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Effects of bombesin and neuromedin-B on the proliferative activity of the rat adrenal cortex

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Summary. Bombesin (BM) and neuromedin-B (NMB) exert similar biological effects, acting via two functionally distinct BM-receptor subtypes. The present study aimed to investigate whether BM and NMB stimulate the proliferation of rat adrenocortical cells and to compare their mode of action. Adult female rats were treated with a single subcutaneous dose of 3 μg BM or NMB. Adrenocortical proliferative activity was assessed by the metaphase-arrest technique. BM administration resulted in a marked increase in the number of metaphases in zona glomerulosa (ZG) and zona fasciculata (ZF), and in the entire cortex. This increase appeared 24 h after injection in the ZG, and after 48 h in the ZF. NMB administration, on the other hand, caused a prompt increase in the number of metaphases in the ZG and entire cortex at 12 h, followed by a subsequent drop below the control level at 24 and 48 h of experiment. These findings indicate that BM and NMB enhance the proliferative activity of rat adrenocortical cells acting via different receptors or different mediators.

Key words: Bombesin, Neuromedin-B, Adrenal growth, Cell proliferation, Rat

Introduction

Bombesin (BM) is a tetradecapeptide originally purified from the skin of the frog *Bombina bombina* (Anastasi et al., 1971). Various BM-related peptides were subsequently isolated and classified into three subfamilies: BM; ranatensin; and litorin (Erspamer et al., 1988). In mammals, two BM-related peptides have been identified: neuromedin-B (NMB) of the ranatensin subfamily; and gastrin-releasing peptide (GRP) of the BM subfamily (McDonald et al., 1979; Minamino et al., 1983).

BM and NMB exert similar biological effects, the latter peptide being frequently found to be less potent (Brown et al., 1978a). High concentrations of BM-like and NMB-like immunoreactivity have been demonstrated in the hypothalamus and pituitary gland of various animal species (Brown et al., 1978a,b; Polak et al., 1978; Panula et al., 1982, 1984; Roth et al., 1982; Major et al., 1983; Ghatei et al., 1984; Minamino et al., 1984; Steel et al., 1988; Larsen et al., 1989). In the pituitary gland, GRP and BM immunoreactivities were localized in corticotropes (Steel et al., 1988; Houben and Denef, 1991). High concentrations of BM immunoreactivity were also found in the adrenal zona medullaris, mainly connected with norepinephrine cells (Lemaire et al., 1986). Such a localization of BM and NMB suggests the possible involvement of these neuropeptides in the regulation of the growth and function of the adrenal cortex.

BM and related peptides can function as tumor promoters in certain cell types (Rozengurt and Sinnett-Smith, 1983) and have been implicated as autocrine growth factors in the pathogenesis of some human small-cell lung carcinomas (Cuttitta et al., 1985). The present study aimed to investigate whether BM and NMB stimulate the proliferation of adrenocortical cells in rats.

Materials and methods

Adult female Wistar rats (110-120g body weight) were employed, and maintained under standardized conditions of lighting (14L:10D) 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 BM or NMB (Sigma, St. Louis, Mo.), and sacrificed after 0, 12, 24 or 48 h (5 rats per each time-point). Two hours before the sacrifice, the rats received (at 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 x400. For each rat 8 medulla-containing sections were analyzed, and metaphases counted

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separately in the zona glomerulosa (ZG), zona fasciculata (ZF) and zona reticularis (ZR). On randomly chosen rats, the mitotic index was estimated by counting 5000 parenchymal cells in the ZF, and these data were correlated with the number of metaphases per section of the ZF; as demonstrated earlier (Stachowiak et al., 1990), there is a close direct correlation between metaphase count 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 and the Multiple Range test of Duncan. The linear correlation between metaphase count 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

As expected, in control rats metaphase-arrested cells were only found in the ZG and ZF (Figs. 2, 3).

BM administration resulted in a marked increase in the number of metaphases in ZG, ZF and the entire cortex. This increase was apparent in the ZG and entire cortex 24 h after neuropeptide injection, and in the ZF after 48 h (Fig. 2).

