NEUROPEPTIDES

Neuropeptides (1994) **27**, 297–303

Evidence that Endogenous Vasoactive Intestinal Peptide (VIP) is Involved in the Regulation of Rat Pituitary-adrenocortical Function: in vivo Studies with a VIP Antagonist

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Abstract—The effect of a subcutaneous bolus injection of 2 μg | Ac,Tyr^1,D-Phe^2 | -GRF(1–29) amide, a specific VIP antagonist (VIP-A), on the hypothalamo-pituitary-adrenocortical (HPA) axis were investigated in both normal and ether- or cold-stressed rats. Blood concentrations of ACTH, aldosterone (ALDO) and corticosterone (B) were measured by specific RIA 1, 2 or 4 h after VIP-A injection. VIP-A administration to normal rats strikingly lowered the plasma concentration of ALDO, without significantly affecting those of ACTH and B. Ether and cold stresses notably raised the blood levels of ACTH, ALDO and B, and these rises lasted unchanged until 4 h. VIP-A did not affect the response of HPA axis to ether stress, but provoked a marked depression of that to cold stress. In light of these findings the following conclusions can be drawn: (i) endogenous VIP does not regulate HPA-axis function under basal conditions, but it plays a pivotal role in the mechanisms involved in the activation of HPA axis induced by cold exposure; and (ii) endogenous VIP exerts a tonic stimulatory action on ALDO secretion, probably by acting directly on the adrenal zona glomerulosa.

Introduction

VIP is a strongly basic octacosa peptide widely distributed in the central and peripheral nervous systems, that at present is recognized as a neurotransmitter involved in neuroendocrine regulation.[1–3]

VIP is present in all components of the hypothalamo-pituitary-adrenal (HPA) axis. In the hypothalamus, the highest concentration of this neuropeptide is found in the suprachiasmaticus nucleus, while in the cells of the parvocellular subdivision of the paraventricular nucleus VIP is co-

Date received 19 March 1994
Date accepted 30 May 1994

localized with CRH.[4–6] These neurons project fibers to the external zone of the median eminence, where VIP can be released into the portal circulation.[7,8] VIP immunoreactivity and VIP mRNA have been demonstrated in the anterior pituitary, and in an in-vitro perfusion system ACTH was found to cause an appreciable release of pituitary VIP.[2,9–12] Adrenal zona medullaris contains a small number of VIP-positive cells, and an abundant network of VIP-ergic fibers is present in zona medullaris, as well as in the adrenal capsule and adjacent zona glomerulosa.[13–18]

The above described localization of VIP strongly suggests its involvement in the regulation of HPA-axis function. Indeed, various investigations confirm a role of VIP in the regulation of ACTH and adrenocortical-steroid release.[2,19] The present study was designed to ascertain whether endogenous VIP plays a role in the functional regulation of rat HPA axis under basal and stress conditions. To achieve this goal, a specific competitive antagonist of VIP (VIP-A), the |Ac-Tyr^1,D-Phe^2|-GRF(1–29) amide,[20] was employed.

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. They were divided into 3 groups, two of which were stressed by ether inhalation or cold exposure. *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 employed 10 min later.[21] *Cold stress*: rats were placed in a wire cage for 20 min at 4°C, and immediately employed. Each of the 3 groups of rats was divided into two subgroups; one subgroup was subcutaneously administered VIP-A (Peninsula, Merseyside, UK) dissolved in isotonic saline (2 μg/rat), the other subgroup received vehicle only and served as a control. All the injections were made at 9:00 a.m., and rats were decapitated at times 0 (no injection), 1, 2 or 4 h after the injection (6 rats for each time point). The trunk blood of each rat was collected in the presence of NaF (1 mg.ml^{-1}), plasma was separated and stored at −20°C.

ACTH was extracted from plasma, and its concentration determined by RIA: ACTHK-RIA kit (Cis Bio International, Gif-sur-Yvette, France). Sensitivity: 10 pg.ml^{-1}. Cross-reactivity: ACTH(1–24), 100%; α-MSH and β-lipotropin, 0.1%; other pituitary hormones, less than 0.001%. Intra-assay and inter-assay variations: 6% and 9%, respectively. Aldosterone (ALDO) and corticosterone (B) were extracted from plasma, and their concentrations measured by RIA, using |1,2,6,7-^{3}H|-ALDO and |1α,2α(n)-^{3}H|-B (Amersham, UK; S.A.: 1.96 TBq.mmol^{-1}) and antisera developed in the rabbit (Sigma, St. Louis, MO, USA). *ALDO-RIA*: sensitivity: 5 pg.ml^{-1}. Cross-reactivity: ALDO, 100%; 17-iso-ALDO and other steroids, less than 0.1%. Intra-assay and inter-assay variations: 5% and 7%, respectively. *B-RIA*: sensitivity: 50 pg.ml^{-1}. Cross-reactivity: B and cortisol, 100%; 11-deoxycorticosterone and progesterone, 2%; other steroids, less than 0.001%. Intra-assay and inter-assay variations: 7% and 9%, respectively. Blood glucose concentration was estimated by a commercial kit (Glucose Hexokinase; Abbott Labs, Chicago, Ill., USA; c.v.: 3%).

