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Effects of bombesin on the morphology and function of the rat adrenal cortex: comparison of the acute and chronic responses

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Summary. The acute and chronic effects of bombesin (BM) on the structure and function of rat adrenal cortex were investigated by morphometric and radioimmunological techniques. An intraperitoneal bolus injection of 2 μg/rat BM markedly raised plasma corticosterone (B) concentration (PBC). The intraperitoneal BM infusion (1 μg/rat.h^{-1}) for 1, 2 or 4 days evoked a notable increase in the number of adrenocortical cells, without inducing apparent changes in either PBC or B output by adrenal quarters. Since proliferation and expression of specialized functions are mutually exclusive states of cells, our findings suggest that the conspicuous stimulation of adrenocortical-cell proliferation evoked by BM infusion may be responsible for the apparent lack of effect of this treatment on B secretion.

Key words: Bombesin, Adrenal cortex, Cell proliferation, Steroidogenesis, Rat

Introduction

Bombesin (BM) and its mammalian counterpart gastric-releasing peptide (GRP) acutely stimulate adrenal glucocorticoid release (Thomas and Sander, 1985; Sander and Porter, 1988; Sander and Thomas, 1991). On the contrary, BM infusion for 7 days notably lowers rat plasma corticosterone (B) concentration and depresses the *in-vitro* capacity of adrenocortical cells to secrete B under basal (but not ACTH-stimulated) conditions (Malendowicz et al., 1991). The present study aimed to follow sequential changes in rat-adrenal cortex structure and function during long-term infusion of BM, and to compare them with the acute effects of this neuropeptide.

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Materials and methods

Animal treatment

Adult female Wistar rats were maintained under standardized conditions of lighting (14 h on: 10 h off) and temperature (22±2° C), with free access to laboratory pellets and tap water.

In the acute studies, two groups of rats (n=8) were intraperitoneally injected with 2 μg BM (Bachem, Bubendorf, Switzerland) or 0.2 ml saline vehicle, and sacrificed 60 min later. In the long-term studies, rats were intraperitoneally infused (Alzet osmotic pumps; Alza, Palo Alto, CA.) with BM (1 μg/rat.h^{-1}), and sacrificed after 1, 2 or 4 days of infusion (6 rats for each time-point). A group of rats (n=6) was infused for 4 days with the saline vehicle.

The animals were decapitated between 9:00 and 10:00 am, and their trunk blood was collected and stored at -20 °C. Pituitary and adrenal glands were promptly removed; neural and intermediate lobes were separated from the anterior pituitary one under the dissecting microscope, and adrenals were freed of adherent fat. Gland weights were recorded.

Morphometry

Left adrenal glands were fixed in Bouin's solution, embedded in paraffin and serially cut at 5-6 μm. Sections were stained with haematoxylin-eosin. Two stages of morphometric analysis were performed. In the 1st stage, using a magnification of x 100 and a square lattice test system, the volume densities (Vv) of the zonae glomerulosa (ZG), fasciculata (ZF) and reticularis (ZR) were estimated by differential point counting (Weibel, 1979). In the 2nd stage, the Vv of parenchymal-cell nuclei and cytoplasm, as well as the number of nuclear profiles per unit area of section were estimated on a screen at a magnification of x 3,000. The volumes of

adrenal zones, and the average volume and number of their parenchymal cells were calculated according to Weibel (1979), as previously detailed (Malendowicz, 1987).

Corticosterone secretion by adrenal slices

The right adrenals were quartered and preincubated for 30 min at 37 °C in 1 ml Krebs-Ringer bicarbonate buffer with 0.3% glucose (KRBG). The incubation medium was discarded and new KRBG with 0.3% bovine serum albumin (Fraction V; Sigma, St. Louis, MO) was added. After 60 min of incubation at 37 °C, with continuous shaking, medium was collected and stored at -20 °C until B assay (Lesniewska et al., 1990).

