

Effects of the prolonged administration of bradykinin on the rat pituitary-adrenocortical axis

L.K. Malendowicz¹, C. Macchi², G.G. Nussdorfer² and A. Markowska¹

¹Department of Histology and Embryology, School of Medicine, Poznan, Poland and

²Department of Anatomy, University of Padua, Italy

Summary. The effects of a prolonged administration of bradykinin (BK) and/or D-Arg, [Hyp³, D-Phe⁷]-BK, a specific antagonist of BK receptors (BK-A) (daily subcutaneous injections of 4 nmol/rat for 6 days) on the function of the pituitary-adrenocortical axis were investigated. BK did not change plasma aldosterone concentration (PAC), but markedly lowered that of corticosterone (PBC) and consequently induced a compensatory hypersecretion of ACTH by the pituitary gland. BK-A did not apparently affect the function and growth of the adrenal gland, but, when administered together with BK, markedly raised both PAC and PBC, and provoked a significant atrophy of the adrenal gland, probably due to loss of parenchymal cells. Taken together, these rather puzzling findings do not appear to provide clear evidence for the involvement of BK in the physiological regulation of adrenocortical growth and steroidogenic capacity in rats.

Key words: Bradykinin, Bradykinin-receptor antagonist, Pituitary-adrenal axis, ACTH secretion, Steroid-hormone secretion, Adrenal cortex, rat

Introduction

It has been recently shown that bradykinin (BK) acutely raises aldosterone (ALDO) and corticosterone (B) secretion of rat adrenals, by a not-yet known BK receptor-independent mechanism not involving corticomedullary paracrine interactions (Malendowicz et al., 1995c). We have also reported that D-Arg, [Hyp³, D-Phe⁷]-BK, a specific antagonist of BK receptors of the B2 subtype (BK-A), despite counteracting BK effects, exerts a marked secretagogue action on the rat adrenal cortex, that we tentatively ascribed to its hypertensive effect probably increasing adrenal blood flow.

The aim of the present study was to investigate the effect of a prolonged BK and BK-A treatment on the pituitary-adrenocortical functions in rats. Although

biological plasma half-life of kinins is very short (Erdos, 1979; Ryan, 1982) we assumed that repeated injections of BK might modify its biological action on the studied system.

Materials and methods

Adult female Wistar rats (150-160 g body weight) were kept under a 10:14 h light-dark cycle (illumination onset at 8:00 a.m.) at 22±2 °C, with free access to standard chow and tap water. Three groups of rats (n=6) were given daily subcutaneous injections of 4 nmol BK (Bachem, Bubendorf, Switzerland), BK-A (Sigma, St. Louis, MO, USA) or BK plus BK-A dissolved in 0.2 ml 0.9% NaCl, for 6 consecutive days. A fourth group of animals received the vehicle only and served as a control. The rats were decapitated 60 min after the last injection, trunk blood was collected, and adrenal glands promptly removed.

The left adrenals were weighed, and then fixed in Bouin's solution, embedded in paraffin and serially cut at 6 µm. Sections were stained with hematoxylin-eosin. The volume density 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 average volumes of zona glomerulosa (ZG), zona fasciculata (ZF) and zona reticularis (ZR) cells were calculated according to Weibel (1979), as previously detailed (Malendowicz, 1987).

The right adrenal of each rat was quartered and preincubated for 30 min at 37 °C with 1 ml of Krebs-Ringer bicarbonate buffer with 0.2% glucose (KRBG). The incubation medium was discarded and new KRBG with 0.3% bovine serum albumin was added (Lesniewska et al., 1990). After 120 min of incubation at 37 °C with continuous shaking, the medium was collected, and ALDO and B concentrations were assayed.

