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Effects of pretreatment with tetradecanoyl phorbol acetate on regulation of growth hormone and prolactin secretion from ovine anterior pituitary cells

Robert S. Boyd and Michael Wallis

Biochemistry Laboratory, School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG, England

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Tetradecanoyl phorbol acetate (TPA) stimulates growth hormone (GH) and prolactin secretion from ovine anterior pituitary cells. Pretreatment of the cells with TPA abolishes this effect, presumably due to down-regulation of protein kinase C. Such pretreatment did not alter effects of thyrotropin-releasing hormone or dopamine on prolaction secretion, suggesting no involvement of protein kinase C. Pretreatment with TPA attenuated actions of GH-releasing hormone on GH release (but not actions on cyclic AMP levels), possibly due to depletion of cellular stores of GH. Such pretreatment also attenuated inhibition of GH release by somatostatin, possibly due to phosphorylation of receptors or associated proteins by protein kinase C.

Dopamine; Growth hormone-releasing hormone; Protein kinase C; Somatostatin; Thyrotropin-releasing hormone; (Pituitary cell, Ovine)

1. INTRODUCTION

Secretion of growth hormone (GH) and prolactin from anterior pituitary cells is under the control of hypothalamic factors. GH secretion is stimulated by GH-releasing hormone (GHRH) and inhibited by somatostatin, while prolactin secretion is stimulated by thyrotropin releasing hormone (TRH) and inhibited by dopamine. Various factors appear to play a role in mediating the actions of these hypothalamic hormones, including cyclic AMP and intracellular [Ca^{2+}]. The phorbol ester tetradecanoyl phorbol acetate (TPA), which increases protein kinase C activity [1,2], stimulates both prolactin and GH release from anterior pituitary cells [3–5]. This suggests that protein kinase C may also be involved in regulating the secretion of these hormones.

Although short-term treatment with phorbol esters is known to activate protein kinase C, in several cell types long-term exposure (3 to 24 h) to TPA reduces both the amount and activity of protein kinase C to very low levels [6,7]. This selective down-regulation of protein kinase C has been employed by a number of investigators to determine the role of the enzyme in the molecular mode of action of hormones [7,8].

Recently, Ohmura et al. [9] have shown that a short pretreatment with TPA can lead to attenuation of the stimulatory actions of GHRH on activated rat pituitary cells. Here we have used cultured sheep pituitary cells to investigate the effects of such down-regulation on responses to GHRH and somatostatin (GH secretion) and TRH and dopamine (prolactin secretion), and thus the role that protein kinase C may play in regulating the actions of these factors.

Correspondence address: R.S. Boyd, Biochemistry Laboratory, School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG, England

2. MATERIALS AND METHODS

2.1. *Materials*

Synthetic somatostatin (1–14), GHRH (1-44-NH_2), dopamine, TRH and TPA were obtained from Sigma Chemical Co., Poole, England. Sera and culture media were obtained

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from Gibco, Paisley, Scotland. Ovine GH (NIH-GH-S9) and ovine prolactin (NIAMDD-oPrl-14) were gifts from Dr A.E. Wilhelmi and the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD, USA.

2.2. *Preparation and culture of pituitary cells*

Cells were prepared and incubated as in [10]. Ovine anterior pituitary cells were dispersed (60 min, 37°C) using collagenase (Boehringer; 1.5 mg/ml), hyaluronidase (Sigma, type-1S; 0.5 mg/ml), deoxyribonuclease I (Boehringer, grade II; 0.25 mg/ml) and bovine serum albumin (Sigma, fraction V; 30 mg/ml) in medium containing NaCl (137 mM), KCl (5 mM), Na_2HPO_4 (0.7 mM), glucose (10 mM) and Hepes (25 mM), adjusted to pH 7.4 with NaOH. Cells were collected by centrifugation (400 × *g* for 2 min) and washed 5 times by resuspension in the Hepes-buffered medium and centrifugation. The cells were pipetted (0.5–1.0 × 10^6 cells/dish) into 3.5 cm culture plates (Sterilin) and incubated in Dulbecco's modified Eagle medium (3 ml/dish) containing 25 mM Hepes, 5% fetal calf serum, 10% horse serum and antibiotics for 72 h at 37°C under 95% air/5% CO_2.

