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Atriopeptin: An Endogenous Corticotropin-Release Inhibiting Hormone.

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Activation of the hypothalamic-pituitary-adrenocortical axis is a major component of the body's response to stress. Current theories on the pathophysiology of disorders associated with hyperfunction of the axis, such as depression and Cushing's disease, are based on the concept that anterior pituitary adrenocorticotropin (ACTH) secretion is stimulated by hypothalamic corticotropin-releasing hormones and inhibited by adrenal corticosteroids. Hypothalamic inhibitory control of pituitary ACTH secretion has been also postulated, but has not gained general acceptance because of the lack of definitive evidence for a corticotropin-release inhibiting hormone. It is shown here that in conscious rats stress-induced secretion of ACTH and corticosterone is markedly enhanced by the immunoneutralisation of atriopeptin. Therefore, we propose that atriopeptin is a physiologically relevant corticotropin-release inhibiting hormone.

Evidence suggesting an inhibitory component of the hypothalamic control of ACTH secretion dates back to the early sixties *cf.* Ref. 1. A considerable body of data now indicates that the mediator of this inhibition may be atriopeptin (atrial natriuretic peptide, ANP) produced in the hypothalamo-hypophyseal pathway to the external zone of the median eminence *cf.* Ref. 2. Although earlier studies have suggested that ANP has no significant action on pituitary hormone release [3,4] more recent systematic analysis has shown that at physiologically relevant subnanomolar concentrations ANP inhibits stimulated ACTH secretion *in vitro* [5-9] as well as *in vivo* [10] by a direct action on the anterior pituitary gland. In anterior pituitary cells ANP suppresses ACTH release by activating particulate guanylyl cyclase, a mechanism distinct from the nuclear receptor-mediated effects of glucocorticoid hormones, the classical inhibitors of ACTH release *cf.* Refs. 6 & 11. Finally, ANP is released into the hypophyseal portal circulation by hypothalamic neurons [12].

The outstanding pivotal question is whether or not endogenous ANP is involved in the control of ACTH secretion? Since no effective antagonist of ANP action is available at present we have addressed this problem by injecting immunoneutralising antisera against ANP into conscious chronically cannulated rats subjected to stress.

Materials and Methods

Animals All procedures with animals were in accordance with the Cruelty to Animals Act 1986 (U.K.). Male Wistar-derived rats (Animal House, Western General Hospital, Edinburgh, 200-250 g BW) were anesthetized with halothane and equipped with an intraarterial catheter in the right external carotid artery. The cannula was exteriorized by suturing to the skin of the neck and flushed daily with 0.3 ml of 5000 IU/ml heparin. Animals were housed in individual cages in a controlled environment (lights on 05.00-19.00 h). At least 48h after the implantation of the cannula an extension tubing was attached for the collection of blood samples. Two h afterwards blood samples (0.25 ml; volume replaced with saline) were obtained in the following order: 10 min and immediately before the stress stimulus. Anti-ANP antisera or pre-immune rabbit serum (0.2 ml/rat) were injected through the carotid cannula at 2min before stress. Stress consisted of brief immobilisation and the intraperitoneal injection of 0.2 ml of 0.9% NaCl. Further blood samples were obtained 5, 10 , 20, 30, and 60 min afterwards. All experiments were performed between 09.00 and 13.00 h. ACTH in plasma was determined by direct radioimmunoassay using antiserum 8514 as described previously, with minor modifications to ensure that the anti-ANP rabbit serum in the samples did not interfere with the measurement of ACTH[10]. Plasma corticosterone was also measured by radioimmunoassay [13].

Sera Antisera against ANP were raised in New Zealand white rabbits using various synthetic rat ANP analogues (Peninsula Labs, St. Helen's, Merseyside, U.K.) conjugated to mammalian purified protein derivative or bovine thyroglobulin with glutaraldehyde. The titer and specificity of the antisera were characterized in radioimmunoassay using ^{125}I-ANP(99-126) as tracer. The sera were also screened for immunoneutralising activity *in vitro* at 1/100 and 1/300 dilutions by examining the effects of antiserum on the ANP-induced accumulation of cyclic guanosine3':5'monophosphate (cGMP) in suspensions of rat anterior pituitary cells as previously described [6]. Four different antisera (Pink, Orange, RD and RU) all directed against the C-terminus of ANP, blocked the effects of ANP on pituitary cGMP accumulation *in vitro* and were selected for *in vivo* studies. Specificity studies with these sera at 1/400-fold dilution showed negligible cross reaction (< 1 %) with human brain natriuretic peptide, somatostatin and vasopressin. None of the antisera recognized ANP (99-109) and all reacted avidly with ANP (111-126). Each antiserum was injected into three rats and the data are reported as a single anti-ANP serum-treated group.

Results

A submaximal stress stimulus (intraperitoneal injection of saline into previously handled rats) was used in order to reveal any facilitation of the ACTH response by anti-ANP serum. Intraperitoneal injection of saline increased plasma ACTH at least 2-fold above basal in 5 out of 9 control rats receiving pre-immune rabbit serum and in 8 out of 12 rats treated with antiserum against ANP, ($p > 0.1$ by 2x2 contingency table and chi-square test) indicating that the threshold of the stress response was not changed by the injection of anti-ANP serum. In the rats that showed an increase of plasma ACTH upon stress the size of the hormonal response at 5 and 10 min after stress was significantly enhanced by the administration of anti-ANP serum (Fig 1a). Overall, the amount of ACTH secreted after stress was three times greater in the group treated with anti-ANP serum (Fig 1a, *inset*). By 60 min plasma ACTH returned to baseline levels in both groups. The plasma corticosterone response in these animals was also significantly enhanced at 5, 10 and 20 min when compared with controls (Fig 1b).

