The role of intracellular messengers in adrenocorticotropin secretion in vitro

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Summary. Adrenocorticotropin (ACTH), an opiomelanocortin peptide, is secreted from anterior pituitary corticotrophs upon stimulation with corticotropin-releasing hormone (CRH), arginine vasopressin (AVP) and several other neuropeptides. CRH, the most potent secretagogue of ACTH, stimulates ACTH secretion and biosynthesis by increasing the production of cyclic adenosine 3',5'-monophosphate (cAMP) within corticotrophs. AVP, which is a weak secretagogue of ACTH but strongly potentiates CRH-stimulated ACTH secretion, operates through the phosphatidylinositol (PI) transduction pathway. Both CRH and AVP increase cytosolic free [Ca^{2+}] within normal corticotrophs indicating a role for Ca^{2+} in ACTH secretion. Glucocorticoids inhibit ACTH synthesis by suppressing transcription of the proopiomelanocortin (POMC) gene and attenuate ACTH release by decreasing cAMP accumulation stimulated by CRH. This review focuses on the roles of these intracellular messengers in ACTH secretion from normal anterior pituitary cells in vitro, and discusses the possible interactions between the cAMP, calcium and PI transduction pathways. Future areas of research are suggested such as identification of protein substrates of cAMP-dependent and Ca^{2+}-dependent kinases within normal corticotrophs and evaluation of their role in ACTH biosynthesis and secretion.

Key words. Opiomelanocortin; cyclic adenosine 3',5'-monophosphate (cAMP); calcium; phosphatidylinositol (PI); glucocorticoids.

Introduction

Several previous reviews have evaluated the role of neuropeptides in the secretion of opiomelanocortin peptides from the anterior pituitary gland[10, 55, 77, 80, 106, 139, 149, 182]. The present review is different in that it focuses on the intracellular pathways which regulate opiomelanocortin secretion from normal anterior pituitary corticotrophs in vitro as well as on the possible interactions between transduction pathways. Experiments on the homogenous corticotrophic AtT-20 mouse tumor cell line, reviewed in full elsewhere[15, 140], will provide further insight when data from normal anterior pituitary cells is insufficient.

Summary of the hypothalamo-hypophysial control of opiomelanocortin secretion

The anterior pituitary gland possesses a heterogenous cell population which includes gonadotrophs, somatotrophs, mammotrophs and thyrotrophs as well as adrenocorticotropin-secreting corticotrophs[120]. Corticotrophs comprise 8–15% of the cells in the rat anterior pituitary gland[121, 194], are located in clusters[115] and most are stellate with processes extending between neighboring cells[92, 120, 194] which are frequently growth hormone cells[120, 160]. This morphology suggests an interaction between corticotrophs and other cells of the anterior pituitary, although this has not been documented.

Adrenocorticotropin (ACTH) belongs to a family of peptides derived from a common precursor protein encoded by the pro-opiomelanocortin (POMC) gene[40, 77, 104, 106, 107]. This gene is strongly expressed in the anterior and intermediate pituitary gland, with lesser amounts found in other tissues including the arcuate nucleus and the adrenal medulla[77]. The bovine[122], human[41, 175], mouse[126, 181] and rat[51] POMC genes have been cloned or sequenced. The human POMC gene is approximately 11.6 kilobases long and consists of three exons, the second and third exons encode the opiomelanocortin peptides[41, 77, 106]. DNA sequences upstream from the POMC gene have been identified as glucocorticoid receptor binding sites[51]. Their occupancy probably accounts for the inhibition of POMC gene transcription by glucocorticoids discussed below. The synthesis and processing of the POMC protein are similar to that described for other proteins[133]. The POMC gene is first transcribed into heteronuclear RNA which is spliced into mature messenger RNA (mRNA)[77]. The POMC mRNA then leaves the nucleus and, as indicated by studies on AtT-20 tumor cells[150], is translated on ribosomes associated with the endoplasmic reticulum (ER) into a pre-POMC protein. The pre-POMC protein enters the ER co-translationally, having its signal sequence cleaved off as it enters and forms the pro-POMC precursor protein[74, 77]. The pro-POMC protein then undergoes several enzymatic modifications including glycosylation of asparagine residues[74, 75, 151] and phosphorylation of serine residues[54]. These modifications lead to the formation of the POMC precursor which is transported to the Golgi apparatus, packaged into secretory granules and cleaved into opiomelanocortin peptides[75, 77, 107, 151]. The cleavage pattern of the POMC precursor protein is tissue specific. In anterior pituitary corticotrophs, it is cleaved into ACTH, β-lipotropin (β-LPH) and small amounts of β-endorphin[40, 77, 104, 106, 107]. In the intermediate lobe and other tissues, the processing continues to form α-melanocyte-stimulating hormone (α -MSH) and corti-

