Role of corticotropin-releasing hormone (CRH) in rapid desensitization of the of the adrenocorticotropin (ACTH) response of anterior pituitary cells to arginine vasopressin (AVP)

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Introduction

Recently we have shown that treatment of ovine anterior pituitary cells with arginine vasopressin (AVP), an important regulator of ACTH secretion, results in desensitization to a subsequent stimulation with AVP[1]. The desensitization was found to be rapid and occurred at low AVP concentrations (e.g. pre-treatment with 5 nM AVP for 5 min caused a significant desensitization). CRH is also an important regulator of ACTH, and the two neurohormones are known to interact in this regulation.

The aims of this study were:

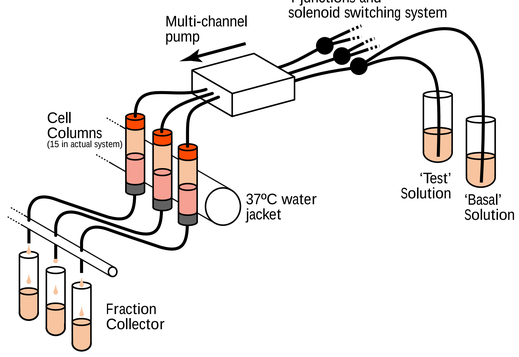
1) to determine the sensitivity to desensitization of the ACTH response to CRH, and
2) to investigate the effect of a low concentration of CRH on the ability of AVP to desensitize anterior pituitary cells to subsequent AVP stimulation.

Methods

General Procedures:

Ovine anterior pituitary cells prepared by collagenase dispersion
⇩
Overnight culture of cells
⇩
Transfer of cells to columns of multi-column perifusion system (see below)
⇩
Perifusion of cells with Krebs Ringer ± CRH and/or AVP and/or KCl
⇩
Collection of 5 or 10 min fractions of perifusate
⇩
Measurement of ACTH concentration in fractions by radioimmunoassay

Multi-column perifusion system



Data Analysis

All data are reported as means ± SEM. Data were analysed by one-way ANOVA. Level of significance is indicated in graphs with asterisks: ⋆ indicates $p<0.05$, ⋆⋆ indicates $p<0.01$.

Results

Effect of CRH pre-treatment on the ACTH response to CRH

In the control columns, perifused anterior pituitary cells were treated with a 5 min, 10 nM CRH pulse at 200 min, resulting in a broad peak of ACTH secretion. (Results from a single representative column are shown in Fig 1a.)

In the test columns, cells were pre-treated with 0.1 or 1.0 nM CRH for either 25 or 50 min immediately prior to the CRH pulse. (Fig 1b, c show the results of two of these pre-treatments.) The two 0.1 nM CRH pre-treatments caused only a slight reduction in the response to a subsequent CRH pulse, neither of which was statistically significant. Further decreases in the responses to the test pulse were seen following pre-treatment with 1.0 nM CRH, but the reduction was only significant with the 50 min pre-treatment (44.9 ± 9.3%, n=9, $p<0.05$).

Results are summarised in Fig 2.

Figure 1

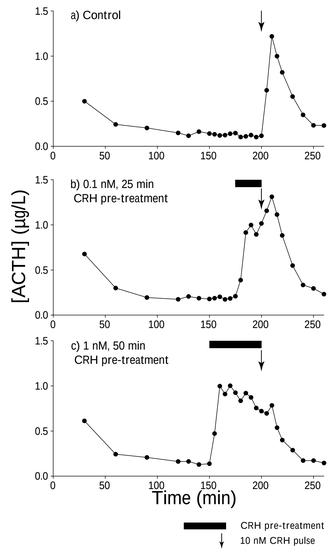
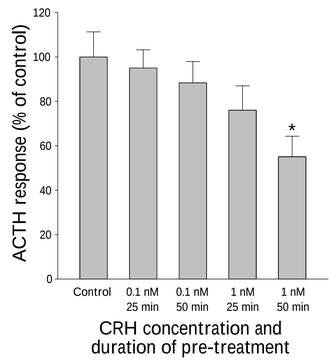


Figure 2



Effect of CRH on AVP-induced stimulation of ACTH

To investigate the effect of CRH on AVP-induced desensitization, perifused cells were treated with 5 min, 100 nM AVP pulses after 120, 200 and 280 min (Fig 3a: control), and were continuously exposed to a low level (0.01 nM) of CRH from 80 min onward (Fig 3b: CRH-treated control). A 15 min, 5 nM AVP pre-treatment immediately preceded the second pulse (Fig 3c, d: AVP pre-treated & CRH treated + AVP pre-treated test columns, respectively).

