The effect of neurotensin on pituitary secretion of thyrotrophin and prolactin in vitro

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Abstract. The effects of neurotensin on thyrotrophin (TSH) and prolactin (Prl) release were studied in two in vitro systems – anterior pituitary cells in culture and perfused anterior pituitary fragments. Neurotensin significantly reduced basal secretion of both TSH and Prl (P < 0.001) from cultured pituitary cells, and abolished thyrotrophin releasing hormone (TRH)-stimulated TSH release. Neurotensin significantly reduced TRH-stimulated TSH and Prl release (P < 0.02) from perfused pituitary fragments. These data indicate that neurotensin has a direct inhibitory effect on TSH and Prl secretion by the anterior pituitary.

Neurotensin (NT), a tridecapeptide originally isolated from bovine hypothalamus (Carraway & Leeman 1973), has numerous biological actions (Bissette et al. 1978; Nemeroff et al. 1980), including modulation of pituitary hormone secretion both in vivo and in vitro. The central administration of neurotensin in vivo suppresses basal and thyrotrophin releasing hormone (TRH)-stimulated thyrotrophin (TSH) (Maeda & Frohman 1978) and basal prolactin (Prl) secretion (Vijayan & McCann 1979), but it is not clear whether neurotensin acts directly on the pituitary, at the hypothalamus, or elsewhere in the central nervous system (CNS). A physiological role for NT in the regulation of TSH secretion is suggested by the observation that T3 stimulates NT release from rat hypothalamus in vitro (Shennan & Sheppard 1983) and changes in thyroid status in the rat alter hypothalamic (Sheppard & Shennan 1983) and anterior pituitary (Goedert et al. 1982) NT concentration. The aim of this study was to determine any direct effect of NT on pituitary secretion in vitro. Two in vitro systems were employed: isolated anterior pituitary cells in culture and perfused pituitary fragments.

Materials and Methods

1 Effect of NT on TSH and Prl secretion in rat anterior pituitary cells in culture

Female Wistar rats (200–300 g) were killed by cervical fracture and anterior pituitary glands removed. Pituitary tissue was chopped into 0.125 mm³ blocks and enzymatically dispersed as described by Daniel & Ramsden (1982). Cell yield was approximately 0.8 × 10⁶ viable cells per pituitary. Cells were plated in flat-bottomed plastic microtitre plates (Nunc-Intermed; Gibco) at a cell density of 10⁴ cells per well in Dulbecco’s modified Eagle”s medium (DMEM; Flow Laboratories Ltd.) supplemented with 10% (v/v) foetal calf serum (FCS) and maintained at 37°C in a humidified atmosphere of 95% air:5% CO₂. After 24 h cells were firmly attached to the plate and culture medium was replaced with DMEM containing 2.5% FCS. Test incubations were carried out after a further 48 h when cells could be seen (under magnification) to be spread out on the surface of the wells. Incubation medium was removed and replaced with: DMEM, DMEM + TRH (28 nM), DMEM + NT (54 nM), or DMEM + TRH + NT. After 2 h of incubation with test substances, medium was removed and stored at –20°C prior to determination of rat TSH (rTSH) and rat Prl (rPrl).

II Perifusion of rat anterior pituitaries

a) Preparation of pituitary fragments for perifusion. Anterior
pituitaries from female rats were removed, bisected and separate halves were chopped into 0.125 mm³ fragments. Tissue fragments were rinsed with ice-cold 10 mM phosphate buffered saline pH 7.4 (PBS), and mixed with sterile polyacrylamide-gel beads (Biogel-P2; Bio-Rad Laboratories). The tissue and Biogel were placed in columns (2 ml), each containing tissue from two hemipituitaries from different animals, and perfused with DMEM containing Hepes (25 mM), glutamate (1.2 mM), antibiotic/antimycotic solution (1%, w/v), bovine serum albumin (BSA, 0.2%, w/v), bacitracin (5.65 units/ml), pH 7.4, at 37°C for an equilibration period of 2 h. Test substances were introduced into the system via a three-way tap. The flow rate was 0.5 ml/min and 4 min fractions (2 ml) were collected and stored at -20°C prior to assay for rPrl and rTSH.