Corticosterone and glucose assays

B concentration in plasma (PBC) and incubation medium was measured by radioimmunoassay, using [1α,2α(n)-^{3}H]-B (1.96 TB9.mmol^{-1}) (Amersham Int., Amersham, U.K.) and a B antiserum developed in rabbit (Sigma). Sensitivity: 125 fmol/tube. Cross-reactivity: B, 100%, 11-deoxycorticosterone, 27%; cortisol, 7.5% aldosterone, 4.6%; testosterone, 4.5%; 11-deoxycortisol, 4.3%; 20α-hydroxyprogesterone, 3.5%; 20ß-hydroxy-progesterone, 2.7%; androstenedione, 1.9%; cortisone and 17-hydroxyprogesterone, 1.2%; other steroids, less than 0.1%. Intra- and inter-assay variations: 6% and 8%, respectively.

Blood glucose concentration was estimated by a commercial kit (Glucose Hexokinase; Abbotts Labs, Chicago, Ill.; C.v.: 3%).

Statistics

Individual results were averaged per group, and SE was calculated. The statistical comparison of the data was performed by ANOVA, followed by the Multiple Range Test of Duncan.

Results

The intraperitoneal bolus administration of BM induced within 60 min a 2-fold rise in PBC (Fig. 1).

Prolonged BM infusion did not affect body, adrenal and anterior-pituitary weights (Fig. 2), nor did it significantly change the volumes of ZG and ZF, and the number of their parenchymal cells (Fig. 3). The volume of ZR, the number of ZR cells and the number of adrenocortical cells in the entire cortex underwent a significant rise in relation to the duration of BM infusion (Fig. 3). The volume of ZR cells did not display significant changes, while that of ZG and ZF showed a transient increase at the 2nd day of BM infusion (Fig. 3). BM infusion did not evoke appreciable changes in either PBC or B production by adrenal quarters (Fig. 4). Glycemia was increased (16%) after 2 days of BM administration (Fig. 5).

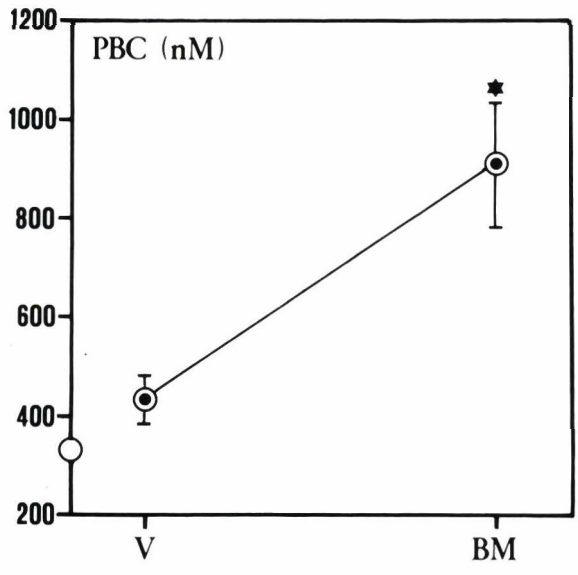

Fig. 1. Acute effect of BM on PBC in rats. Note that the moderate stress evoked in control rats by the intraperitoneal injection of vehicle (V) raises PBC over the basal value (indicated on the ordinate). Data are means±SE (n=8). *: p< 0.01 from V-group.