2.3. *Experimental incubations*

After 72 h the incubation medium was discarded and the cells were washed three times with the incubation medium without serum (in which subsequent incubations were also carried out). The cells were preincubated for 5 h with or without TPA, washed and then incubated for 30 min with or without test substances. At the end of the experimental incubation the media were centrifuged (400 × *g*, 2 min) and the supernatants stored at −20°C until assayed for hormone content. The cellular cyclic nucleotides were extracted with 10% (w/v) trichloroacetic acid (2 h at 4°C) and stored frozen at −20°C prior to assay.

2.4. *Assays*

The radioimmunoassay procedures for ovine GH and prolactin were based on methods using specific antisera for these hormones, raised in rabbits, as in [10]. Hormones were iodinated with $Na^{125}I$ as in [10]. All samples, standards etc. were dissolved in assay buffer containing sodium phosphate (0.05 M) pH 7.6, merthiolate (0.6 mM), bovine serum albumin (0.05%) and Triton X-100 (0.05%). cAMP in cell extracts was measured, following acetylation, as in [11].

3. RESULTS

TPA (100 ng/ml) stimulated secretion of GH and prolactin from ovine anterior pituitary cells as shown previously [5,12]. Pretreatment of the cells with 0.1–1000 ng/ml TPA for 5 h resulted in a dose-dependent inhibition of this stimulatory effect (fig.1). Pretreatment with 1000 ng/ml TPA for 5 h completely inhibited the ability of 100 ng/ml TPA to stimulate prolactin and GH release.

TRH (100 nM) stimulated prolactin release 2–3-fold and, as expected, dopamine inhibited this stimulated prolactin release (fig.2). Pretreatment of the pituitary cells with TPA had no significant effect on TRH-stimulated prolactin release or the ability of dopamine (100 nM) to inhibit TRH-stimulated prolactin release.

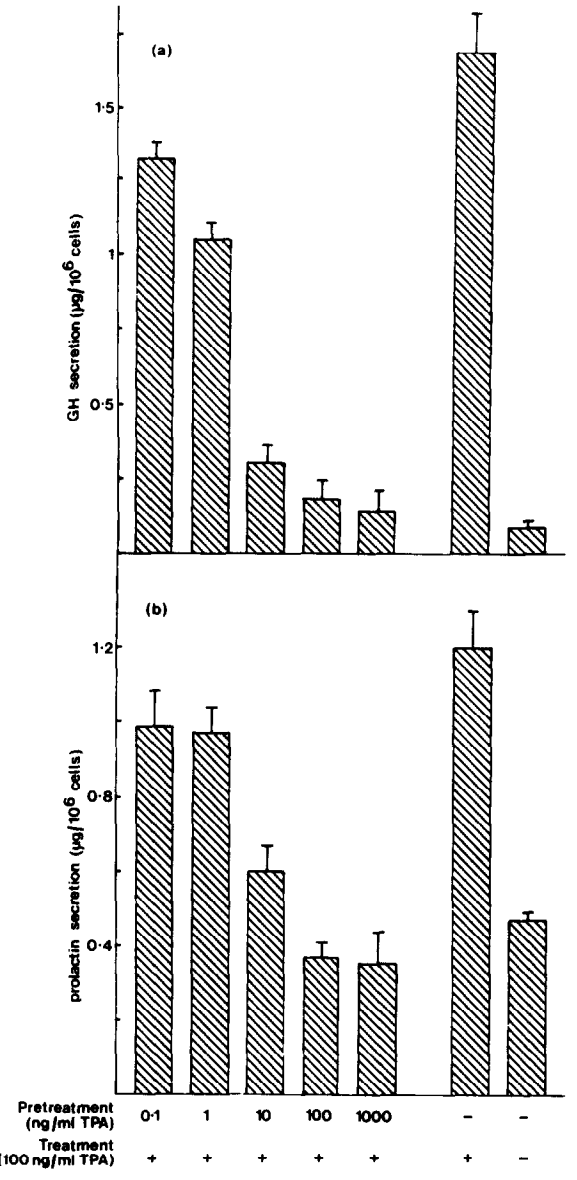

Fig.1. Effects of pretreatment with TPA on the secretion of GH (a) and prolactin (b) by sheep pituitary cells stimulated with 100 ng/ml TPA. Cultured cells were pretreated for 5 h with the concentration of TPA shown. Medium was then changed and secretion during a 30 min incubation in the presence of 0 or 100 ng/ml TPA was measured. Values shown are means ± SE (n = 3). Similar results were obtained in 2 experiments.