The extent of desensitization caused by the pre-treatment was assessed by expressing the response to the second pulse as a percentage of the mean of the responses to the first and third pulses, which acted as controls. (In the absence of pre-treatment the response to the second pulse was comparable in magnitude to the mean of the responses to the first and third pulses.)

Following pre-treatment with AVP the ACTH response to the second AVP pulse was reduced (Fig 3c, d†). The degree of this desensitization was similar when cells were pre-treated with AVP in either the presence (Fig 3d) or absence (Fig 3c) of CRH. The responses in the pre-treated columns were 60.5 ± 2.7% (CRH present) and 65.6 ± 7.3% (CRH absent)(n=3) of the appropriate controls. Results are summarised in Fig 4.

†(Controls in which the second AVP pulse was replaced by a 5 min pulse of 100 mM KCl showed that the desensitization seen in Fig 3c, d was not due to depletion of ACTH [data not shown]).

Figure 3

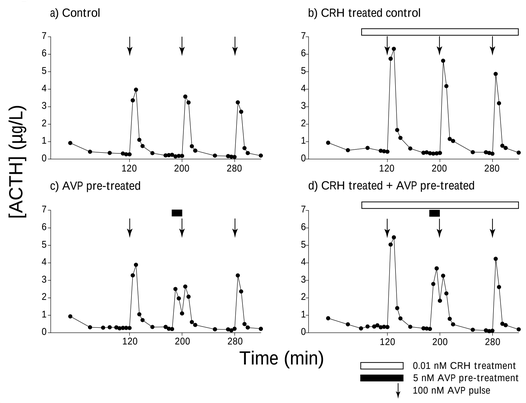
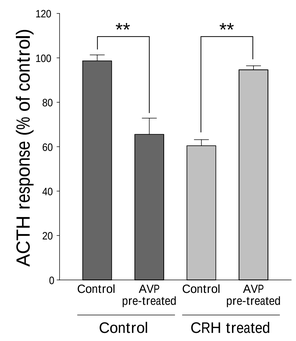


Figure 4



Discussion

The hypothalamic peptides, CRH and AVP, are released into the hypophyseal portal circulation in a pulsatile fashion. We have shown previously[1] that desensitization of the ACTH response to AVP occurs following pre-treatment of ovine anterior pituitary cells with AVP pulses that are similar in concentration and duration to those seen in vivo.

In contrast we show here that the ACTH response of these cells is relatively less sensitive to desensitization with CRH than AVP. In the sheep, endogenous pulses are typically of low concentration (0.02 – 0.25 nM) and short duration (less than approximately 20 min)[2]. Our results, showing that a pulse of 1 nM CRH for 25 min is insufficient to cause significant desensitization, suggest that endogenous CRH pulses would not be of high enough concentration or of long enough duration to elicit desensitization in vivo.

ACTH secretion is under multi-factorial control in vivo, with AVP and CRH being the primary physiological stimulators. The relative importance of these two hypothalamic peptides appears to be influenced by many factors including species studied, the type of stress, individual animal variation and, for in vitro studies, the techniques and preparations used. It has been suggested[3] that the primary drive for ACTH secretion following activation of the hypothalamic-pituitary-adrenal axis is enhanced AVP secretion with CRH acting either in a permissive or dynamic manner to set corticotroph gain.

In view of this, and also of the results of our research investigating desensitization of the ACTH response to either CRH or AVP alone, it was of interest to determine whether CRH can modify AVP-induced desensitization. Our results indicate that a low level "background" of CRH neither protects the ACTH response of ovine anterior pituitary cells from AVP-induced desensitization nor enhances the desensitization process.

References

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Abstract
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