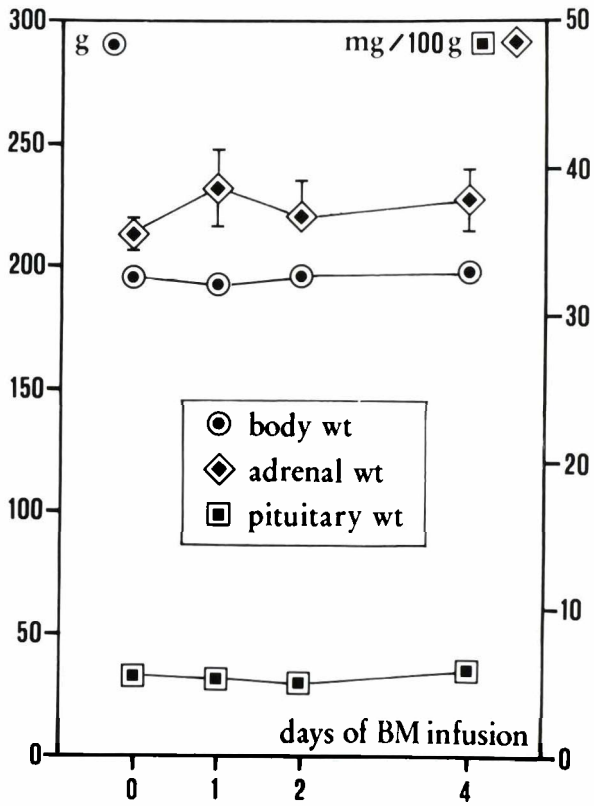

Fig. 2. Lack of effect of BM infusion on body, adrenal and pituitary weights in rats. Data are means±SE (n=6).