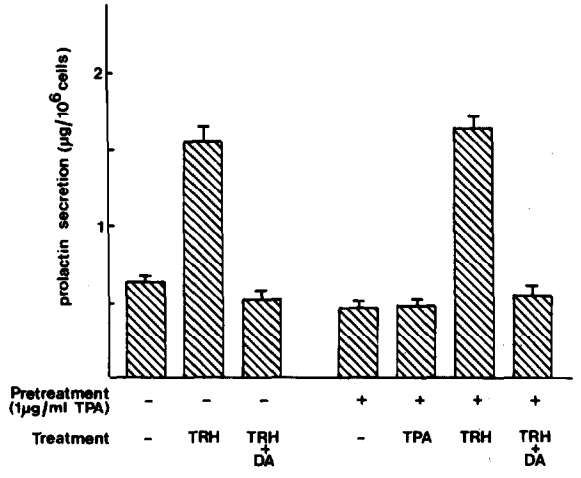

Fig.2. Effects of pretreatment with TPA on the secretion of prolactin in response to TRH in the presence or absence of dopamine (DA). Cultured ovine pituitary cells were pretreated for 5 h with or without 1 μg/ml TPA, as indicated. Medium was then changed and secretion was measured during a 30 min incubation in the presence or absence of TPA (100 ng/ml), TRH (100 nM) or TRH plus dopamine (100 nM each). Values shown are means ± SE (n = 3). Similar results were obtained in 3 experiments.

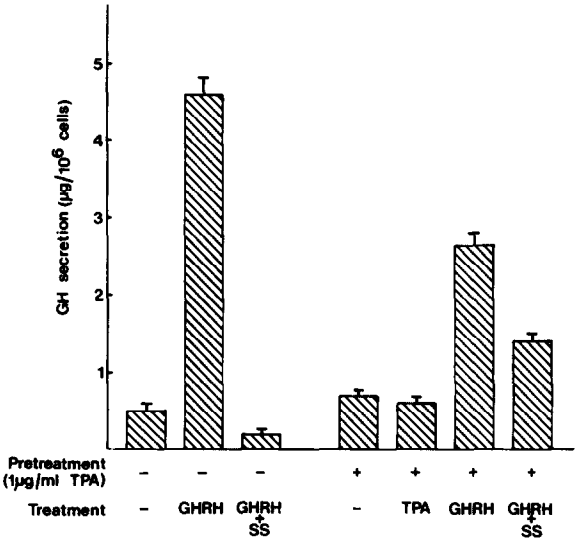

Fig.3. Effects of pretreatment with TPA on the secretion of GH in response to GHRH in the presence or absence of somatostatin (SS). Cultured ovine pituitary cells were pretreated for 5 h with or without 1 μg/ml TPA, as indicated. Medium was then changed and secretion was measured during a 30 min incubation in the presence or absence of TPA (100 ng/ml), GHRH (1 nM) or GHRH (1 nM) plus somatostatin (100 nM). Values shown are means ± SE (n = 3). Similar results were obtained in 4 experiments.

GHRH (1 nM) stimulated GH release 5–10-fold and somatostatin (100 nM) inhibited this (fig.3). Pretreatment of the anterior pituitary cells with TPA (1000 ng/ml) for 5 h lowered the ability of GHRH to stimulate growth hormone release by 44 ± 5% (n = 12). Somatostatin (100 nM) blocked the ability of GHRH to stimulate GH secretion in cultured pituitary cells (fig.3), but this effect was markedly attenuated in cells that had been pretreated with TPA.

