Mechanism of rapid desensitization of the adrenocorticotropin (ACTH) response to arginine vasopressin (AVP)

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Introduction

Recently, we have shown that treatment of ovine anterior pituitary cells with AVP, an important regulator of ACTH secretion, results in desensitization to a subsequent stimulation with AVP[1]. This desensitization was found to be rapid and readily reversible, characteristics which suggested that it may be mediated by phosphorylation of the pituitary AVP (V1b) receptor. This mechanism of desensitization is common amongst G protein-coupled receptors (GPCRs).

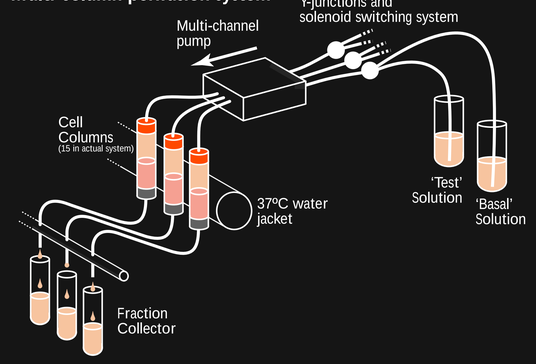
The aim of this study was to investigate whether this desensitization involves protein kinase C (PKC) and/or casein kinase 1α (CK1α), two kinases known to be involved in desensitization of GPCRs.

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 ± AVP and/or pharmacological agents
⇩
Collection of 5 or 10 min fractions of perifusate
⇩
Measurement of ACTH concentration in fractions by

Multi-column perifusion system



Data Analysis

All data are reported as means ± SEM. Unless otherwise indicated statistical significance of differences between means was assessed using Student's t-test. Level of significance is indicated in graphs with asterisks: * indicates p<0.05, *** indicates p<0.001.

Results

Effect of pre-treatment with 1,2-dioctanoyl-sn-glycerol on the ACTH response to AVP

Perifused anterior pituitary cells were treated with 5 min 100 nM AVP pulses after 100, 180 and 260 min of perifusion, resulting in three similar peaks of ACTH secretion (Results from a single, representative perifusion column are shown in Fig 1a).

Pre-treatment with 10 nM AVP prior to the second pulse resulted in a reduction in response of 48.6 ± 1.6% (n=6, p<0.0001) compared to the mean of the responses to the first and third (i.e. control) pulses (Fig 1b).

In contrast, pre-treatment with the specific PKC activator 1,2-dioctanoyl-sn-glycerol (DiC_8) did not cause a reduction in response. In fact there was a 51.3 ± 4.4% (n=6, p<0.0001) increase in ACTH secretion following pre-treatment with 300 μM DiC_8 for 15 min (Fig 1c). Results are summarized in Fig 2.

Figure 1

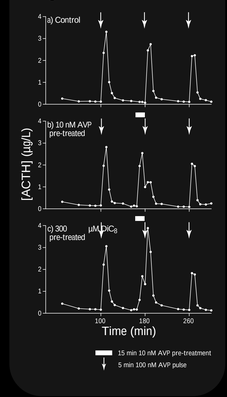
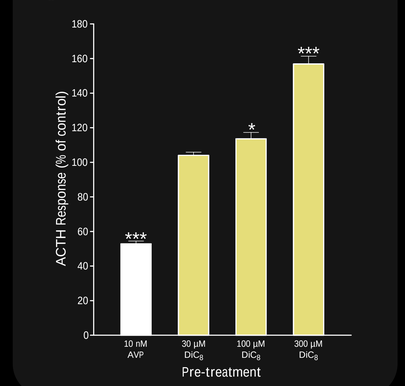


Figure 2



Effect of pre-treatment with Ro 31-8220 on desensitization of the ACTH response to AVP

Involvement of PKC in the desensitization of the ACTH response to AVP was also investigated using the specific PKC inhibitor Ro 31-8220[2]. The response of cells stimulated with a 5 min 100 nM AVP pulse preceded by a 15 min pre-treatment with 10 nM AVP was compared to the response of controls which were not pre-treated.

The presence of 2 μM Ro 31-8220 in the perifusion medium did not reduce the magnitude of desensitization observed following pre-treatment: pre-treatment with 10 nM AVP in combination with Ro 31-8220 reduced the response to a 100 nM AVP pulse to 11.2 ± 4.5% (n=4, p<0.001)(Fig 3c, d) of control compared with 30.2 ± 2.4% (n=3, p<0.005) when Ro 31-8220 was not present (Fig 3a, b). Results are summarized in Fig 4.