ACTH was extracted from plasma, and its concentration determined by radioimmunoassay: ACTH-RIA kit (Cis Bio International, Gif-sur-Yvette, France). Sensitivity: 10 pg/ml. Cross-reactivity: ACTH(1-24),

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100%; α -MSH and β -lipotropin, 0.1%; other pituitary hormones, less than 0.001%. Intra- and interassay variations: 6% and 9%, respectively. ALDO and B were extracted from plasma and incubation media and their concentrations were measured by RIA as previously detailed (Malendowicz et al., 1993). Intra- and interassay variations were: ALDO, 7% and 8%; B, 8% and 9%, respectively.

Data were expressed as means \pm SE, and their statistical comparison was done by ANOVA, followed by the Multiple Range Test of Duncan.

Results

Neither BK nor BK-A treatment affected body and adrenal weights; however, the simultaneous administration of BK and BK-A decreased them (-15% and -34%, respectively) (Fig. 1). In BK-treated rats ZG cells were smaller (-12%) and ZF cells larger (11%) than in control rats; BK-A and BK plus BK-A increased the average cell volume in all adrenocortical zones (ZG, 8-10%; ZF, 11-18%; ZR, 17-18%) (Fig. 2).

BK markedly raised ACTH blood level (2.4-fold), did not affect plasma ALDO concentration (PAC) and decreased plasma B concentration (PBC) (-57%). BK-A did not induce significant changes in hormonal plasma levels, but, when simultaneously administered with BK, notably enhanced both PAC (78%) and PBC (2.1-fold) (Fig. 3).

Adrenals slices obtained from all experimental groups of rats secreted similar basal amounts of ALDO. Adrenal slices from BK-treated rats produced less B than those of control animals (-30%); conversely, slices from

both BK-A and BK plus BK-A groups secreted markedly higher amounts of B (29-42%) (Fig. 4).

Discussion

Taken together, our present findings appear very puzzling and apparently rather conflictive with those obtained in the acute experiments, where BK was found to raise both PAC and PBC (Malendowicz et al., 1995c).

At variance with a single *bolus* injection, repeated

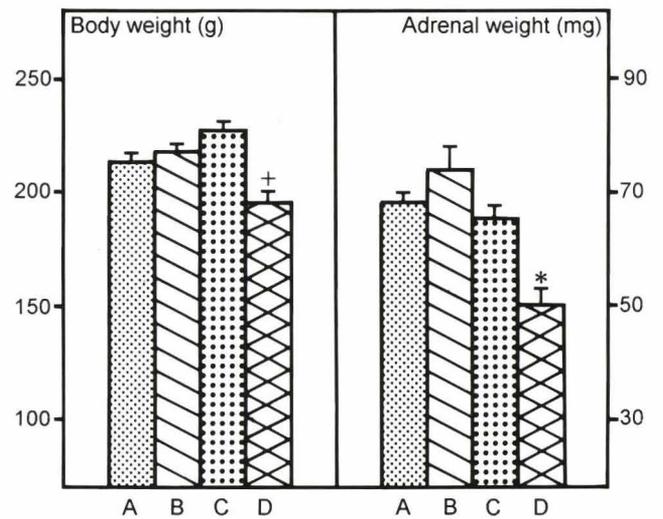


Fig. 1. Effects of a prolonged treatment with BK and/or BK-A on body and adrenal weights in adult rats. A: controls; B: BK; C: BK-A; D: BK plus BK-A. Data are means \pm SE (n= 6). +: P<0.05; *: P< 0.01 from group A.