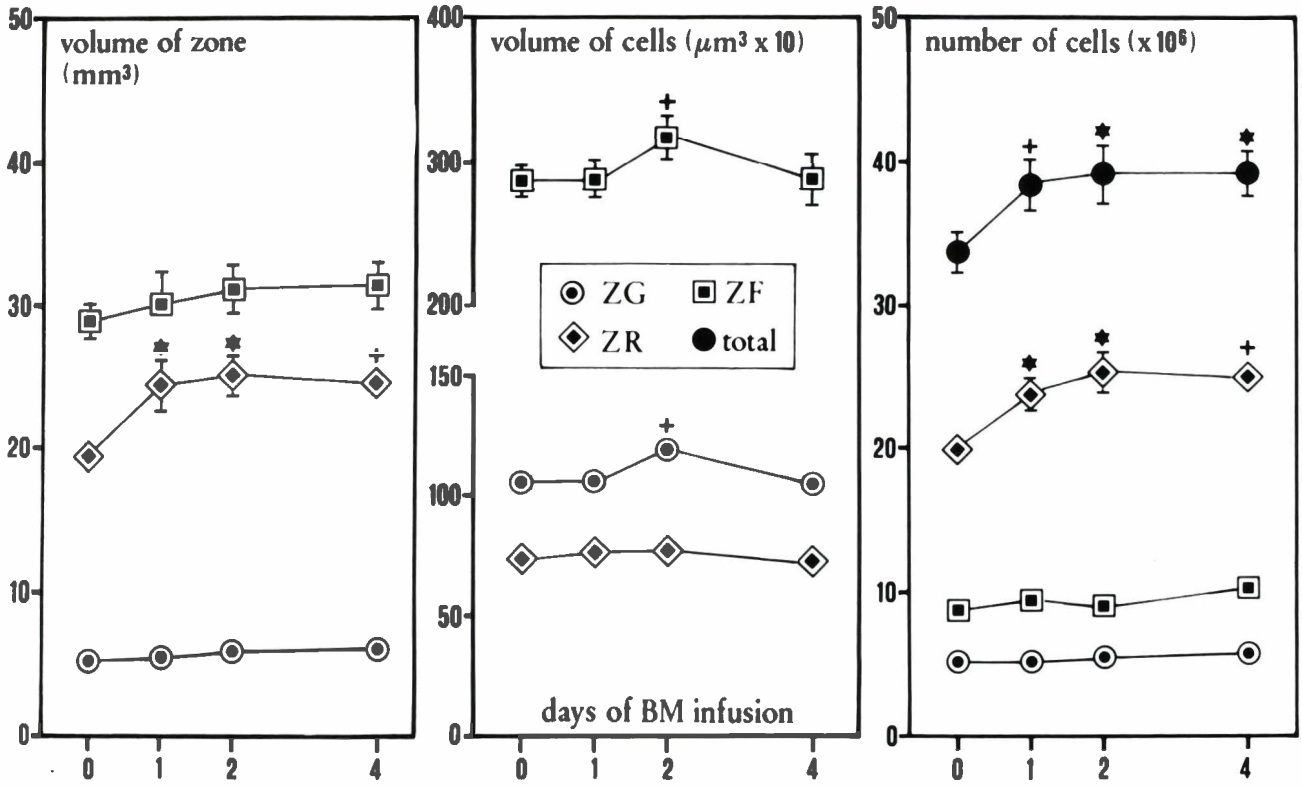

Fig. 3. Effects of BM infusion on the morphometric parameters of rat adrenal cortex. Data are means±SE (n=6). +: p<0.05 and; *:p<0.01 from 0-group.

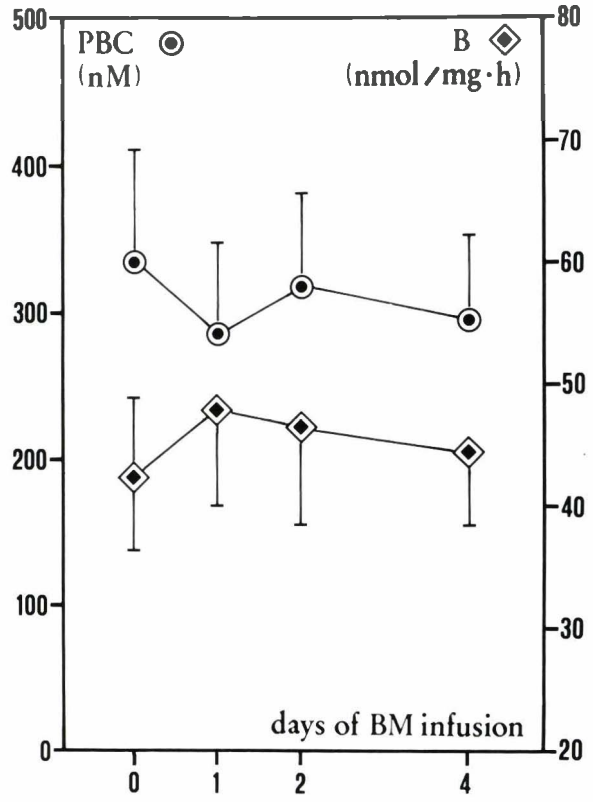

Fig. 4. Lack of effect of BM infusion on PBC and B output by adrenal quarters. Data are means±SE (n=6).

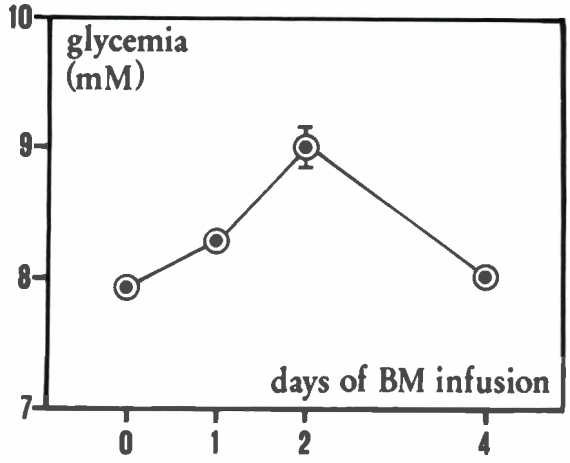

Fig. 5. Effect of BM infusion on glycemia in rats. Data are means±SE (n=6). *:p<0.01 from 0-group.

