

Effects of proadrenomedullin N-terminal 20 peptide on the rat pituitary-adrenocortical axis under basal and stressful conditions

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Abstract. Proadrenomedullin N-terminal 20 peptide (PAMP) derives, along with adrenomedullin (AM), from prepro-AM. AM has been reported to modulate the activity of the hypothalamic-pituitary-adrenal (HPA) axis, and this study aimed at ascertaining whether PAMP exerts similar effects. PAMP was subcutaneously administered to non-stressed and stressed rats, and the plasma concentrations of ACTH and corticosterone were measured by radioimmune assay. In non-stressed rats, PAMP raised ACTH and corticosterone blood levels at 60 min, and ACTH plasma concentration at 120 min. Ether and cold stresses increased the plasma levels of both ACTH and corticosterone, and PAMP dampened HPA axis response to cold stress, without affecting that to ether stress. The conclusion is drawn that PAMP i) stimulates rat HPA axis, through a mechanism similar to that of ether stress; and ii) interferes with the neural pathways involved in the cold stress-induced activation of HPA axis.

Introduction

Proadrenomedullin N-terminal 20 peptide (PAMP) is produced, together with adrenomedullin (AM), by the post-translational proteolytic cleavage of a prohormone, named prepro-AM. PAMP and AM are both hypotensive peptides, and share several biological activities, among which vasodilatory action and potent suppressive effect on adrenal aldosterone secretion (reviewed in refs. 1-4).

Evidence has been provided that AM modulates the activity of the hypothalamic-pituitary-adrenal (HPA) axis. To summarize: AM was found to inhibit basal and CRH-

stimulated release of ACTH from cultured rat pituitary corticotrophs (5) and, when systemically administered, to lower plasma concentrations of ACTH and cortisol in sheep (6). However, the intracerebroventricular administration of AM has been reported to stimulate HPA axis in sheep and rats (7-9).

The effects of PAMP on the HPA axis have been far less investigated, the only finding available is that this peptide inhibits ACTH secretion from cultured pituitary cells (10). Therefore, it seemed worthwhile to study the effects of the systemic administration of PAMP on the rat HPA axis and its responses to stress.

Materials and methods

Animals and reagents. Adult female Wistar rats (150-160 g body weight) were kept under a 12:12 h light/dark cycle (illumination onset 8:00 a.m.) at 23°C, and maintained on a standard diet and tap water *ad libitum*. The study protocol was approved by the local Ethics Committee for Animal Studies. PAMP was purchased from Bachem (Bubendorf, Switzerland). All other chemicals were provided by Sigma-Aldrich Corp. (St. Louis, MO).

Ether and cold stress. Stress was induced as previously described (11). Ether stress: rats were individually placed for 2 min in a 10 l-glass jar, in which 10 ml of ethyl ether had been previously poured, and sacrificed 10 min later. Cold stress: rats were placed in a wire cage for 20 min at 4°C, and decapitated immediately thereafter.

Experimental design. Rats received daily subcutaneous (s.c.) injections of 0.2 ml 0.9 NaCl for 10 days to dampen injection- and handling-induced stress. On day 11 (time 0), two groups of animals (n=12) were given a s.c. injection of 1 or 3 nmoles/100 g of PAMP dissolved in 0.2 ml saline. Rats were injected at 9:00 a.m., and divided into two subgroups (n=6), which were decapitated 60 or 120 min after the injection. Control rats received a s.c. injection of vehicle only. Other two groups of rats (n=12) were given a s.c. injection of 3 nmoles/100 g of PAMP or vehicle, then were divided into two subgroups, which underwent ether or cold stress 110 and

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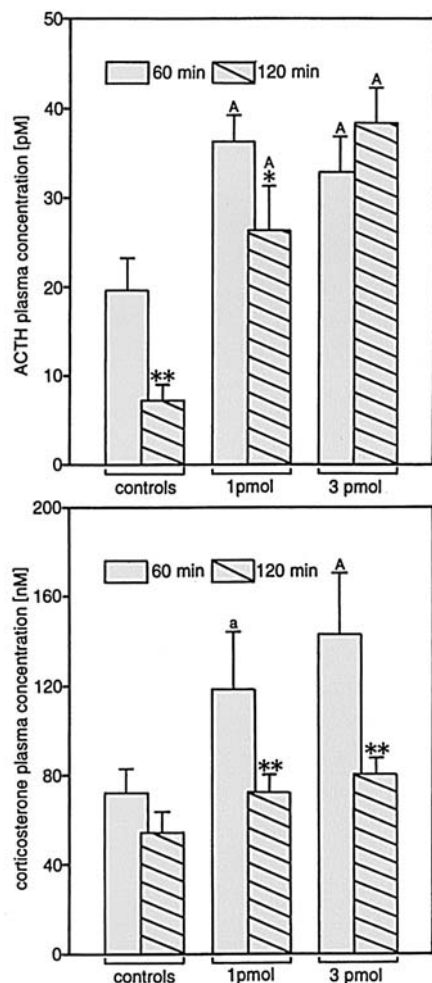


Figure 1. Effects of PAMP on ACTH and corticosterone plasma concentrations in rats. Baseline values are: ACTH, 11.2 ± 0.9 pM; and corticosterone, 62.4 ± 8.2 nM. Bars are means \pm SEM of 6 separate experiments. * $p < 0.05$ and ^A $p < 0.01$ versus the respective control value; ** $p < 0.05$ and ** $p < 0.01$ versus the respective 60-min value.