cotropin-like intermediate lobe peptide from ACTH and γ-LPH and β-endorphin from β-LPH[77,106,107]. Other peptides such as γ-MSH are cleaved from the N-terminal portion of the POMC precursor[77,107,122] and are thought to potentiate ACTH-induced glucocorticoid synthesis in the adrenal cortex[103]. Opiomelanocortin peptides are stored within membrane-bound granules located mainly along the plasma membrane[92,115,120,194]. Upon stimulation with secretagogues, opiomelanocortin peptides are released concomitantly in equimolar amounts[9,183].

ACTH secretion in vivo is induced by various physiological and psychological perturbations including hemorrhage[60], inflammation[27], hypoglycemia[78,135] and several types of stress[62,148,174]. ACTH release in response to stress is mediated by several hypothalamic peptides including corticotropin-releasing hormone (CRH), arginine vasopressin (AVP) and oxytocin (OXT), which are released into the portal blood following restraint, cold and ether stress[62]. Further indicating that CRH and AVP stimulate ACTH secretion during stress, immunoneutralization of CRH or AVP decreases ACTH release induced by restraint or ether stress by 75% and 50%, respectively[147,178]. ACTH induces the release of glucocorticoids from the adrenal cortex which, in turn, act at multiple sites to alleviate the physiological condition which stimulated ACTH release[83,85,117]. In addition to acting on numerous peripheral targets[83], glucocorticoids feed back onto the hypothalamus and anterior pituitary gland to attenuate ACTH secretion[45,57,85,158] (fig. 1). Glucocorticoids also bind to limbic and autonomic centers in the brain[110], areas which project to the PVN[80]. The mechanisms of action of glucocorticoids are discussed in a later section.

ACTH secretion in vitro is also stimulated by CRH, AVP and OXT[10,17,23,55,77,80,106,139,149,182,184]. CRH, a 41 residue peptide, is the physiological hypothalamic-releasing factor for opiomelanocortin peptides from the anterior pituitary gland[184]. CRH is synthesized albeit not exclusively, in the parvocellular paraventricular nucleus (PVN) of the hypothalamus[14,124,130,173]. These neurons project to the median eminence[28,124,130] and release CRH into the portal vessels[63] (fig. 1). The concentration of CRH detected in the portal blood of anesthetized rats is 0.1 nM[63]. This concentration is identical to that necessary for significant stimulation of ACTH secretion by CRH in vitro[184]. Several neurotransmitters, including acetylcholine, serotonin and catecholamines, have been proposed to stimulate CRH release from the PVN[80], but the precise role each plays in ACTH secretion is unknown. The best evidence is for the catecholamines which recently have been shown to stimulate CRH release from cultured hypothalamic cells from new born rats through β-adrenergic mechanisms[200]. Radiolabeled CRH binds to anterior pituitary cells of rats[7,47,91,204] and humans[48] with a K_d of approximately 1 nM. CRH appears to bind exclusively to corti-