Discussion

High concentrations of BM-like immunoreactivity (ir) have been demonstrated in the hypothalamus and pituitary gland of several animal species (Brown et al., 1978; Polak et al., 1978; Villareal and Brown, 1978; Ghatei et al., 1984; Minamino et al., 1984; Larsen et al., 1989). In the pituitary gland GRP-like ir is found in corticotropes, and in the adrenal gland elevated concentrations of BM-like ir are connected with nor-

epinephrine-secreting cells (Lemaire et al., 1986; Houben and Denef, 1991). Evidence is available that both BM and GRP potentiate CRH-stimulated ACTH release by pituitary corticotropes, and that a short-term infusion of BM raises ACTH blood level (Hale et al., 1984; Thomas and Sander, 1985; Familiari et al., 1987; Gunion et al., 1989; Sander and Thomas, 1991; Olsen et al., 1992). On the contrary, a prolonged infusion of BM has no effect on ACTH plasma concentration (Malendowicz et al., 1991). As mentioned in the Introduction, BM-induced changes in the level of circulating ACTH are paralleled by analogous changes in the plasma concentration of glucocorticoids (for review, see Malendowicz 1993).

Our present results show that BM acutely stimulates B secretion; however, BM infusion does not affect either PBC or B output by adrenal quarters. The increase of glycemia after 2 days of BM infusion provides a proof that in the osmotic minipumps this neuropeptide retains its biological activity. Having no effect of B secretion, prolonged BM administration induces a marked proliferation of adrenocortical cells, an effect appreciable throughout the whole experiment and in keeping with previous findings (Markowska et al., 1993). In this connection, it must be noted that BM and related peptides can function as tumor promoters and have been implicated as autocrine growth factors in the pathogenesis of some human small-cell lung carcinomas (Rozengurt and Sinnet-Smith, 1983; Cuttitta et al., 1985).

In light of these findings, it seems legitimate to suggest that the differences between the acute and chronic effects of BM on B secretion in the rat are caused by the striking adrenocortical proliferogenic effect of this neuropeptide in long-term experiments. It is generally accepted that proliferation and the expression of specialized functions are mutually exclusive states of the cells, a biological principle that has been clearly demonstrated in *in-vitro* cultured adrenocortical cells after ACTH exposure (Masui and Garren, 1971; Ramachandran and Suyama, 1975). Thus, the stimulation of the proliferative activity of adrenal cortex induced by prolonged BM infusion may be responsible for the apparent lack of effect of this treatment on B secretion.

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References

Brown M., Rivier J., Kobayashi R. and Vale W. (1978). Neurotensin-like and bombesin-like peptides: CNS distribution and action. In: Gut Hormones. Bloom S.R. (ed). Churchill Livingstone. Edinburgh. pp 550-558.

Cuttitta F., Carney D.N., Mulshine J., Moody T.W., Fedorko J., Fischler A. and Minna J. (1985). Bombesin-like peptide can function as autocrine growth factors in human small-cell lung cancer. Nature 316, 823-826.

Familiari M., Funder J.W. and Giraud A.S. (1987). Bombesin potentiation of CRF stimulated ACTH release is dependent on presence of glucocorticoids. Regul. Pept. 19, 107-112.

Ghatei M.A., Bloom S.R., Langevin H., Mac Gregor G.P., Lee Y.C., Adrian T.E., O'Shaghnessy D.J., Blank M.A. and Uttenthal L.O. (1984). Regional distribution of bombesin and seven other regulatory peptides in the human brain. Brain Res. 293, 101-109.

Gunion M.W., Tache Y., Rosenthal M.J., Miller S., Butler B. and Zib B. (1989). Bombesin microinfusion into the rat hypothalamic paraventricular nucleus increases blood glucose, free fatty acids and corticosterone. Brain Res. 478, 47-58.

Hale A.C., Price J., Ackland J.F., Doniach I., Ratter S., Besser G.M. and Rees L.H. (1984). Corticotrophin-releasing factor mediated adrenocorticotrophin release from rat anterior pituitary cells is potentiated by C-terminal gastrin-releasing peptide. J. Endocrinol. 102, 121-123.

Houben H. and Denef C. (1991). Evidence for the presence of gastrin-releasing peptide immunoreactivity in rat anterior pituitary corticotrophs and lactotrophs, AtT_{20} cells, and H_3 cells: failure to demonstrate participation in local control of hormone release. Endocrinology 128, 3208-3218.