b) Validation of the isolated perfused pituitary system. In order to establish that the perfusion system used here was appropriate for the investigation of pituitary hormone responses to stimulus in vitro, two pilot studies were carried out. In study 1, perfused pituitary fragments were stimulated once with TRH for 2, 4 or 8 min (total TRH dose, 28, 56, 112 pmoles). Effluent fractions were collected at -20°C for TSH determinations. In study 2, pituitary fragments from thyroidectomized or euthyroid control rats were perfused and stimulated with TRH for 2 min (28 pmoles). Blood samples from each animal were also taken at the time of sacrifice for serum TSH measurement.

c) Determination of the effect of Neurotensin on TSH secretion by perfused pituitary fragments. Perfusion columns, each containing material from two hemipituitaries, were prepared as described above. Following the initial equilibration period a standard dose of TRH (28 nM) was introduced into the column for 8 min (total TRH dose: 112 pmoles). One hour later either TRH (28 nM) or NT (27 nM) plus TRH (28 nM) were administered for 8 min. Medium was collected for a further 20 min after the second stimulus. One control and one NT column were run in parallel on each occasion.

d) Calculation of TSH and Prl responses. The integrated pituitary hormone response to stimuli was calculated from the area under curve in response to stimulus after subtraction of basal hormone release.

III Assays
Rat TSH and Prl were assayed by radioimmunoassay using materials supplied by NIAMDD Pituitary Hormone Distribution Program, standards being diluted in the same cell culture or perfusion medium as samples. TSH assay sensitivity was 0.008 µg/tube (reference preparation NIADDK-rTSH-RP-1) and rPrl assay sensitivity was 0.16 ng/tube (reference preparation NIADDK-rPrl-

<table>
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<tr>
<th>Table 1. Effects of NT on basal Prl and TSH secretion and on stimulated TSH secretion by pituitary cells in culture.</th>
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<tbody>
<tr>
<td>Control medium</td>
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<tr>
<td>TSH (µg/ml)</td>
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<tr>
<td>Prl (µg/ml)</td>
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</table>

| | Control medium | NT (54 nM) | TRH (28 nM) | TRH (28 nM) + NT (54 nM) |
| TSH (µg/ml) | 0.144 ± 0.030 (8) | 0.095 ± 0.066 (8) | 0.304 ± 0.026 (8)** | 0.139 ± 0.037 (8) |

Results expressed as mean ± SEM, number of experiments in parentheses.
* P < 0.001 cf. control (Student’s t-test for unpaired data).
** 2P < 0.01 cf. control (Dunnett’s multiple comparison test).
RP-3). Inter- and intra-assay coefficients of variation for TSH and rPrl assays were less than 10%.

IV Statistics
Results obtained with pituitary cells in culture were tested for significance using Student's t-test for unpaired data or Dunnett's test for multiple comparisons. All other data were analysed using non-parametric statistical tests (Mann-Whitney U-test for unpaired data and Wilcoxon signed-rank sum test for paired data).

Results
Effect of NT on TSH and Prl secretion in cultured pituitary cells
NT (54 nM) significantly reduced the basal secretion of both TSH and Prl from pituitary cells in culture ($P < 0.001$) (Table 1A). In the second set of experiments using fewer wells (Table 1B) basal TSH secretion was also reduced but this reduction did not quite reach significance. TRH significantly stimulated TSH secretion compared with control values ($P < 0.02$) but this elevation was abolished by NT.

Validation of perifused pituitary fragments
A 4-fold increment in TRH stimulus gave rise to a 3-fold increase in TSH response and a 2-fold increase in TRH stimulus led to a 4-fold increase in Prl response (Table 2). Serum TSH concentration and mean TSH responses to TRH were significantly higher in hypothyroid animals compared to controls (Table 3).

<table>
<thead>
<tr>
<th>TRH (total dose: pmoles)</th>
<th>TSH (µg)</th>
<th>Prl (µg)</th>
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<tr>
<td>a) 28</td>
<td>1.74 ± 0.27</td>
<td>0.28 ± 0.07</td>
</tr>
<tr>
<td>b) 56</td>
<td>2.6 ± 1.16</td>
<td>1.02 ± 0.42</td>
</tr>
<tr>
<td>c) 112</td>
<td>5.38 ± 1.03</td>
<td>4.22 ± 0.6</td>
</tr>
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Results expressed as mean ± SEM, number of experiments in parentheses.
* Significantly greater than (a) $P < 0.01$.
** Significantly greater than (a) $P < 0.002$.
(Mann-Whitney U-test for unpaired data).

