

## Different effects of neurotensin and neuromedin-N on the proliferative activity of rat adrenal cortex

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**Summary.** Evidence indicates that neurotensin (NT) and neuromedin-N (NMN) exerts an adrenocorticotrophic effect in the rat. The present study aimed to investigate whether these neuropeptides are able to stimulate the proliferation of rat adrenocortical cells *in vivo* and to compare their mode of action. Adrenocortical proliferative activity was assessed by the metaphase-arrest technique and metaphases were counted per medulla-containing adrenal section. A bolus administration of NT (3 µg/rat) resulted in a significant increase in the number of metaphases in both zona fasciculata and the entire cortex, an effect observed 48 h after the injection. The administration of NMN (3 µg/rat) induced a notable rise in the number of metaphases in the zona fasciculata and the entire cortex within 12 h, followed by a subsequent drop after 24 h and a return to normal values at 48 h. These findings indicate that NT and NMN enhance rat adrenal growth *in vivo* acting via different mediators.

**Key words:** Neurotensin, Neuromedin-N, Adrenal cortex, Cell proliferation, Rat

### Introduction

Neuromedin N (NMN) is a hexapeptide originally isolated from porcine spinal cord by Minamino et al. (1984). The carboxy-terminal tetrapeptide sequence of NMN is identical to that of neurotensin (NT). In fact, NT and NMN are encoded by the same gene, and NT/NMN-precursor protein consists of 169-170 highly conserved aminoacids, C-terminal region of which contains one copy of both NT and NMN (Dobner et al., 1987, 1992; Kislauskis et al., 1988). Thus, the same cell may produce and secrete both NT and NMN; however, the marked variations in the relative distribution of these neuropeptides in discrete brain areas and in other organs suggest differential processing of their common

precursor molecule (Shaw et al., 1990; Kitabgi et al., 1991).

Recent data indicate that NMN, although less efficiently than NT, specifically activates NT receptors (NT-R), while the presence of specific NMN receptors (NMN-R) has never been demonstrated (Hermans et al., 1992). Accordingly, NMN evokes NT-like effects, such as analgesia, hypothermia, spontaneous motor activity or neuron activation. In general, NMN is less potent than NT, and this is due to the higher sensitivity of NMN to the enzymatic degradation (Minamino et al., 1984; Checler et al., 1986; Rompre and Gratton, 1992).

The involvement of NT and NMN in the regulation of the growth, structure and function of the rat adrenal cortex is well known (for review, see Malendowicz, 1993). Recently, we demonstrated that bombesin and neuromedin-B differentially stimulated proliferative activity of the rat adrenal cortex (Markowska et al., 1993). The present study aimed to investigate whether NT and NMN exerted similar effects on the mitotic activity of rat adrenocortical cells.

### Materials and methods

Adult female rats (110-120 g body weight) were employed. They were maintained under standard conditions of light (14 h on: 10 h off) and temperature (22 ± 2 °C), with free access to laboratory pellets and tap water. The rats were given a single subcutaneous injection of 3 µg NT or NMN (Sigma, St. Louis, Mo.), and sacrificed after 0, 12, 24 or 48 h (6 rats for each time-point). Two hours before the sacrifice all rats received (9:00 a.m.) 0.1 mg vincristin (Gedeon-Richter, Budapest, Hungary) intraperitoneally. Adrenal glands were promptly removed, fixed for 24 h in Bouin's solution, and embedded in paraffin. Medulla-containing sections were stained with haematoxylin-eosin.

The number of metaphase-arrested cells was evaluated at x 400. For each rat 8 medulla-containing adrenal sections were analyzed, and metaphases counted in the zona glomerulosa (ZG), zona fasciculata (ZF) and

zona reticularis (ZR). On randomly chosen rats, the mitotic index was estimated by counting 5,000 parenchymal cells in the ZG and ZF, and these data were correlated with the number of metaphases per section of the ZG and ZF. As demonstrated earlier (Stachowiak et al., 1990), there is a close direct correlation between metaphase number per adrenal section and mitotic index (Fig. 1).

