increase in the amount of NPY mRNA transcripts compared with 1 day of dexamethasone treatment (Fig. 2), and $91 \pm 1\%$ of the cells displayed intense labeling (Figs. 1 and 3). ISH for insulin mRNA revealed labeling of all cells, and there was no obvious difference in labeling intensity between dexamethasone-treated and control cells (data not shown). ISH for glucagon, somatostatin, PP, or PYY revealed no labeling for respective mRNA in dexamethasone-treated or control cells during the 5-day culture period. NPY immunoreactivity, located in the cell cytoplasm, was detected in a minor population of cells treated with dexamethasone for 2–5 days (Fig. 4A). In contrast,
control cells (Fig. 4B) and cells cultured with dexamethasone for only 1 day did not stain for NPY. In all groups studied glucagon, somatostatin, PP, or PYY immunoreactivities could not be detected, whereas a subpopulation of cells displayed insulin immunoreactivity.

**Dexamethasone Increases Release of NPY and Decreases Release of Insulin from RINm5F Cells**—The release of NPY from dexamethasone-treated RINm5F cells increased in a time-dependent manner concomitantly with a decreased insulin release (Table I). Thus, the content of NPY in the medium after a 5-day culture period with dexamethasone was increased 58-fold compared with 1 day of culture with dexamethasone \( (p < 0.001) \). In contrast NPY could not be detected in the medium of control cells (Table I). Insulin was detected in the medium of both control and dexamethasone-treated cells. In the medium of control cells, the insulin content increased gradually during the first 3 days of culture and then leveled off, whereas the insulin content in the medium of dexamethasone-treated cells was significantly lower compared with controls \( (p < 0.001) \) (Table I) at all time points throughout the 5-day culture period. During the 5-day culture period, the cells displayed a steady increase in number. However, treatment with dexamethasone decreased cell recovery by approximately 30% at day 5 compared with control cells (data not shown). When calculating the release of insulin and NPY per cell for 24 h, insulin release from control and dexamethasone-treated cells decreased with time, and by day 5 had decreased to approximately 35% from control cells \( (p < 0.001) \) and approximately 5% from dexamethasone-treated cells \( (p < 0.001) \) compared with cells cultured for 1 day (Fig. 5). In contrast, the release of NPY displayed a time-dependent increase from cells cultured with dexamethasone by a 22-fold increase \( (p < 0.001) \) at day 5 compared with cells cultured with dexamethasone for 1 day (Fig. 5).

**Dexamethasone Impairs D-Glyceraldehyde- and KCl-stimulated Increase of \([Ca^{2+}]_i\)—Base-line \([Ca^{2+}]_i\) levels in RINm5F cells were 89 \pm 3 nM. D-Glyceraldehyde (10 mM) induced a gradual increase in \([Ca^{2+}]_i\). At 1 day after initiation of culture, \([Ca^{2+}]_i\) increased to 212 \pm 4 nM at 300 s after addition of D-glyceraldehyde \( (p < 0.001) \). A similar pattern of influence was observed at days 3 and 5 after initiation of culture (Fig. 6). When the cells were cultured in the presence of dexamethasone for 1 day, the D-glyceraldehyde-induced increase in \([Ca^{2+}]_i\) was the same as in control cells, both in pattern and magnitude (Fig. 6). However, the D-glyceraldehyde-induced increase in \([Ca^{2+}]_i\), was markedly impaired at day 3 when cultured with dexamethasone and more pronounced at day 5. In fact after 5 days of culture with dexamethasone, D-glyceraldehyde failed to increase \([Ca^{2+}]_i\). In contrast, base-line \([Ca^{2+}]_i\), was not affected by dexamethasone. Depolarization by KCl (20 mM) markedly increased \([Ca^{2+}]_i\), due to opening of voltage-sensitive \(Ca^{2+}\) channels (Fig. 6). After 5 days of culture in the presence of dexamethasone, this depolarization-induced increase in \([Ca^{2+}]_i\), was reduced by approximately 50% \( (p < 0.001) \).