<p>| TSH responses to TRH in perifused pituitary fragments from euthyroid or hypothyroid rats. |
|-----------------------------------------------|-----------------------------------------------|</p>
<table>
<thead>
<tr>
<th>TSH response (µg)</th>
<th>Serum TSH (µg/ml)</th>
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<tr>
<td>Euthyroid</td>
<td>Hypothyroid</td>
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<tr>
<td>1.80 ± 0.26</td>
<td>0.19 ± 0.08</td>
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<tr>
<td>(6)</td>
<td>(6)</td>
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<tr>
<td>3.92 ± 0.39</td>
<td>4.76 ± 0.36</td>
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<td>(10)*</td>
<td>(10)**</td>
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TRH stimulus administered: 28 pmoles.
Results expressed as mean ± SEM, number of experiments in parentheses.
* $P < 0.002$. ** $P < 0.001$ cf. euthyroid.
(Mann-Whitney U-test for unpaired data).

Effect of NT on TSH and Prl secretion in perifused pituitary fragments
Mean TSH and Prl responses to an initial TRH stimulus (28 nM) followed by a second identical stimulus in the presence or absence of NT are shown in Table 4. For statistical analysis first and second TSH and Prl responses (expressed in µg) in the same column were compared. No significant differences were observed between first and second control TSH and Prl responses to TRH. The presence of NT significantly reduced both TSH and Prl response to a second TRH stimulus when compared to an initial control response in the same column ($P < 0.02$).

Discussion
Two in vitro systems were used to clarify the effects of NT on the anterior pituitary. We have previously demonstrated enhanced basal and TRH-stimulated TSH release from hypothyroid cell cultures and suppressed release from hyperthyroid cells compared with euthyroid cultures (Daniel &
Table 4. TSH and Prl responses to TRH in perifused pituitary fragments in the presence or absence of NT.

<table>
<thead>
<tr>
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<th>Column A</th>
<th>Column B</th>
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<tbody>
<tr>
<td>(TRH) response</td>
<td>(TRH) response</td>
<td>(TRH) response</td>
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<tr>
<td>(TRH) response</td>
<td>(TRH + NT) response</td>
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<tr>
<td>(1)</td>
<td>(2)</td>
<td>(3)</td>
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A) TSH (n = 8)

Mean TSH (μg) ± SEM 4.14 ± 0.82 4.73 ± 1.23 6.62 ± 1.86 3.82 ± 0.82*

B) Prl (n = 7)

Mean Prl (μg) ± SEM 4.5 ± 0.48 4.11 ± 1.35 4.25 ± 1.14 1.83 ± 0.59*

(1) and (2) are mean TSH and Prl responses to first and second control TRH (112 pmoles) stimuli in the same column. (3) is mean TSH and Prl responses to an initial control TRH stimulus, (4) is mean TSH and Prl responses to a second stimulus in the presence of NT (27 nM). (3) and (4) and paired responses in the same column.

* Significantly different from response to TRH stimulus (3).

P < 0.002 (Wilcoxon signed rank sum test).

In the perifusion system a TRH dose-related response could be demonstrated for TSH and Prl secretion, and increased responses were observed from hypothyroid tissue compared with euthyroid tissue for a given TRH stimulus. Both these systems therefore respond in a manner analogous to the in vivo situation. NT was shown to have a suppressive effect on TRH-stimulated TSH release from pituitary cells and fragments; a suppressive effect on basal TSH release was demonstrated using cultured cells. Prolactin release in response to TRH was inhibited in the perifusion system and basal Prl release was inhibited in the cell cultures. The levels of NT required to inhibit TRH-stimulated release were similar in molar terms to that of the TRH initiating the release i.e. in the perifusion system the ratio of the molar concentrations of NT to TRH was 1:1 and in the cultured cells 2:1.

Our results are in accord with those quoted previously where NT was shown to suppress TSH secretion when administered centrally in vivo (Maeda & Frohman 1978). It should however, be pointed out that there is one report of increased serum TSH levels following high doses of NT administered peripherally (Maeda & Frohman 1978), and two other reports of enhanced release of Prl following exposure of anterior pituitary material in vitro to higher levels of NT than used here (Enjalbert et al. 1982; Vijayan & McCann 1979). Further work is therefore required to elucidate any dose-related effects of neurotensin on the pituitary. Nevertheless our initial data support the hypothesis that NT has a direct inhibitory effect on the anterior pituitary at a similar molar concentration to the dose of TRH which stimulates TSH and Prl release.

Acknowledgment

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References


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