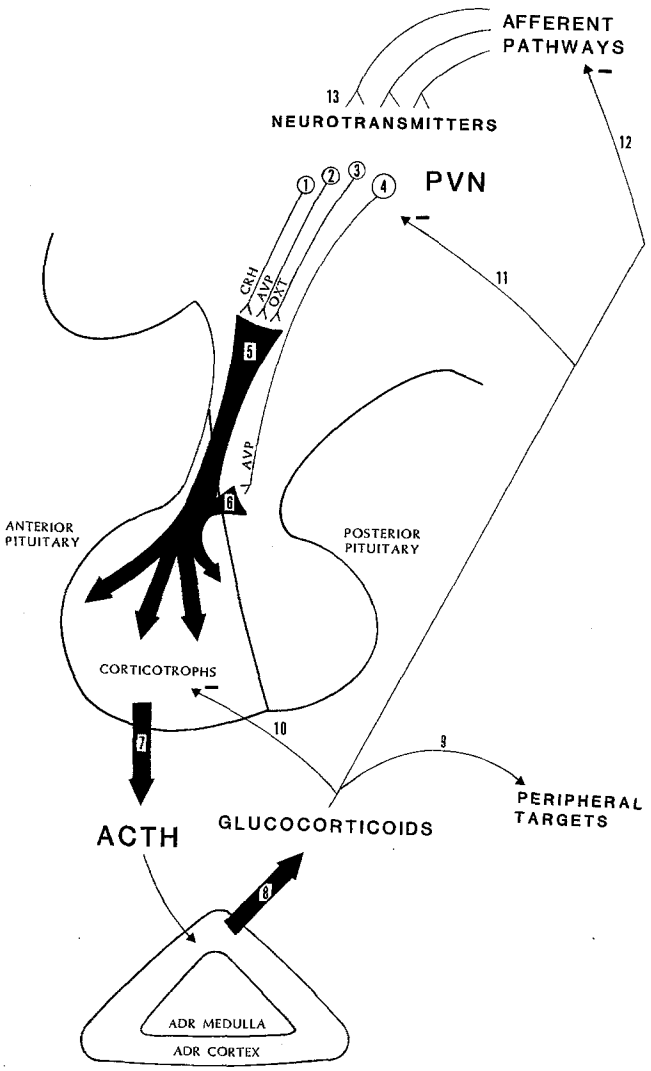

Figure 1. Hypothalamic control of ACTH secretion from the anterior pituitary gland. Numbers in figure refer to numbers in brackets cited below. Corticotropin-releasing hormone (CRH) [1], arginine vasopressin (AVP) [2] and oxytocin (OXT) [3] are synthesized in the parvocellular paraventricular nucleus (PVN) of the hypothalamus[14,28,49,124,130,155,156,173,188,187,205], and are released from nerve terminals in the median eminence into the portal blood [5][62,63,129]. AVP is also synthesized in magnocellular neurons of the PVN [4][49,186,187,205], which project to the posterior pituitary[30,145], and may release AVP into short portal vessels. Some of these may join the long portal vessels at the base of the infundibular stalk [6] (Baertschi, unpublished observations). AVP and OXT are co-localized with CRH within cells of the PVN, but the degree of co-localization is controversial[155,156,196]. The small contribution of catecholamines in portal blood in the control of ACTH secretion[80] is not represented. The release of CRH, AVP and OXT from the PVN is regulated by several neurotransmitters, including acetylcholine, serotonin and catecholamines, which are released from impinging afferent fibers [13][80]. CRH, AVP and OXT stimulate ACTH secretion from anterior pituitary corticotrophs [7][10,17,23,55,77,106,139,149,182,184]. ACTH induces the release of glucocorticoids from the adrenal cortex [8][83,85,117] which, in addition to acting on peripheral targets [9][83], feed back onto anterior pituitary corticotrophs [10] and the hypothalamus [11] to inhibit ACTH secretion[43,57,83,85,110,117,158]. Glucocorticoids also bind to limbic and autonomic centers in the brain [12][110], areas which give rise to afferent pathways which terminate on the PVN[80]. The minor contribution of peripheral catecholamines[78], the possible role of lymphokines[27,77] and control of intermediate lobe corticotrophs are not represented.

cotrophs[98,195] and to induce the accumulation of cyclic adenosine 3',5'-monophosphate (cAMP)[66,67,93,95] through the recruitment of membrane-bound guanine nucleotide-binding proteins (G-proteins)[7,134].