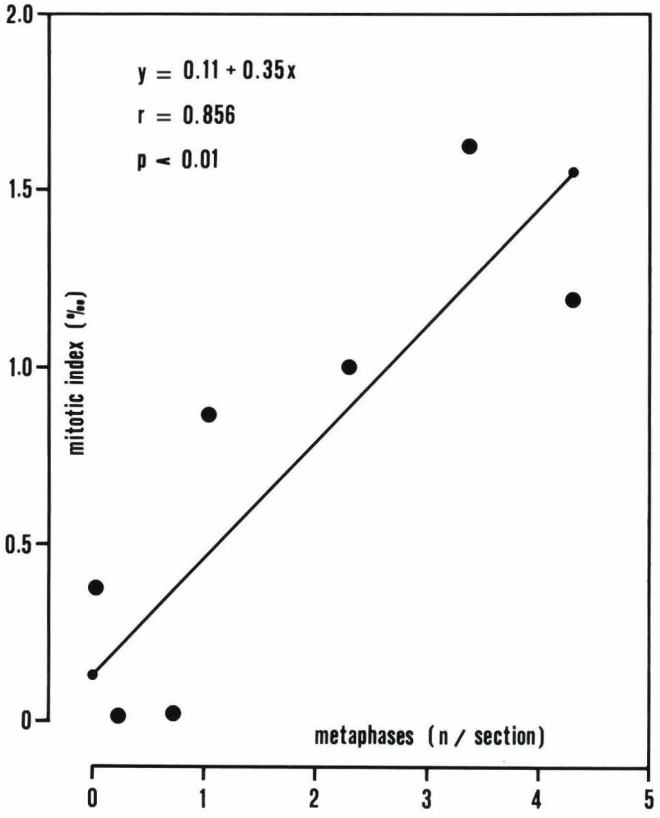

Fig. 1. Correlation between mitotic index and number of metaphases per section in the ZF of randomly chosen rats from both experiments.

NMB administration induced a significant increase in the number of metaphases in the ZG and entire cortex within 12 h, followed by a subsequent drop after 24 and 48 h. The number of metaphases in the ZF did not undergo any significant change (Fig. 3).

Discussion

The present findings clearly demonstrate differences in the proliferative activity of rat adrenocortical cells after the administration of a single dose of BM and NMB. Both neuropeptides markedly increased the number of metaphase-arrested adrenocortical cells, however NMB effect was faster and of lower magnitude than that evoked by BM. This suggests that BM and NMB stimulate adrenal proliferative activity via different receptors or via different mediators.

Available evidence indicates the presence of at least two functionally distinct BM-receptor subtypes: a GRP-preferring BM receptor and an NMB-preferring BM receptor. Each of them is expressed in different subsets of brain regions and in other tissues, and both subtypes belong to the G-protein coupled-receptor superfamily (von Schrenk et al., 1989, 1990; Wada et al., 1990, 1991; Battey and Wada, 1991). Thus, the differences in the

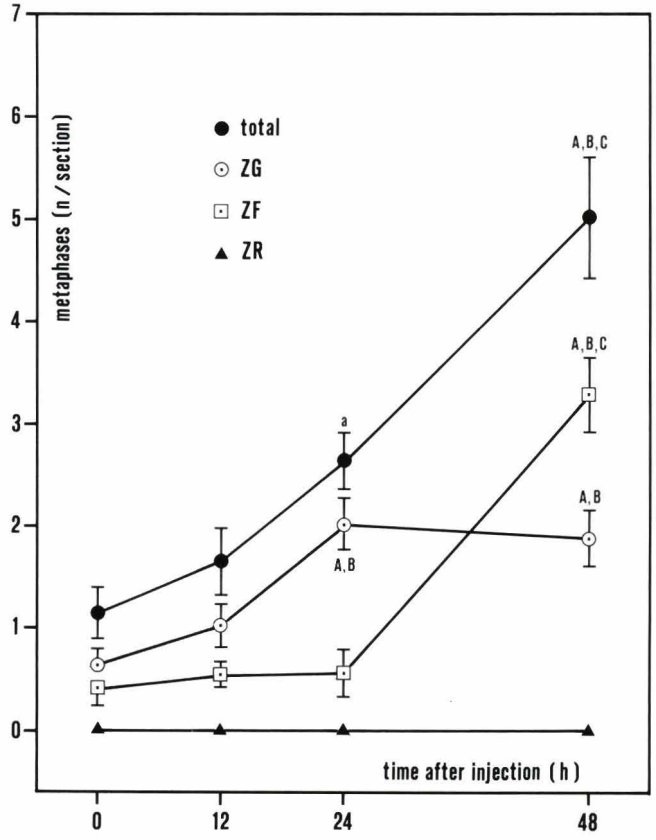

Fig. 2. Effect of BM administration on the proliferative activity of rat adrenal cortex. Each point represents the group mean ± SE (n=5). a and A, P <0.05 and P <0.01 from group 0 (control rats); B, P <0.01 from group 12; C, P <0.01 from group 24.