100 min after the injection, respectively. In each stress experiment a group of rats was not stressed (basal group). Trunk blood was collected in the presence of EDTA (1 mg/ml), and plasma was separated and stored at -36°C until hormone assay.

Hormone assays. ACTH was extracted from plasma, and its concentration was determined by radioimmune assay (RIA) (12), using the ACTH double antibody kit (Diagnostic Products, Los Angeles, CA). Sensitivity: 8 pg/ml. Cross-reactivity: ACTH₁₋₂₄, 100%; α -MSH, 0.2-0.4%; and other peptides (Met⁵-enkephalin, Leu⁵-enkephalin, neurotensin, β -endorphin, substance-P and somatostatin), 0%. Intra- and interassay CVs: 6% and 9%, respectively. Corticosterone was assayed by RIA, as previously detailed (13,14). Sensitivity: 50 pg/ml. Cross-reactivity: corticosterone and cortisol, 100%; 11-deoxycorticosterone and progesterone, 2%; and other steroids, $<0.001\%$. Intra- and interassay CVs: 7% and 9%, respectively.

Statistics. Data were expressed as mean \pm SEM ($n=6$), and their statistical comparison was done by ANOVA, followed by the multiple range test of Duncan.

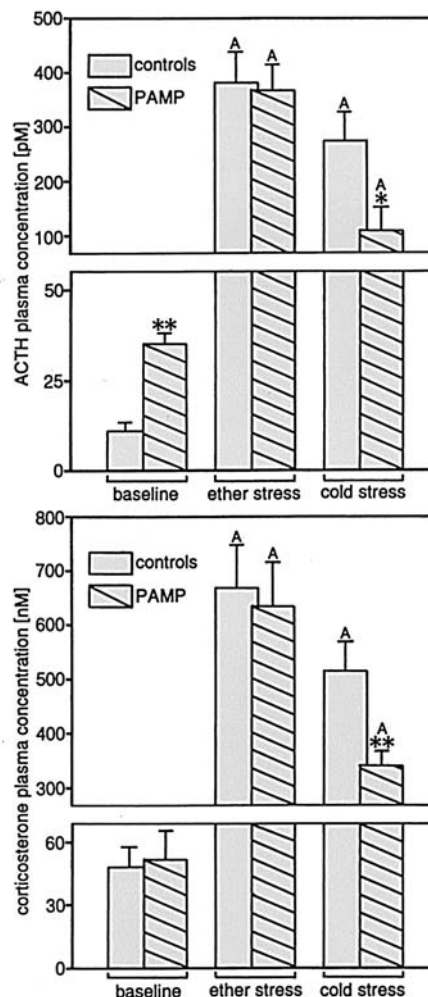


Figure 2. Effects of PAMP (3 nmoles/100 g) on ACTH and corticosterone plasma concentrations in non-stressed and ether- or cold-stressed rats. Bars are means \pm SEM of 6 separate experiments. ^A $p < 0.01$ from the respective baseline value; * $p < 0.05$ and ** $p < 0.01$ from the respective control value.

Results

Sixty minutes after vehicle injection, ACTH, but not corticosterone, plasma concentration was higher than after 120 min. PAMP, independently of the dose administered, raised ACTH and corticosterone blood levels at 60 min. At 120 min, PAMP evoked a significant increase only in the ACTH blood concentration (Fig. 1).

Both ether and cold stresses raised the levels of circulating ACTH and corticosterone, and the response to the ether stress was higher than that to cold stress. As expected (Fig. 1), PAMP at 120 min increased ACTH, but not corticosterone, plasma concentration in non-stressed rat. PAMP did not affect HPA-axis response to ether stress, but significantly dampened that to cold stress (Fig. 2).

Discussion

Our study confirms that, despite the prolonged (10-day) accustoming period of animals to handling and s.c. injections, a novel manipulation and injection evokes an acute and short-lasting activation of the HPA axis, resulting in $\sim 90\%$ rise in the blood level of ACTH, but not corticosterone,



SPANDIDOS. Moreover, our findings indicate that PAMP, HPA axis. The effect is more evident at 60 min, thereby indicating an acute short-lived action of this peptide.

As expected, ether and cold stresses were found to elicit an intense HPA activation (~30-fold and 10-14-fold increases in ACTH and corticosterone blood levels, respectively). The HPA axis response to ether stress was not affected by PAMP, which in contrast dampened the response to cold stress. This finding suggests that the same mechanism underlies the ether stress- and PAMP-induced activation of the HPA axis in rats, while PAMP may somehow interfere with the neural pathways responsible for stimulation of ACTH release by cold stress. In this connection, it is to be recalled that different effects on ether and cold stresses have been reported for other regulatory peptides: galanin potentiates ACTH response to ether, but not to cold stress (15); substance P potentiates ACTH response to ether stress and dampens that to cold stress (16); leptin depresses ACTH response to ether stress and potentiates that to cold stress (17); orexin-A potentiates ACTH response to cold, but not to ether stress (18); and finally beacon suppresses ACTH response to ether, but not to cold stress (11).

Taken together, our present findings provide the first evidence that PAMP, like AM (7-9), affects the function of rat HPA axis *in vivo*. Further studies are underway to ascertain the hypothalamic-pituitary locus of action of PAMP, as well as the physiological relevance of this novel effect of this peptide.

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