Individual results were averaged per group and standard error (SE) was calculated. The statistical comparison of the data was performed by ANOVA followed by the Multiple Range Test of Duncan. The linear correlation between metaphase number per adrenal section and mitotic index was tested by the *r* coefficient of Pearson, and the regression line was obtained by the least square method.

**Results**

NT administration resulted in a significant increase in the number of metaphase-arrested cells in ZF and entire cortex, which was apparent 48 h after neuropeptide injection (Fig. 2). NMN administration induced a notable rise in the number of metaphase-arrested cells in the ZF and entire cortex within 12 h, followed by a drop after 24 h and a return to normal values after 48 h (Fig. 3).

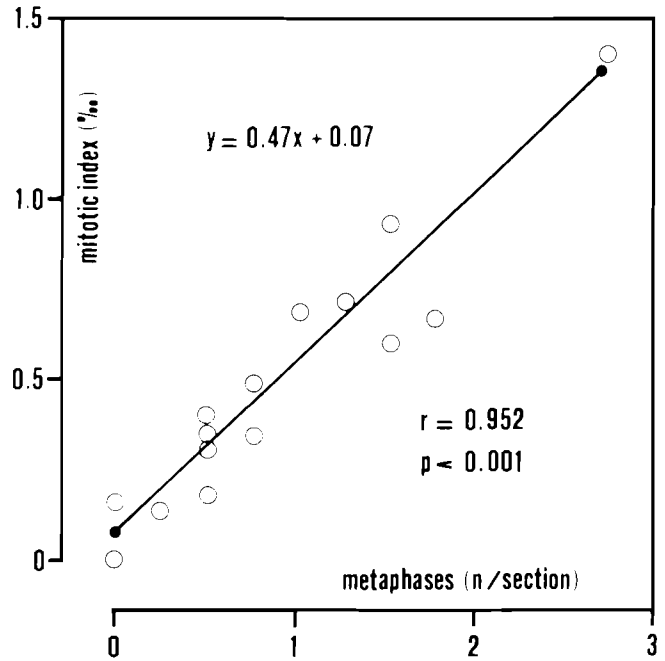


Fig. 1. Direct linear correlation between mitotic index and number of metaphases per section in the ZG and ZF of randomly-chosen rats from both experiments.

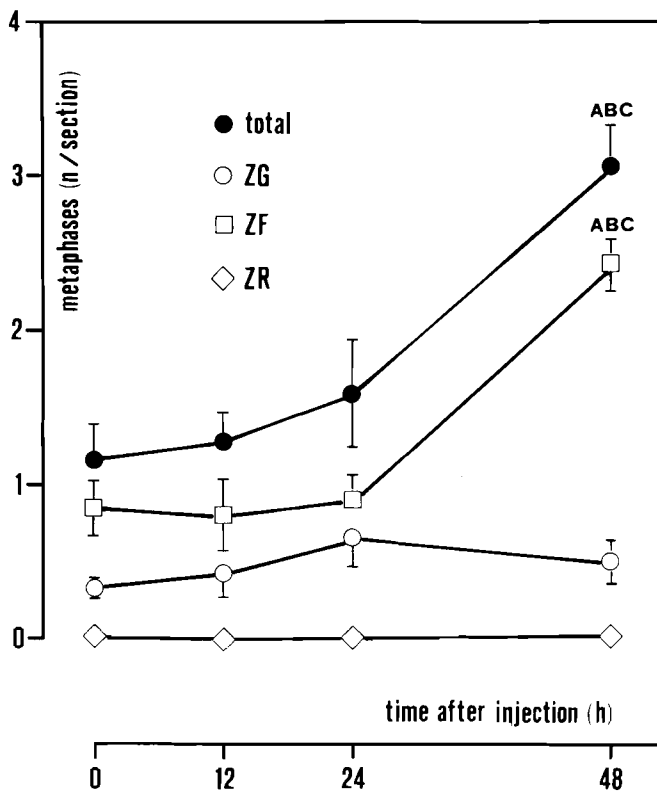


Fig. 2. Effect of NT administration on the proliferative activity of rat adrenocortical cells. Each point represents mean  $\pm$  SE (n= 6). A, B and C,  $P < 0.01$  from 0 h, 12 h and 24 h groups, respectively.