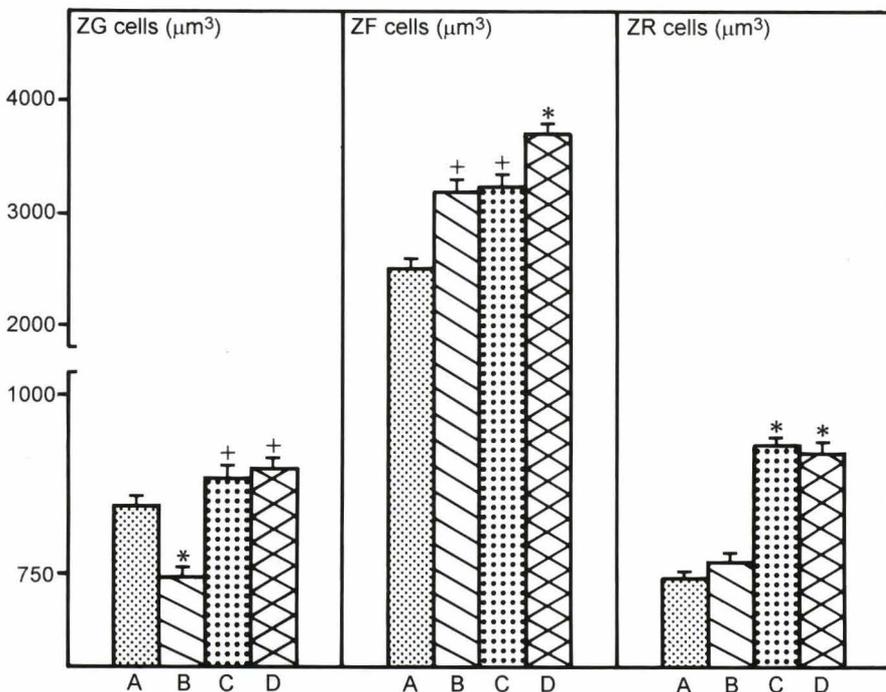


Fig. 2. Effect of a prolonged treatment with BK and/or BK-A on the average volume of adrenocortical cells in adult rats. A: Controls; B: BK; C: BK-A; D: BK plus BK-A. Data are means \pm SE (n= 6). +: P<0.05; *: P< 0.01 from group A.

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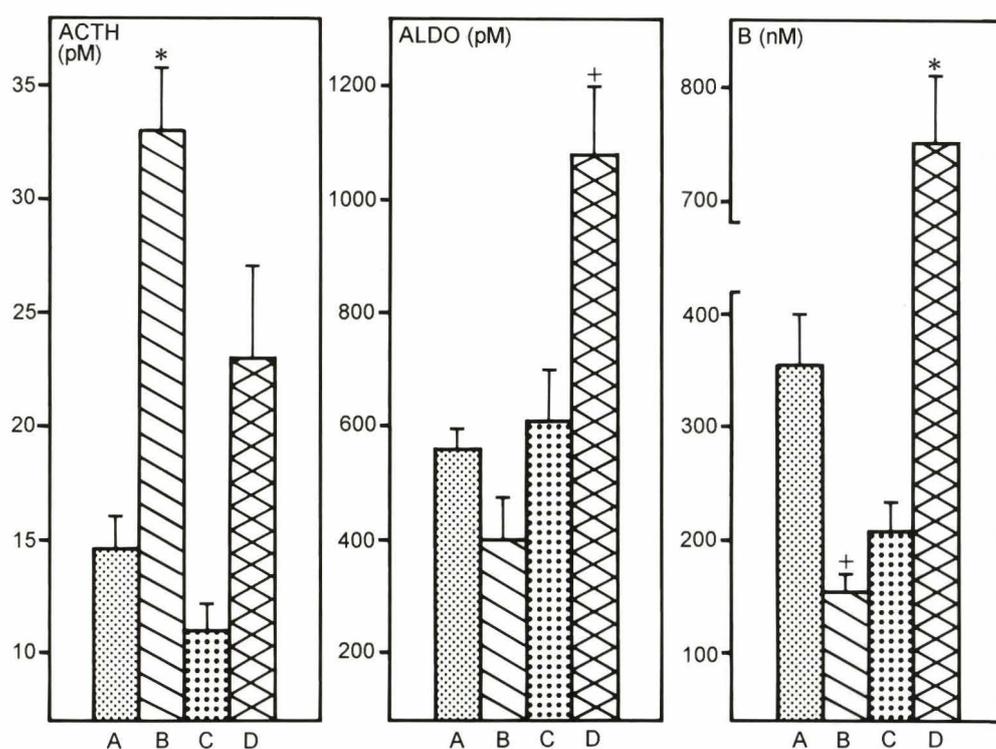


Fig. 3. Effects of a prolonged treatment with BK and/or BK-A on the plasma concentrations of ACTH, ALDO and B in adult rats. A. Controls; B. BK; C. BK-A; D. BK plus BK-A. Data are means \pm SE (n=6). †: $p < 0.05$; *: $p < 0.01$ from group A.