AVP and OXT are synthesized within cells of the magnocellular hypothalamic nuclei as well as the parvocellular PVN[49,155,156,186,187,205]. In general, the parvocellular neurons project to the median eminence[49,186,187,205] releasing AVP and OXT into the portal blood[62,129], while magnocellular neurons project to the posterior pituitary gland[30,145] (fig. 1). Posterior pituitary AVP may reach the anterior pituitary gland[16,19], possibly through short portal vessels which join the long portal vessels at the base of the infundibular stalk (fig. 1) (Baertschi, unpublished observations). The distribution of AVP, OXT and CRH neurons within the hypothalamus shows some overlap[28,72,82,155,156], but there is little evidence for co-localization of CRH with either AVP or OXT within hypothalamic cell bodies in normal rats[155]. In rats treated with colchicine, which enhances peptide staining in cell bodies, OXT and CRH are co-localized in a small number of supraoptic neurons and in 40% of the oxytocin neurons of the anterior magnocellular PVN[155,156]. The degree of co-localization of AVP and CRH is controversial. One group identifies less than 3% double-stained cell bodies[155,156], while another group reports that AVP and CRH are co-localized in about half of the CRH neurons within the PVN[196]. This latter group also finds AVP and CRH within the same axons and terminals in the median eminence of normal rats[197,198]. The reasons for the discrepancy are not clear, but may be due to different antisera used. Adrenalectomy, which increases the number of detectable CRH and AVP neurons in the PVN[154–156,167], does not influence the co-localization of OXT and CRH, but increases the number of PVN cells double-stained for CRH and AVP from 3% to 70%[154]. This is presumably due to the removal of glucocorticoid feedback which suppresses CRH and AVP synthesis in the hypothalamus[85,154]. The co-localization of CRH and AVP or OXT is significant because co-release of AVP or OXT with CRH would potentiate ACTH secretion (see below).

Tritium-labeled AVP binds to a single class of specific receptors on anterior pituitary cells with a K_d of approximately 1 nM[87,90,166], and as discussed below, stimulates ACTH secretion through the phosphatidylinositol (PI) transduction pathway[71,138,179]. As determined with the reverse hemolytic plaque assay, 80–90% of plaque-forming cells (ACTH cells) and approximately 20% of the nonplaque-forming cells from the anterior pituitary gland bind AVP[39]. These results suggest that most corticotrophs express the AVP receptor. Anterior pituitary AVP receptors are functionally distinct from AVP receptors identified in other tissues[13,17,87,88], suggesting that pituitary AVP receptors are of a novel (V_3) type. Biochemical evidence for different receptor subtypes is not yet available. OXT competitively binds to AVP receptors with a K_d of 4–8 nM[13,90] and at 0.05 nM significantly stimulates ACTH secretion[17,23] through the PI pathway[179].

Several other peptides modulate ACTH secretion from anterior pituitary corticotrophs as well. Angiotensin II (AII) binds to specific receptors on corticotrophs with a K_d of 2 nM[132] and at 1 nM stimulates PI turnover[71]. It is thought that 10 nM AII potentiates CRH-stimulated ACTH release[3,159], although it appears to be much less effective than AVP or OXT[17]. Catecholamines, at 5 nM, also stimulate ACTH secretion from cultured anterior pituitary cells, but these effects appear to be additive to and not synergistic with CRH[65]. Nanomolar concentrations of vasoactive intestinal peptide[193] and somatostatin[143,146] stimulate and inhibit ACTH release from AtT-20 cells respectively but, concentrations of 10 nM and 200 nM, have no significant effects on normal cells[8,17]. The existence of a hypothalamic peptide inhibitor of ACTH secretion from normal anterior pituitary corticotrophs has been proposed by Sayers and colleagues[157], but such a peptide has not yet been conclusively identified. An atrial natriuretic factor, α-ANF[1–28][86], and delta-sleep-inducing peptide[128] inhibit CRH-stimulated ACTH secretion from normal anterior pituitary cells, but further studies are necessary to prove a physiological role for these peptides.

