THE POSSIBLE ROLE OF ENDOGENOUS BRADYKININ IN THE REGULATION OF THE RESPONSE OF RAT PITUITARY-ADRENOCORTICAL AXIS TO STRESSES

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ABSTRACT

The role played by endogenous bradykinin (BK) on the hypothalamo-pituitary-adrenal (HPA) axis was investigated in the rat. Normal and ether-stressed (2 min ether-vapor inhalation) or cold-stressed (20 min at 4°C) animals were given a *bolus* subcutaneous injection of 20 nmol of D-Arg,[Hyp³, D-Phe⁷]-BK, an antagonist of B₂ subtype of BK receptors (BKA); the blood concentrations of ACTH, aldosterone (ALDO) and corticosterone (B) were measured by specific RIA, 1, 2 or 4 h after the injection. BKA did not evoke significant changes in the basal plasma levels of the three hormones. Ether and cold stresses notably raised the blood concentrations of ACTH, ALDO and B, being maximal responses observed after 1 or 2 h. BKA markedly enhanced the responses of ACTH and B, but not that of ALDO to ether stress. BKA magnified ALDO and B responses to cold stress, but it strikingly depressed ACTH one. In light of these findings, it may be concluded that (i) endogenous BK does not affect basal activity of rat HPA axis, but it exerts a general inhibitory action on its response to the stresses; and (ii) different mechanisms are involved in the cold and ether stress-induced activation of the HPA axis, which could explain the quantitative and qualitative differences observed in the inhibitory action of endogenous BK according to the kind of stress employed.

Bradykinin (BK) is a potent vasodilatory peptide, whose synthesis from kininogens and degradation are catalyzed by kallikreins and kinase II, respectively. BK acts via at least two subtypes of receptors, B₁ and B₂; B₂ receptor is widely distributed in the body tissues, while B₁ expression seems to be mainly restricted to the aorta (for review, see 1).

The presence of the kallikrein-kinin system in the hypothalamus and pituitary gland (16–18), and in the adrenal gland (8, 14, 19) suggests the possible involvement of BK in the physiological regulation of the function of the hypothalamo-pituitary-adrenal (HPA) axis. The recent demonstration that a *bolus* subcutaneous injection of BK markedly raises in the rat plasma aldosterone (ALDO) and corticosterone (B) concentrations within 60 min (10) lends support to this contention.

It therefore seemed worthwhile to investigate the

role of endogenous BK in the regulation of the function of HPA axis of rats under basal and stressful conditions. To this task we examined the effects of the acute administration of the specific B₂-receptor antagonist (BKA) D-Arg,[Hyp³, D-Phe⁷]-BK (6) on the basal activity of rat HPA axis and on its response to ether and cold stresses.

MATERIALS AND METHODS

Adult female Wistar rats (weighing 190–210 g) were kept under a 10:14 h light-dark cycle (illumination onset at 8 00 a.m.) at 22 ± 1°C. The animals were given daily subcutaneous injections of 0.2 ml 0.9% NaCl for 7 days, in order to damp the response of their HPA axis to handling. On the 8th day, rats were divided into two groups, which were given a subcutaneous injection of 20 nmol · kg⁻¹

Fig. 1 Effect of BKA on plasma ACTH concentration of normal rats, and ether- or cold-stressed rats. S, time of application of the stress. Data are means $\pm$ SE ($n = 6$). $^aP < 0.05$ and $^AP < 0.01$ from 0-h group; $^+P < 0.05$ and $^*P < 0.01$ from the respective control (vehicle-injected) group

Fig. 2 Effect of BKA on plasma ALDO concentration of normal rats, and ether- or cold-stressed rats. S, time of application of the stress. Data are means $\pm$ SE ($n = 6$). $^aP < 0.05$ and $^AP < 0.01$ from 0-h group; $^*P < 0.01$ from the respective control (vehicle-injected) group

BKA (Sigma, St. Louis, U.S.A.) or 0.2 ml saline vehicle. Immediately after the injection, each group of animals was divided into 3 subgroups, one of which was not further treated; the other two subgroups underwent to ether or cold stress. Ether stress: rats were individually placed for 2 min in a 10 l-glass jar, in which 10 ml ethyl ether had been previously poured, and employed 10 min later (9). Cold stress: rats were placed in a wire cage for 20 min at 4°C, and immediately employed. All the injections were made at 9 00 a.m., and rats were decapitated at times 0 (just after the injection), S (just after stress), 1, 2 or 4 h after the injection (6 rats for each time point). The trunk blood was collected in the presence of EDTA, plasma was separated and stored at -30°C .