Larsen P.J., O'Hare M.M.T., Vangsted A. and Mikkelsen J.D. (1989). Gastrin-releasing peptide (GRP) is present in a GRP (1-27) form in anterior pituitary cells of the guinea pig. Peptides 10, 815-818.

Lemaire S., Chouinard L., Mercier P. and Day R. (1986). Bombesin-like immunoreactivity in bovine adrenal medulla. Regul. Pept. 13, 133-146.

Lesniewska B., Nowak M., Nussdorfer G.G. and Malendowicz L.K. (1990). Sex-dependent effect of melatonin on the secretory activity of rat and hamster adrenal gland in vitro. Life Sci. 47, 241-245.

Malendowicz L.K. (1987). Sex differences in adrenocortical structure and function. XXIV. Comparative morphometric studies on adrenal cortex of intact mature male and female rats of different strains. Cell Tissue Res. 249, 443-450.

Malendowicz L.K. (1993). Involvement of neuropeptides in the regulation of growth, structure and function of the adrenal cortex. Histol. Histopath. 8, 173-186.

Malendowicz L.K., Lesniewska B., Baranowska B., Nowak M. and Majchrzak M. (1991). Effect of bombesin on the structure and function of the rat adrenal cortex. Res. Exp. Med. 191, 121-128.

Markowska A., Nussdorfer G.G. and Malendowicz L.K. (1993). Effect of bombesin and neuromedin-B on the proliferative activity of the rat adrenal cortex. Histol. Histopath. 8, 359-362.

Masui H. and Garren L.D. (1971). Inhibition of replication in functional mouse adrenal tumor cells by adrenocorticotropic hormone mediated by adenosine 3':5'-cyclic monophosphate. Proc. Natl. Acad. Sci. USA 68, 3206-3210.

Minamino N., Kangawa K. and Matsuo H. (1984). Neuromedin B is a major bombesin-like peptide in rat brain: regional distribution of neuromedin B and neuromedin C in rat brain, pituitary and spinal cord. Biochem. Biophys. Res. Commun. 124, 925-932.

Olsen L., Knigge U. and Warberg J. (1992). Gastrin-releasing peptide stimulation of corticotropin secretion in male rats. Endocrinology 130, 2710-2716.

Polak J.M., Ghatei M.A., Wharton J., Bishop A.E., Bloom S.R., Solcia E., Brown M.R. and Pearse A.E.G. (1978). Bombesin-like immunoreactivity in the gastrointestinal tract, lung, and central nervous system. Scand. J. Gastroenterol. 13, S149-S156.

Ramachandran J. and Suyama A.T. (1975). Inhibition of replication of mouse adrenocortical cells in culture by ACTH. Proc. Natl. Acad. Sci. USA 72, 113-117.

Rozengurt E. and Sinnet-Smith J. (1983). Bombesin stimulation of DNA synthesis and cell division in cultures of Swiss $3T_3$ cells. Proc. Natl. Acad. Sci. USA 80, 2936-2940.

Sander L.D. and Porter J.R. (1988). Influence of bombesin, CCK, secretin and CRF on corticosterone concentration in the rat. Peptides 9, 113-117.

Sander L.D. and Thomas R.M. (1991). Gastrin-releasing peptide, but not pentagastrin, stimulates ACTH and cortisol secretion in conscious dogs. Regul. Pept. 35, 127-133.

Thomas R.M. and Sander L.D. (1985). Influence of CCK and bombesin on ACTH and cortisol secretion in the conscious dog. Peptides 6, 703-707.

Villareal J. and Brown M. (1978). Bombesin-like peptides in hypothalamus: chemical and immunological characterization. Life Sci. 23, 2729-2738.

Weibel E.R. (1979). Stereological methods. 1. Practical Methods for Biological Morphometry. Academic Press. London. pp 1-415.

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