Supplement Methods and Data

Cell Culture

Pheochromocytoma PC12 cells were grown at 37°C/6% CO₂ in 10-cm plates or six-well plates and Dulbecco's modified Eagle's high-glucose medium supplemented with 5% fetal bovine serum, 10% horse serum, 100 U/mL penicillin, and 100 µg/mL streptomycin (1). Promoter and secretion experiments were performed on cells plated onto 12-well poly-L-lysine (Sigma)-coated Costar plates.

KCl-Mediated Norepinephrine Release

Norepinephrine secretion was monitored as previously described (2). PC12 cells were plated on poly-l-lysine coated 12-well dishes and were labeled for 3 hours with 1 µCi of L-[³H] norepinephrine (PerkinElmer Life Sciences, Waltham, MA) in 1 mL of PC12 growth medium. The cells were then washed twice with basal medium and then with basal secretion buffer [150 mm NaCl, 5 mm KCl, 2 mm CaCl₂, and 10 mm HEPES (pH 7.0)] for 15 min. The cells were then treated for 30 mins at 37°C with 1 µM Ucn 2 in cell membrane depolarization (55 mmol/L KCl) solution (KCl + Ucn 2) or with cell membrane depolarization solution alone (KCl). Cells were also 'Mock' treated for 30 mins with secretion buffer alone. The release buffer for experiments involving KCl as secretagogue had NaCl reduced to 100 mmol/L for maintenance of tonicity. After 30 mins, the supernatant was collected, and the cells were lysed with basal secretion buffer containing 0.1% Triton X-100 for 10 min. Release buffer and cell lysates were assayed for L-[³H] norepinephrine by liquid scintillation counting, and results were expressed as percent secreted (Amount Released/[Amount Released+Amount in Cell Lysate] x100- basal % secreted).

In vitro TH Promoter Studies
PC12 pheochromocytoma cells were transfected (at 60-80% confluence) with 1 µg/well (12-well plate) of full-length human TH promoter (3). Twenty-four hours after transfection, triplicate wells of cells were treated with and without Ucn 2 peptide (1 nM, 10 nM and 100 nM) for 24 hours in normal growth media. Cells were harvested and luciferase activity normalized to cellular protein content (BIO-RAD).

**Statistics**

For TH promoter and norepinephrine release studies each experiment included at least 3 replicates and the experiment was repeated to confirm results. Results were expressed as mean ± SEM. Statistical significance was calculated using Student’s t-test. A value of P<0.05 was considered statistically significant.

**Figure Legends**

**Supplement Figure 1.** Acute Ucn 2 effect on KCl-induced secretion of norepinephrine from PC12 (rat pheochromocytoma) cells. Cells were mock treated or treated for 30 minutes with 1 µM Ucn 2 in the presence of KCl (55 mmol/L) or treated with KCl alone. After 30 minutes of treatment, the cells were harvested for measurement of norepinephrine secretion. Release buffer and cell lysates were assayed for L-[³H]norepinephrine by liquid scintillation counting, and results were expressed as percent secreted (Amount Released/[Amount Released+Amount in Cell Lysate]x100-basal % secreted). Bars represent mean S.E.M. of triplicate samples. Ucn 2 significantly decreased KCL stimulated secretion of L [³H]norepinephrine (*P= 1.5 X 10⁻⁵).

**Supplement Figure 2.** PC12 cells were transfected with 1 µg Ucn 2 dose-dependently decreased TH promoter activity *in vitro*. Bars represent mean S.E.M. of triplicate samples of luciferase
activity/µg/protein well compared to untreated control (Control) cells (1 nM Ucn 2 vs. NT, P= 0.09; 10 nM Ucn 2 vs. NT, P= 0.028 *; 100 nM Ucn 2 vs. NT, P= 0.028 **).

Supplemental References


Supplemental Figure 1

Net [H] Norepinephrine Release (%)

Control (Mock)  KCl  KCl + Ucn 2

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Supplemental Figure 2

Luciferase Activity/μg/Protein/Well

Control

Ucn 2 (1 nM)

Ucn 2 (10 nM)

Ucn 2 (100 nM)

0 1000 2000 3000 4000 5000 6000 7000 8000

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