ACTH was extracted from plasma, and its concentration assayed by RIA: ACTH-RIA kit (Cis Bio International, Gif-sur-Yvette, France). Sensitivity: $10 \text{ pg} \cdot \text{ml}^{-1}$. Cross-reactivity: ACTH (1–24), 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 their concentrations measured by RIA, using

[1,2,6,7- ^3H]-ALDO and [1α , 2 α (n)- ^3H]-B (Amersham, U.K.; S.A.: $1.96 \text{ Tbq} \cdot \text{mmol}^{-1}$) and antisera developed in rabbit (Sigma). ALDO-RIA: sensitivity: $5 \text{ pg} \cdot \text{ml}^{-1}$. Cross-reactivity: ALDO, 100%; 17-iso-ALDO and other steroids, less than 0.1%. Intra- and interassay variations: 5% and 7%, respectively. B-RIA: sensitivity: $50 \text{ pg} \cdot \text{ml}^{-1}$. Cross-reactivity: B and cortisol, 100%; 11-deoxycorticosterone and progesterone, 2%; other steroids, less than 0.001%. Intra- and interassay variations: 7% and 9%, respectively.

Data were expressed as means $\pm$ SE ($n = 6$), and their statistical comparison was done by ANOVA, followed by the Multiple Range Test of Duncan.

RESULTS

Non-Stressed Rats

Vehicle injection did not result in any significant change in the plasma concentrations of ACTH, ALDO or B at 1 h, when the maximal response of HPA axis to handling stress is usually observed (11). BK did not evoke appreciable alterations in the basal values of these three parameters (Figs. 1–3).

Fig. 3 Effect of BKA on plasma B concentration of normal rats, and ether- or cold-stressed rats. S, time of application of the stress. Data are means $\pm$ SE ($n = 6$). ^A $P < 0.01$ from 0-h group; * $P < 0.05$ and * $P < 0.01$ from the respective control (vehicle-injected) group

Ether Stress

Ether stress induced an immediate rise in the blood levels of ACTH (3.3-fold), ALDO (2.8-fold) and B (4.5-fold), that was not apparently affected by BKA (Figs. 1–3). ACTH-response peaked at 2 h (8.0-fold rise). BK increased ACTH response at 1 and 4 h (2.0-fold and 4.2-fold), depressed it at 2 h (-36%) (Fig. 1). ALDO response remained unchanged until 4 h, and BK did not affect it (Fig. 2). B response did not display marked time-related changes, and BK magnified it at 1 h (30%) (Fig. 3).

Cold Stress

Cold stress caused an immediate increase in the plasma concentrations of ACTH (5.5-fold), ALDO (50%) and B (2.9-fold), that was not apparently affected by BK (Figs. 1–3). ACTH response showed a peak at 2 h (18.0-fold increase), that was abolished by BK (Fig. 1). ALDO response reached its maximum at 1 h (3.4-fold), and BK maintained it elevated at 2 and 4 h (73% and 97%) (Fig. 2). B response did not evidence significant time-related changes, though manifesting an oscillatory trend; BK increased B response over the respective control value at 2 and 4 h (80% and 24%) (Fig. 3).

DISCUSSION

The existence of a kallikrein-kinin system in the hypothalamus and pituitary gland of various species is well documented. It has been suggested that BK may modulate the local cerebral microcirculation and may enhance vasopressin secretion (2, 7), which could account for its centrally mediated hypertensive effect (2, 3). Moreover, findings indicate that BK enhances the pituitary release of prolactin, β -endorphin and ACTH (4, 12, 15). These last results, coupled with the demonstration of a potent *in vivo* B and ALDO secretagogue effect (10), strongly suggest that BK stimulates HPA axis.

The physiological significance of this effect of BK remains, however, to be proved, inasmuch as the *in vivo* B secretagogue effect of BK is not reversed by BKA, which would indicate a B_2 -receptor-independent mechanism (10). Our present results clearly show that endogenous BK does not exert any tonic stimulatory influence on the basal activity of HPA axis in the rat. In contrast, and rather unexpectedly, they indicate that endogenous BK plays a role in damping the response of HPA axis to stressful conditions. In fact, BKA appears to markedly magnify glucocorticoid response to stress, thereby suggesting that endogenous BK reduces it.

A very interesting but intriguing finding is that endogenous BK exerts opposite effects on ACTH response to ether and cold stress. In fact, BKA enhances ACTH response to ether stress, but it depresses that to cold stress. In other words, it appears that the inhibitory action of endogenous BK on adrenal response to cold exposure is independent of any suppressive effect on ACTH release. This finding strongly suggests that different mechanisms underly the hypothalamo-pituitary response to ether and cold stresses. In this connection, we want to recall that galanin was previously found to enhance in the rat ACTH and B responses to ether stress, but only B one to cold stress (11).

Another puzzling result concerns the effect of BKA on ALDO response to stresses. It seems that endogenous BK does not affect ALDO response to ether stress, while it markedly lowers that to cold stress. This finding may be related to a well-known side-effect of BKA: the stimulation of catecholamine release from adrenal medulla (1, 13). Since catecholamines enhance ALDO production by zona glomerulosa in a paracrine manner (for review, see 5), it seems reasonable to conceive that

this side-effect of BKA may influence ALDO secretion only when the body response to stress does not involve a marked activation of adrenal medulla. Consequently, it may be tentatively hypothesized that cold stress elicits a catecholamine response much more higher than ether stress.

In conclusion, our present study demonstrates that endogenous BK plays an important role in the modulation of the response of rat HPA axis to stresses. Moreover, they clearly indicate that caution must be used in comparing pituitary and adrenal responses to different kinds of stressful conditions.

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