**Different Secretory Pathways for Insulin and NPY—Acute stimulation with 10 mM D-glyceraldehyde or 20 mM KCl for 60 min significantly increased insulin release from both control and dexamethasone-treated RINm5F cells \( (p < 0.001) \). However, the insulin secretory response to D-glyceraldehyde and KCl from cells treated with dexamethasone was much lower than the response from control cells \( (p < 0.001) \) (Table II). Removal of extracellular \(Ca^{2+}\) abolished the KCl-stimulated release of insulin from both control and dexamethasone-treated cells (Table II). In contrast neither stimulation with D-glyceraldehyde or KCl nor removal of \(Ca^{2+}\) from the incubation medium affected the release of NPY from cells cultured with dexamethasone as determined with radioimmunossay (Table II). NPY could not be detected in the incubation medium from cells cultured without dexamethasone.

**DISCUSSION**

Although NPY is widely distributed in the nervous system, it has been demonstrated also in pancreatic endocrine cells in the hamster (8), mouse (9), human (18), and rat when treated with glucocorticoids (10–12) and during fetal development (19). In the dexamethasone-treated rat, we recently showed that NPY is expressed in insulin-producing beta cells (11, 12). Glucocorticoids administered in vivo are known to induce peripheral insulin insensitivity with elevated plasma insulin and glucose levels associated with hypertrophy and proliferation of the beta cells (20); for review see Ref. 21. Since NPY has been shown to inhibit glucose-stimulated insulin secretion (6, 7), it is possible that the expression of NPY in islet cells after dexamethasone
In the present study we found that following culture of RINm5F cells in dexamethasone-containing medium for 5 days, the release of insulin upon stimulation with d-glyceraldehyde and KCl was markedly reduced compared with control cells. Still, however, insulin secretion was Ca\(^{2+}\)-dependent and abolished by removal of extracellular Ca\(^{2+}\). The d-glyceraldehyde-induced raise in [Ca\(^{2+}\)]\(_i\) was impaired after dexamethasone, suggesting impairment either of the closure of ATP-dependent K\(^+\) channels or of the opening of the \(\omega\)-conotoxin-sensitive Ca\(^{2+}\) channels. Also the increase in cytosolic [Ca\(^{2+}\)]\(_i\) when stimulated with KCl was lowered by dexamethasone, suggesting an inhibitory influence of dexamethasone also on the L-type Ca\(^{2+}\) channels. Hence, dexamethasone markedly impaired the raising of [Ca\(^{2+}\)]\(_i\) in RINm5F cells. Considering the importance of cytosolic Ca\(^{2+}\) for insulin secretion (29), this might explain the impairment of d-glyceraldehyde- and KCl-stimulated insulin secretion after dexamethasone treatment, although the exact nature of the inhibited insulin secretion after dexamethasone treatment of RINm5F cells remains to be established.

In other insulin-producing cell lines, no similar study on the influence of long term dexamethasone treatment on insulin secretion has been performed. However, several short term studies exist with divergent results on the influence of dexamethasone on insulin secretion using insulin-producing cell lines and isolated rodent islets. For example, in the hamster insulinoma cell line HIT T-15 insulin biosynthesis and secretion are reduced by glucocorticoids (30), whereas RINm5F cells and isolated rodent islets treated with dexamethasone for 48–72 h respond with increased insulin mRNA levels but decreased insulin secretion (31, 32). Recently Goodman et al. (33) demonstrated that the human insulin promoter is also a negative glucocorticoid-responsive element, and the beta cells have previously been demonstrated to express glucocorticoid receptors (25). Altogether this strongly indicates a functional role for glucocorticoids in the physiology of beta cells with regard to both insulin formation and secretion although the effects might be different in different types of insulin-producing cells.