Figure 3

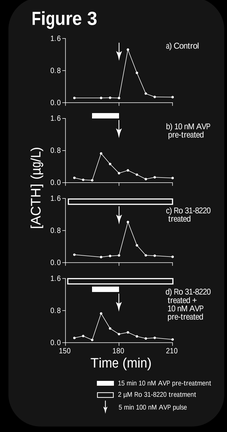
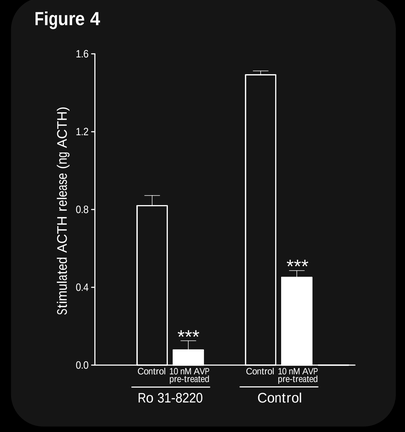


Figure 4



Effect of pre-treatment with CK1-7 on desensitization of the ACTH response to AVP

The involvement of casein kinase 1α in the desensitization of the ACTH response to AVP was investigated using the specific casein kinase 1 inhibitor CK1-7. Cells were perifused with 5 min 100 nM AVP pulses after 100, 180 and 260 min of perifusion.

Pre-treatment before the second pulse with 10 nM AVP for 25 min in combination with CK1-7 at concentrations of up to 100 μM had no effect on the magnitude of desensitization observed as determined by Dunnett's test (Fig 5b, c). Pre-treatment with 30 μM CK1-7 alone did not significantly alter the response to the second 100 nM AVP pulse compared with the control pulses (Fig 5a). Results are summarized in Fig 6.

Figure 5

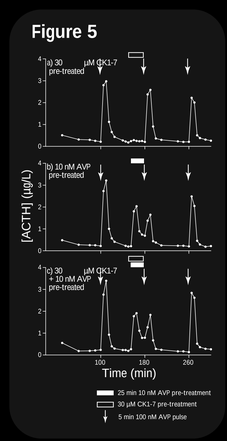
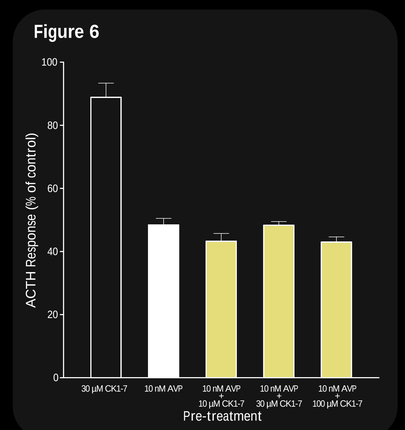


Figure 6



Discussion

Taken together, results from experiments using the PKC activator DiC_8 and the PKC inhibitor Ro 31-8220 indicate that desensitization of the ACTH response to AVP is not mediated by PKC. Pre-treatment with DiC_8 did not result in desensitization to a subsequent stimulation with AVP. Similarly, presence of Ro 31-8220 in the perifusion medium did not reduce the extent of AVP-induced desensitization to a subsequent stimulation with AVP.

This observation suggests vasopressin receptor-subtype specific differences in susceptibility to regulatory phosphorylation and desensitization: the vascular V1a vasopressin receptor has been shown to be desensitized through PKC. These differences in desensitization may play an important role in the regulation of responsiveness to vasopressin in different tissues.

The increase in response to AVP observed following pre-treatment with DiC_8 may be a result of PKC modulation of voltage-stimulated calcium channel activity, enhancing Ca^{2+} entry and therefore ACTH secretion.

Treatment with the specific casein kinase 1 inhibitor CK1-7 in combination with AVP had no effect on desensitization, suggesting that CK1α, which has been shown to be involved in desensitization of the m3-muscarinic receptor, is not involved in the desensitization of the ACTH response to AVP.

Although these results indicate that neither PKC or CK1α are involved in this desensitization process it remains likely that an intracellular kinase—such as a G protein-coupled receptor kinase—is involved in regulation of the V1b receptor.

These results do not support the involvement of either PKC or CK1α in the desensitization of the ACTH response to AVP.

Notes and References

1. Hassan, A.M.A. & Mason, D.R. (1999) Rapid desensitization and recovery of the adrenocorticotrophin response of anterior pituitary cells to arginine vasopressin. Proceedings of the Endocrine Society of Australia **42**: 242

2. Ro 31-8220 was a kind gift from the Roche Research Centre, 40 Broadwater Road, Welwyn Garden City, Hertfordshire AL7 3AY, United Kingdom.

Abstract
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