Data were expressed as means ± 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 resulted in transient significant increases in the plasma concentrations of ACTH (2-fold), ALDO (68%) and B (2.3-fold) at 1 h, followed by return to the baseline at 4 h (Figs 1–3). Glycemia was not affected (Fig. 4).

VIP-A administration did not affect plasma ACTH level at 1 h, though raising it over the respective control value at 2 h (75%) and 4 h (3-fold) (Fig. 1). VIP-A induced a notable drop in ALDO blood level at 1 h (−54%), 2 h (−51%) and 4 h (−43%) (Fig. 2). B blood level and glycemia were not significantly changed by VIP-A (Figs 3 and 4).

Stressed rats

As expected, both ether and cold stresses notably raised the blood levels of ACTH (6.5-fold and 8.5-fold, respectively), ALDO (3.6-fold and 3.7-fold), B (6.7-fold and 8.8-fold) and glucose (12% and

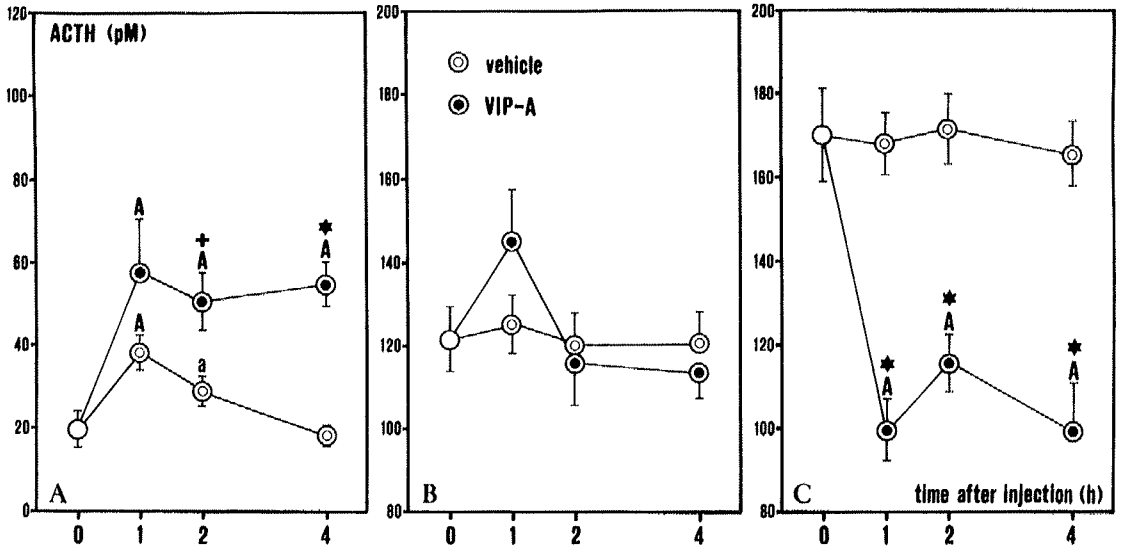

Fig. 1 Effect of VIP-A on ACTH plasma concentration of normal (A), ether-stressed (B) and cold-stressed (C) rats. Data are means ±SE (n = 6). $^{a}P < 0.05$ and $^{A}P < 0.01$ from 0-h group; $^{+}P < 0.05$ and $^{*}P < 0.01$ from the respective control (vehicle-injected) group.