In contrast to insulin, RINm5F cells cultured in control medium and subsequently stimulated with d-glyceraldehyde or KCl did not release NPY. This is in accordance with the finding that control RINm5F cells do not express the peptide or its mRNA. However, following 5 days of dexamethasone treatment NPY was abundantly released from the cells during the subsequent 60-min incubation in accordance with the abundant NPY mRNA expression. The NPY release from the cells was of similar magnitude as that of insulin, illustrating the marked induction of NPY expression by dexamethasone. In contrast to the release of insulin from dexamethasone-treated cells the release of NPY was not augmented by d-glyceraldehyde or KCl nor inhibited by removal of extracellular Ca\(^{2+}\). This indicates that the mechanisms of insulin and NPY secretion differ. Insulin secretion exhibits several characteristics of a regulated secretion (stimulation by nutrient secretagogue and depolarization and inhibition by removal of extracellular Ca\(^{2+}\)) whereas NPY secretion does not seem to be at all regulated. Rather, NPY secretion from dexamethasone-treated RINm5F cells displays more characteristics of constitutive secretion. In a constitutive release process the formed protein bypasses the sorting in the Golgi network and packaging into secretory granules, and therefore escapes the regulation of exocytosis, e.g. in response to raised intracellular Ca\(^{2+}\) (34); Ca\(^{2+}\) is required for the sorting of proteins for the regulated pathway (35). Our results therefore indicate that NPY is released by a constitutive rather than a regulated mechanism. It is worth noticing in this context that RINm5F cells are known to possess...
both constitutive and regulated pathways. Previous studies have demonstrated that RINm5F cells express the prohormone convertase PC2 (36, 37), which is specific for the regulated pathway (38, 39). Also the processing enzyme furin, which is specific for the constitutive pathway (40), has been detected in RINm5F cells (41). Analogous with our present results the insulin-producing cell line INS-1 has been found to express and secrete NPY in a constitutive manner since the cells did not respond with increased release of NPY to stimulation with glucose (42). Furthermore, of the islet regulatory peptides, islet amyloid polypeptide (known to be co-localized with insulin in beta cell secretory granules and co-released with insulin (43)) has been found to be released from rat neonatal beta cells during inhibition of the regulated pathway by removal of extracellular Ca\(^{2+}\), suggesting release of islet amyloid polypeptide also through a constitutive pathway (44). Thus, different types of insulin-producing cells exhibit characteristics for both types of secretion.

In conclusion, our findings demonstrate a steroid-sensitive plasticity of the RINm5F cells causing induction of NPY expression after long term treatment with dexamethasone in conjunction with impaired insulin secretion and inhibited in-

**Fig. 6. Effects of dexamethasone on cytoplasmic Ca\(^{2+}\) content in RINm5F cells.** A. [Ca\(^{2+}\)], in cell suspensions of RINm5F cells in a HEPES medium at 3.3 mM glucose in the presence of 1.28 mM Ca\(^{2+}\). At 200 s d-glyceraldhe (GA) (10 mM) was added to the cuvette and at 900 s KCl (20 mM) was added. Two representative parallel experiments out of three to four are shown at each time point when the cells had been incubated in the presence or absence of dexamethasone for 1 day (top panel), 3 days (middle panel), or 5 days (bottom panel). Solid line, dexamethasone; dotted line, control. B. [Ca\(^{2+}\)], in cell suspensions of RINm5F cells in a HEPES medium at 3.3 mM glucose in the presence of 1.28 mM Ca\(^{2+}\) under baseline conditions at 300 s after addition of d-glyceraldhe (GA) (10 mM) and at 10 s after addition of KCl (20 mM). The results are presented as mean ± S.E. for parallel experiments when the cells had been incubated in the presence or absence of dexamethasone for 1 day (top panel, n = 3), 3 days (middle panel, n = 4), or 5 days (bottom panel, n = 4). Asterisks indicate the probability level of difference with and without dexamethasone. *, dexamethasone;**, control **, p < 0.01; and ***, p < 0.001.

**Table II**

| GA (10 mM) | KCl (20 mM) | Ca\(^{2+}\) (1.28 mM) | Insulin | NPY |
|------------|-------------|------------------------|---------|-----|
|            |             |                        | Control | Dexamethasone | Control | Dexamethasone |
| Control    | 1.79 ± 0.13 | 0.09 ± 0.06             | ND*     | 0.37 ± 0.03 |
| Dexamethasone |         |                        | ND      | 0.41 ± 0.02 |
| Control    | 9.10 ± 0.38 | 0.31 ± 0.04             | ND      | 0.40 ± 0.03 |
| Dexamethasone |         |                        | ND      | 0.41 ± 0.04 |
| Control    | 8.88 ± 0.79 | 0.43 ± 0.02             | ND      | 0.42 ± 0.03 |
| Dexamethasone |         |                        | ND      | 0.42 ± 0.03 |

*ND, not detectable.
crease in cytosolic Ca^{2+}. In contrast to the secretion of insulin, NPY secretion from RINm5F cells is not affected by α-glycerophosphate or KCl but inhibited by removal of extracellular Ca^{2+}. Thus, whereas insulin is released by the regulated pathway, NPY is released by a mechanism showing characteristics of the constitutive pathway. The plasticity of RINm5F cells is evident not only by its sensitivity for dexamethasone to markedly increase in cytosolic Ca^{2+}.

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Ulrika Myrsén-Axcrorna, Sven Karlsson, Frank Sundler and Bo Ahrén

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