Administration of ANP antiserum to unstressed rats

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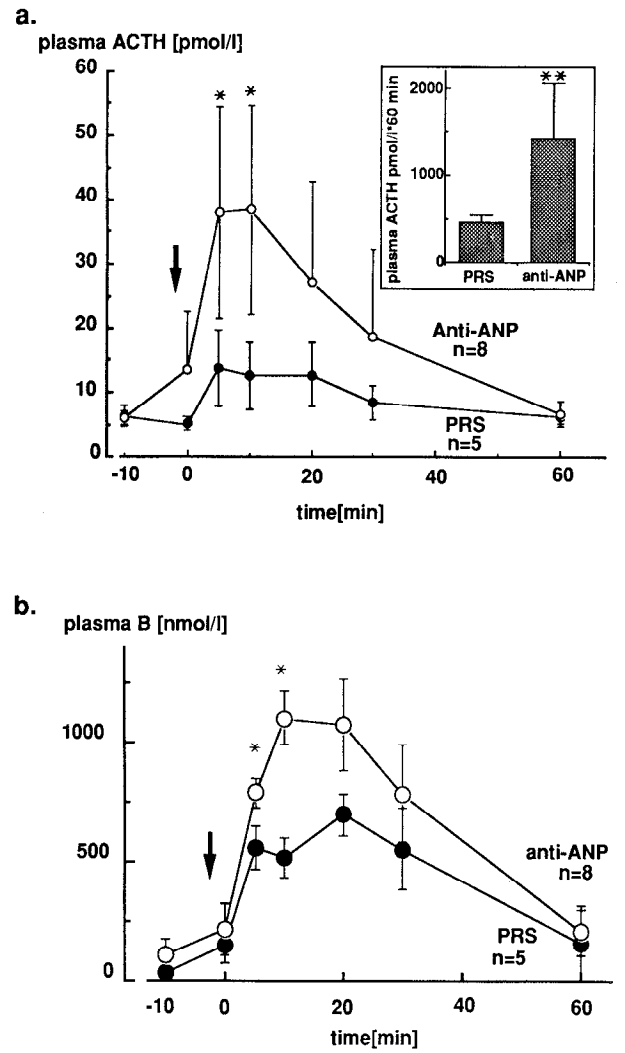

Figure 1. a.) Effect of pre-immune rabbit serum (PRS) and anti-ANP sera (anti-ANP) on stress-induced ACTH secretion in conscious cannulated male rats. Sera (0.2 ml) were given intraarterially at -2 min (arrow). Data are geometric means bars show SEM; * $p<0.05$, Student's t-test performed after log-transformation of the data which was necessary to achieve homogeneity of variances. *Inset:* Data from the same experimental series shown as area under the curve between 0 and 60 min. Data are geometrical means bars show SEM; ** $p<0.01$, compared with pre-immune serum group, Student's t-test after log transformation of data. *b.)* Plasma corticosterone in the same groups of animals as in a. Data are geometric means bars show SEM; * $p<0.05$, Student's t-test performed after log-transformation of the data. Data are geometrical means bars show SEM.

caused no marked changes in plasma ACTH or corticosterone showing that a stress stimulus is required to reveal the effect of endogenous ANP (Data not shown).

Discussion

These data demonstrate that endogenous ANP plays an important role in determining the size of the pituitary-adrenocortical response to stress. With respect to the origin of ANP involved in the regulation of ACTH secretion two sources deserve consideration: the heart *cf.* Refs. 14 & 15 and the hypothalamo-hypophyseal system[16-18]. Atriopeptin in the peripheral circulation originates largely from the heart, but ANP levels in peripheral blood are normally very low (0.001-0.01 nM) and generally do not change in response to acute stressful stimuli *cf.* Ref. 19. Atriopeptin is released from the hypothalamo-hypophyseal pathway into hypophyseal portal blood attaining concentrations (0.1-1 nM) that are relevant for ANP mediated inhibition of pituitary ACTH secretion *in vitro* [5,6] as well as *in vivo* [10]. Moreover, the release of ANP by hypothalamic cultures is enhanced by glucocorticoids suggesting that adrenocortical feedback inhibition of the hypothalamic-pituitary-adrenocortical axis may involve an action of steroids on the hypothalamic ANP system [20].

In summary, the present data show that endogenous ANP is a physiologically relevant inhibitory factor in the control of pituitary ACTH secretion during stress. The site of action of endogenous ANP, whether at the pituitary gland or in the hypothalamus, is not resolved by the present study. However, on the basis of previous evidence it seems reasonable to suggest that this ANP originates from the hypothalamo-hypophyseal tract to the external zone of the median eminence [12,16-18], acts directly at the pituitary level to suppress ACTH secretion [6,8,10,21] and thus constitutes a hypothalamic corticotropin-release inhibiting pathway.

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