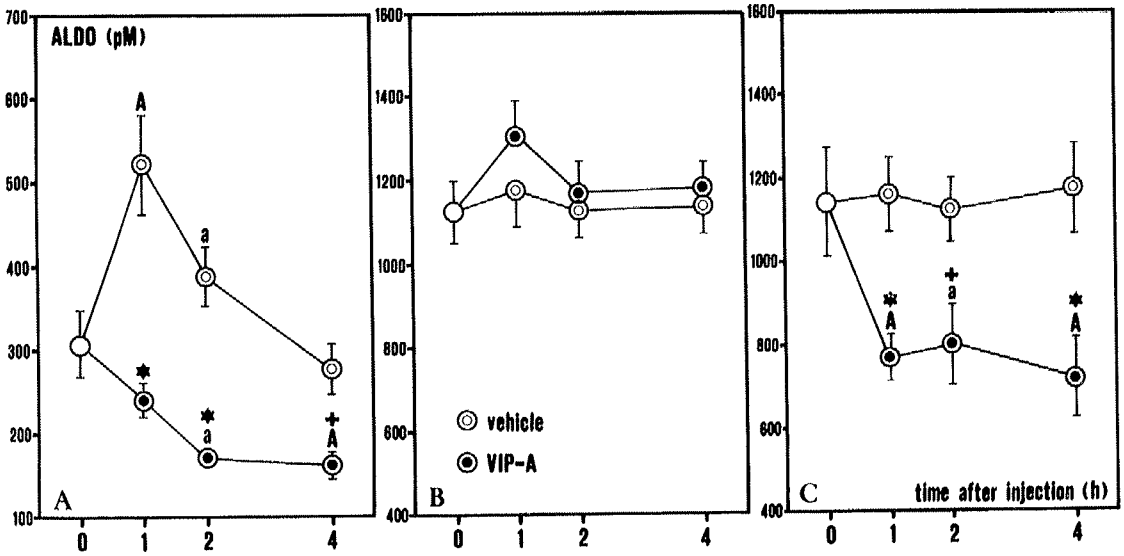

Fig. 2 Effect of VIP-A on ALDO plasma concentration of normal (A), ether-stressed (B) and cold-stressed (C) rats. Data are means ±SE (n = 6). $^{a}P < 0.05$ and $^{A}P < 0.01$ from 0-h group; $^{+}P < 0.05$ and $^{*}P < 0.01$ from the respective control (vehicle-injected) group.

10%). These raises lasted without significant changes until 4 h, and were not modified by the vehicle injection (Figs 1–4).

VIP-A did not affect ACTH and ALDO responses to ether stress, though small non-significant increases were observed at 1 h (Figs 1 and 2). B response to ether stress was not significantly modified at 1 h and 2 h, but it underwent to a slight potentiation (46%) at 4 h (Fig. 3). Conversely, VIP-A administration strikingly depressed ACTH and ALDO responses to cold stress at 1, 2 and 4 h (about −32/−42%, and −30/−40%, respectively), and

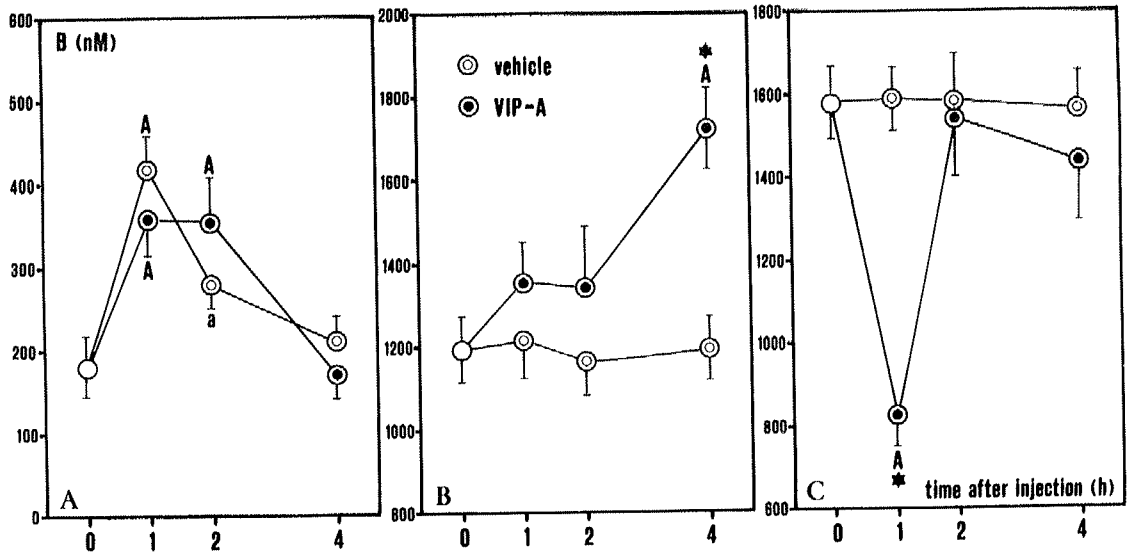

Fig. 3 Effect of VIP-A on B plasma concentration of normal (A), ether-stressed (B) and cold-stressed (C) rats. Data are means ±SE (n = 6). °P < 0.05 and ^P < 0.01 from 0-h group; *P < 0.01 from the respective control (vehicle-injected) group.