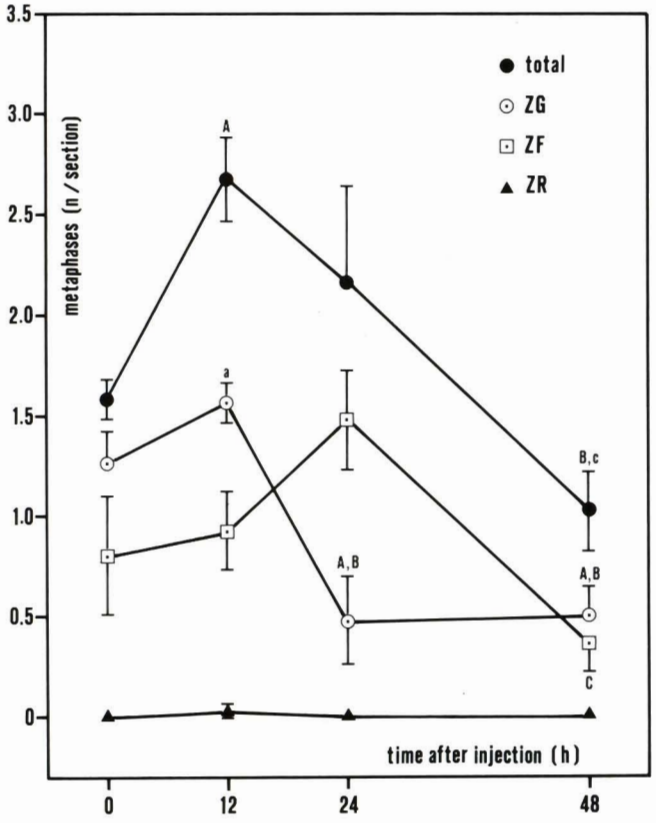

Fig. 3. Effect of NMB administration on the proliferative activity of rat adrenal cortex. Each point represents the group mean ± SE (n=5). a and A, P<0.05 and P<0.01 from group 0 (control rats); B, P<0.01 from group 12; c and C, P<0.05 from group 24.

mode of stimulation of adrenocortical-cell proliferation of BM and NMB may depend on their binding to different receptors. In this connection, it should be emphasized that BM is able to act directly on isolated rat adrenocortical cells, inhibiting their basal corticosterone output (Malendowicz et al., 1991).

As is well known, both neuropeptides provoke striking changes in the function of hypothalamo-pituitary-adrenocortical axis. BM potentiates CRH-stimulated ACTH release by the pituitary corticotropes, and after short-term infusion raises ACTH blood level (Thomas and Sander, 1985; Knigge et al., 1987; Familiari et al., 1988; Sander and Porter, 1988; Sander and Thomas, 1991). Hence, it may be conceived that the BM-induced stimulation of proliferation of rat adrenocortical cells is mediated by ACTH. This contention is supported by the finding that the ACTH-evoked rise in the number of metaphases in rat adrenal cortex is usually observed 48 h after its administration (Szkudlinski et al., 1987). Another factor responsible for the MB-induced stimulation of adrenocortical-cell proliferation may be vasopressin, which is co-localized with CRH in the same hypothalamic neurocytes (Antoni, 1986; Sawchenko, 1987). After a single injection of vasopressin a burst of mitoses in rat adrenal cortex is observed after 48 h (Payet and Lehoux, 1980). This assumption may be supported by the demonstration that various neuropeptides, including neurotensin and many tachykinins, are able to stimulate vasopressin secretion (Massi et al., 1991; Mazzocchi et al., 1993).

As far as the mediator(s) of NMB action on adrenocortical cell proliferation is (are) concerned, obtained data suggest that it (they) is (are) different from ACTH or vasopressin. At present, we are searching for this (these) factor(s).

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