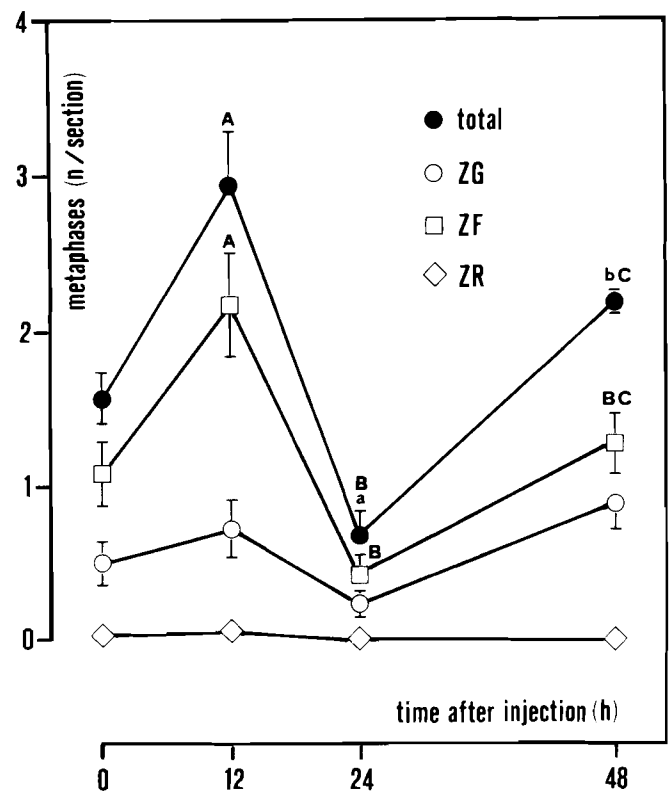


Fig. 3. Effect of NMN administration on the proliferative activity of rat adrenocortical cells. Each point represents mean  $\pm$  SE (n= 6). a and A,  $p < 0.05$  and  $p < 0.01$  from 0 h group; b and B,  $P < 0.05$  and  $P < 0.01$  from 12 h group; C,  $P < 0.01$  from 24 h group.

## Discussion

The present study clearly shows that a single bolus injection of both NT and NMN is able to enhance proliferative activity of rat adrenocortical cells. As mentioned in the Introduction, both NMN and NT are said to exert their biological effects via NT-R. However, this does not seem to be the case under our experimental conditions, inasmuch as the proliferative effect of NMN is faster (12 h after injection) than that of NT (48 h after injection). Thus, it is conceivable that the proliferative responses of rat adrenocortical cells to NT and NMN are mediated by different mechanisms. It should be emphasized that analogous differences in the time of onset of adrenocortical cell proliferation were also observed after bombesin and neuromedin-M bolus injections (Markowska et al., 1993).

Both NT and NMN provoke striking changes in the function of the hypothalamo-pituitary-adrenocortical axis, and a stimulating effect of these neuropeptides on CRH and ACTH release is well documented (Malendowicz et al., 1991, 1992, 1993; Nussdorfer et al., 1992). However, the ACTH-evoked proliferative burst in the rat adrenal cortex usually occurs 48 h after its administration (Szkudlinski et al., 1987). Therefore, ACTH may mediate only the proliferative effect of NT. Another factor responsible for this effect of NT may be vasopressin, which has been reported to induce a burst of mitoses in the rat adrenal cortex about 48 h after its administration (Payet and Lehoux, 1980). This last contention is also supported by the demonstration that some NT effects on rat adrenal cortex (e.g. aldosterone secretagogue action) are mediated by vasopressin (Lesniewska et al., 1992; Mazzocchi et al., 1993).

As far as the mediator(s) of adrenocortical proliferative action of NMN is(are) concerned, our present data indicate that it(they) must be different from ACTH or vasopressin. It could be hypothesized that the effect of NMN is mediated by angiotensin-II, which exerts a prompt and potent stimulatory effect on rat adrenocortical cells (Gill et al., 1987; Szkudlinski and Lewinski et al., 1989; Pawlikowski et al., 1990; Mazzocchi et al., 1992). Preliminary experiments indicate that the proliferative effect of NMN on the rat adrenal cortex is prevented by saralasin, a specific competitive antagonist of angiotensin-II.

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