Table 1

Effects of GHRH with or without TPA pretreatment on cAMP levels in cultured ovine pituitary cells

Pretreatment	Experimental treatment	cAMP concentration (pmol/10^6 cells)
30 min, medium	control	1.95 ± 0.17
30 min, medium	GHRH	10.35 ± 0.18
30 min, TPA	GHRH	22.60 ± 0.25
5 h, medium	control	1.54 ± 0.04
5 h, TPA	control	1.50 ± 0.11
5 h, medium	GHRH	6.21 ± 0.10
5 h, TPA	GHRH	13.80 ± 0.18

Cells were preincubated for 30 min or 5 h with or without TPA (1 μg/ml), and then incubated for 30 min with or without GHRH (1 nM). Values shown are means ± SE (n = 3). Similar results were obtained in 3 experiments

The total cellular content of GH was reduced by 78 ± 7% (n = 6) by TPA pretreatment, which could underlie the decreased secretory response. TPA pretreatment had no significant effect on basal cAMP levels, but led to significantly enhanced stimulation of cAMP concentration by GHRH (table 1). Somatostatin had no effect on cAMP levels, irrespective of TPA pretreatment, in accordance with previous results in ovine pituitary cells [12,13].

4. DISCUSSION

Pituitary protein kinase C has been well characterized [14,15]. The down-regulation of this enzyme on prolonged exposure to TPA has been observed in many tissues and may be due to its proteolytic degradation [6]. In rat pituitary tumour cell lines (GH cells), binding of TRH to its receptor results in activation of phospholipase C and the

generation of inositol trisphosphate and diacylglycerol [16], probably leading to a rapid rise in intracellular free [Ca^{2+}] causing an initial peak of prolactin release [16,17], and activation of protein kinase C giving a more-sustained release of the hormone [3,16,18,19].

That protein kinase C plays a part in mediating the actions of TRH on prolactin secretion in normal pituitary cells is less clearly established. TPA does stimulate prolactin secretion (fig.1) and the effect is abolished by pretreatment with TPA. However, TPA pretreatment did not attenuate the stimulation of prolactin secretion caused by TRH (fig.2), suggesting that in this sheep pituitary cell system protein kinase C is not involved in mediating the effects of this peptide.

The role of protein kinase C in the mode of action of GHRH is unclear. GHRH and TPA have an additive effect on GH secretion, indicating that they may act via independent stimulatory pathways [4,20]. Pretreatment with TPA attenuated the action of GHRH on GH secretion, in agreement with studies on rat pituitary cells [9]. This could be due to down-regulation of protein kinase C, but it seems more likely that the large depletion of the cellular content of GH caused by TPA pretreatment results in a reduced pool of releasable GH and a decreased response to GHRH. The observation that TPA pretreatment did not decrease the ability of GHRH to stimulate cAMP levels in these experiments (table 1) tends to support such a conclusion. In fact TPA pretreatment increased the effects of GHRH on intracellular cAMP levels, possibly due to the activation of protein kinase C prior to its down-regulation. This observation contrasts with results obtained with rat pituitary cells [9] where TPA-pretreatment led to a decreased stimulation of cAMP levels in response to GHRH.

The ability of TPA pretreatment to block partly the inhibition by somatostatin of GHRH-stimulated GH release (fig.3) is striking. It is unlikely to be due to depletion of cellular GH content or to mediation of the inhibitory actions of somatostatin by protein kinase C, given that short-term treatment with TPA stimulates GH release. Possibly activation of protein kinase C (prior to down-regulation) leads to phosphorylation of the somatostatin receptor or some component of the associated effector system. Preliminary observations have indicated that a short preincubation (30 min) with TPA leads to a greater attenuation of the inhibitory effect of somatostatin, which would agree with such a mechanism. An inhibitory effect of TPA on binding of somatostatin has been observed previously for the pancreatic somatostatin receptor [21]. It is notable that TPA pretreatment did not significantly block the ability of dopamine to inhibit TRH-stimulated prolactin release.

In conclusion, the experiments presented here gave no indication that prolonged pretreatment with TPA altered the ability of sheep anterior pituitary cells to respond to TRH or dopamine, acting on prolactin secretion. This suggests that protein kinase C is not involved as a mediator or modulator of the actions of these factors. TPA pretreatment did lead to decreased stimulation of GH secretion by GHRH, but this could be due to depletion of cellular GH stores. Pretreatment with TPA partially blocked the inhibition of GHRH-stimulated secretion by somatostatin, possibly because treatment with TPA activates protein kinase C prior to down-regulation, and the enzyme phosphorylates and partially inactivates the receptors and/or effector systems for somatostatin.

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