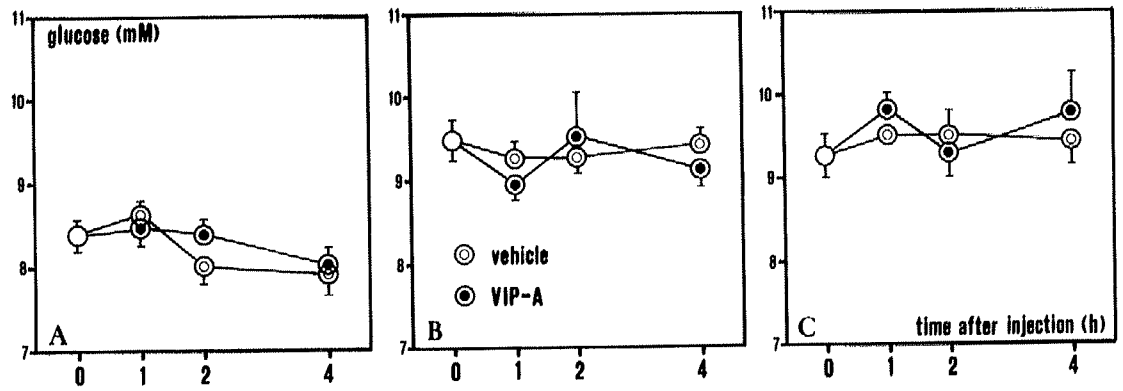

Fig. 4 Effect of VIP-A on glycemia of normal (A), ether-stressed (B) and cold-stressed (C) rats. Data are means ±SE (n = 6).

that of B at 1 h (−48%) (Figs 1–3). The increase in glycemia induced by either kinds of stress was not modified by VIP-A (Fig. 4).

Discussion

The effects of VIP on the secretion of ACTH and glucocorticoid hormones were the object of several investigations, whose results are not always in complete agreement.

Evidence indicates that VIP stimulates ACTH secretion of the atT20 clonal strain of mouse pituitary tumor cells and human pituitary adenoma cells,[22–24] but not of normal corticotropes.[25–30] However, VIP potentiates CRH-induced ACTH release by superfused rat anterior-pituitary fragments.[30] Contrasting results are reported in vivo: Nussdorfer and Mazzocchi[31] did not find any effect of VIP on the ACTH plasma concentration of dexamethasone-suppressed rats, while Pralong et al[32] reported a 10-fold rise in ACTH blood level in rats after 30 min of intravenous VIP infusion. VIP was reported to enhance glucocorticoid secretion in hypophysectomized dogs[33] and intravenously

infused rats,[32] but not in dexamethasone-suppressed rats;[31] conversely a prolonged VIP administration appears to lower plasma B concentration in normal rats and to decrease B output by adrenal slices.[19,34] A direct effect of VIP on inner adrenocortical cells has been shown, but it seems to be due to the agonistic interaction of VIP with ACTH receptors.[35,36]

Our present results showing that VIP-A does not significantly affect the transient rises in ACTH and B plasma levels induced by the vehicle injection, cast doubts about the involvement of endogenous VIP in the physiological regulation of basal HPA-axis function in rats. However, they clearly show that VIP-A suppresses ACTH and B responses to cold stress, without notably affecting those to ether stress.

These last findings indicate that different mechanisms underly the effects of the two types of stress, and strongly suggest that endogenous VIP plays a pivotal role in the mediation of cold-induced activation of rat HPA axis. It may be tentatively suggested that cold-evoked signals, but not ether inhalation-evoked ones, acting at the hypothalamic levels induce the release into the portal circulation (see Introduction) of VIP, that stimulates pituitary corticotropes to secrete ACTH, which in turn enhances glucocorticoid secretion. Our data also rule out the possibility that changes in blood glucose concentration may participate in the cold stress-induced activation of HPA axis mediated by endogenous VIP.

Rather intriguing results were obtained as to the effects of VIP-A on plasma ALDO concentration: in fact, VIP-A not only depresses ALDO response to cold-stress, a finding in keeping with the above discussed ones, but also markedly lower basal plasma concentration of ALDO. Two lines of evidence may explain this last result: (i) adrenal glucocorticoid secretion is almost exclusively ACTH dependent, while mineralocorticoid secretion undergoes a complex multifactorial control;[37–39] and (ii) VIP exerts a direct action on rat-adrenal zona glomerulosa. To summarize: rat zona-glomerulosa cells possess specific VIP receptors,[40] and VIP exerts a strong ALDO secretagogue action on perfused rat capsule-zona glomerulosa preparations,[41] adrenal perfused in situ[42,43] and adrenal slices.[44] Thus, our findings may be interpreted as indicating that, under basal conditions, endogenous VIP exerts a tonic direct stimulatory action on ALDO secretion of rat zona glomerulosa.

In conclusion, the use of a specific VIP-receptor antagonist has provided proofs that endogenous VIP is involved in the regulation of basal mineralocorticoid secretion in rats, and that a VIP-ergic mechanism plays an essential role in the cold stress-induced activation of HPA axis in this species.

Acknowledgement

This study was supported by a grant from the State Committee for Scientific Research (Poland), and performed in the frame of the Polish-Italian Agreement of Scientific and Technical Cooperation.

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