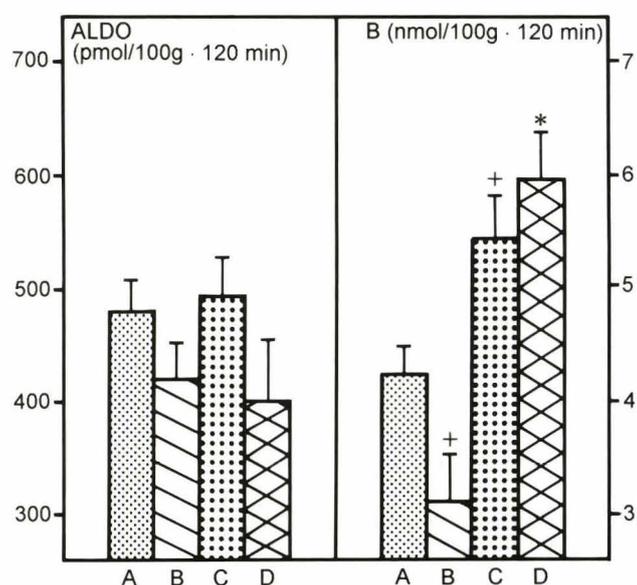


Fig. 4. Effects of a prolonged treatment with BK and/or BK-A on basal ALDO and B secretion by adrenal quarters in vitro. A. Controls; B. BK; C. BK-A; D. BK plus BK-A. Data are means \pm SE (n=6). †: $p < 0.05$; *: $p < 0.01$ from group A.

BK administrations do not affect ALDO secretion. At first glance, it could be suggested that accommodation of the system(s) involved in the regulation of mineralocorticoid secretion might have engendered this discrepancy. Another possibility stems from the well-known «aldosterone escape» phenomenon occurring during prolonged ACTH treatment (for review, see

Abayasekara et al., 1993). It seems reasonable to assume that BK-induced chronic rise in the level of circulating ACTH (see below) may partially suppress ALDO secretion, thus counteracting the stimulatory effect of the kinin. However, it must be noted that the «aldosterone escape» phenomenon is usually coupled with hypertrophy of ZG cells (Mazzocchi et al., 1986; Riondel et al., 1987), while BK provokes a moderate ZG cell atrophy.

In contrast with the acute glucocorticoid secretagogue effect of BK, the prolonged administration of the kinin evokes a net decrease in both PBC and B outputs by adrenal slices. This may obviously result in the compensatory hypersecretion of pituitary ACTH, that in turn induces ZG-cell hypertrophy. It is very difficult to reconcile these findings with the demonstration that BK is able to directly stimulate basal glucocorticoid secretion by ZF/ZR cells *in vitro* via a B2 receptor-mediated mechanism (Rosolowsky and Campbell, 1992; Malendowicz et al., 1995a). It has been previously demonstrated that BK is also able to inhibit ACTH-stimulated steroid secretion by a receptor-independent mechanism probably involving the interference of the kinin molecule with the intracellular mechanism(s) transducing the ACTH secretagogue signal (Malendowicz et al., 1995a,b). It is not inconceivable that this last effect of BK, during its chronic administration, may suppress the action of ACTH required for the maintenance of a normal basal glucocorticoid secretion. Obviously, this hypothesis entails that different intracellular mechanisms are involved in the transduction of the secretagogue and

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trophic signals of ACTH, inasmuch as the BK-induced elevated level of circulating ACTH provokes a clearcut hypertrophy of ZF cells (see above). However, the current evidence indicates that the activation of adenylyl-cyclase (and cAMP generation) mediates both the secretory and trophic actions of ACTH on adrenocortical cells (for review, see Nussdorfer, 1986; Simpson and Waterman, 1992). Therefore, it appears more probable that the observed drop in B secretion is indirectly caused by factor(s) induced by BK and not by the kinin *per se*. Among these factors a major role could be played by changes in the rate of adrenal blood flow, which is known to modulate adrenal glucocorticoid secretion (for review, see Vinson and Hinson, 1992): the BK-evoked hypotension (Bathon and Proud, 1991), by reducing adrenal blood flow, may be responsible for the decrease in PBC. However, this mechanism does not explain the drop in B output by adrenal quarters *in vitro*.

Taking into account the potent *in vivo* stimulating action of BK-A on ALDO and B secretions (Malendowicz et al., 1995c), our present results concerning the prolonged effect of this antagonist on the pituitary-adrenocortical axis are difficult to explain. The administration of BK-A alone does not change adrenal weight, PAC, PBC and ALDO output by adrenal quarters; however, it raises the average volume of adrenocortical cells and B secretion by adrenal quarters. It has been previously demonstrated that BK-A does not affect either ALDO or B secretion of dispersed rat adrenocortical cells (Malendowicz et al., 1995a,b). However, this antagonist, via a BK receptor-independent mechanism, enhances catecholamine release by adrenal chromaffin cells, thus raising blood pressure (for references, see Malendowicz et al., 1995c) and renin secretion (Bierwaltes et al., 1988; Mulinari et al., 1988; Bathon and Proud, 1991). Catecholamines do not seem to be involved in the mediation of the acute effect of the kallikrein-kinin system on adrenals (see Malendowicz et al., 1995c), but the renin-angiotensin system is surely one of the main regulators of mineralocorticoid secretion of ZG in rats (for review, see Quinn and Williams, 1992). Hence, the presently observed lack of effect of prolonged BK-A administration on the pituitary-adrenocortical function could suggest that such a treatment leads to unresponsiveness of rats to BK-A and/or to a shift in adrenal response to this kinin.

As reported in the acute experiments (Malendowicz et al., 1995c), also in the present chronic ones BK-A, when simultaneously administered with BK, induces a striking increase in both PAC and PBC. Unexpectedly, these effects are coupled with a marked atrophy of the adrenal gland, whose parenchymal cells are, however, increased in volume: a morphological finding which strongly suggests a loss of adrenocortical cells. A possible explanation of these intriguing data may be that the high level of circulating glucocorticoids exerts a direct inhibitory effect on the growth of the adrenal cortex (Nussdorfer and Mazzocchi, 1970; Kahri, 1973), via an apoptotic mechanism that does not alter the

secretory function of the remaining cells (Kerr, 1972; Wyllie et al., 1973; and for review, see Kerr and Harmon, 1991). Be that as it may, the synergic action of BK and BK-A on rat adrenocortical function still remains to be elucidated.

To summarize, the prolonged administration of BK does not affect mineralocorticoid secretion, but it inhibits glucocorticoid production, thus eliciting a compensatory hypersecretion of ACTH. This effect of BK is not conceivably mediated by B2 receptors, since BK-A, instead of blocking it, enhances both ALDO and B secretions, although inducing atrophy of the adrenal gland. However, BK-A, when administered alone, does not apparently affect adrenocortical function and growth. In conclusion, the present findings cast doubts on the involvement of BK in the physiological regulation of the adrenal growth and steroidogenic capacity in rats, and suggest that the role of the intra-adrenal kallikrein-kinin system is probably restricted to the fine acute tuning of steroid secretion.

Acknowledgements. This study was supported by a grant from the State Committee for Scientific research of Poland (N.6 P207 071 07), and performed in the frame of the Polish-Italian Agreement of Scientific and Technical Cooperation.

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Accepted February 5, 1996