The hypothalamo-hypophysial control of opiomelanocortin secretion from normal anterior pituitary corticotrophs is summarized in figure 1. In view of the fact that CRH and neurohypophysial peptides are the most potent modulators of POMC synthesis and opiomelanocortin secretion, this review focuses on the intracellular pathways intitiated by these peptides.

Role of the cAMP pathway

Since its discovery in hepatic tissue over 30 years ago[170], cAMP has been detected in numerous cell types[171]. Its role is that of a classic second messenger: to initiate a cascade of intracellular events leading to a physiological response[123,152,170]. cAMP is synthesized from ATP by adenylate cyclase, an enzyme located predominantly on the inner face of the plasma membrane[33,152]. The activity of adenylate cyclase is modulated by receptors through the agency of G-proteins[33]. Guanine nucleotides apparently decrease the binding affinity of G-protein-linked receptors for hormone by altering the equilibrium between high- and low-affinity forms of the receptor[33]. This can be used to ascertain whether a receptor is linked to a G-protein and, in fact, has been used to demonstrate that CRH receptors are linked to a G-protein which stimulates adenylate cyclase, presumably G_S[7,134]. CRH binding to bovine and monkey anterior pituitary membranes is decreased 75% by Gpp(NH)p, a nonhydrolyzable guanine nucleotide[134].

The first demonstration that cAMP may be involved in ACTH secretion was by Fleischer and colleagues[56] who

stimulated ACTH release from rat anterior pituitary halves by 2-fold with a 1-h exposure to 5 mM theophylline, an inhibitor of the phosphodiesterase responsible for cAMP degradation[170]. cAMP accumulation within the anterior pituitary fragments increased by 3-fold over baseline during the same 1-h period, suggesting that cAMP-dependent processes were responsible for the ACTH release. Other investigators have reported similar results in culture, using 0.6–10 mM theophylline or 50–400 μM 3-isobutylmethylxanthine[164,182,183]. Since methylxanthines at 5 μM have subsequently been shown to be purinergic (P1) receptor antagonists[31], these results should be evaluated with caution. Dibutyryl-cAMP[56] and 8-bromo-cAMP[7,164,182], cAMP analogs that penetrate the plasma membrane, stimulate ACTH release by 3–5-fold over 3 h of incubation, demonstrating that cAMP can initiate ACTH secretion. Providing further evidence for the involvement of the cAMP transduction pathway, forskolin[20,164], a direct activator of adenylate cyclase, and cholera toxin[7], an activator of G_s, also stimulate ACTH release from cultured anterior pituitary cells.

At nanomolar concentrations, CRH stimulates adenylate cyclase activity and cAMP accumulation in cultured anterior pituitary cells[44,67,93,95,164]. Intracellular cAMP levels increase by 1–2.5-fold within 60 s of CRH application, reach a maximum of between 4–13-fold within the next 10 min and then slowly decrease, leveling off at 1–6-fold basal levels over the next 3 h[44,67,93,95,164]. cAMP accumulation stimulated by CRH is increased by GTP[7,93] supporting the hypothesis that CRH receptors are linked to adenylate cyclase through a G-protein, presumably G_s. CRH also stimulates cAMP accumulation in isolated plasma membranes from rat anterior pituitary homogenates[7,93,95], suggesting that CRH increases cAMP accumulation by stimulating production and not by decreasing degradation. ACTH secretion stimulated by CRH in vitro is biphasic with a rapid rate of secretion, about 3–6-fold basal, for the first 30–60 min, followed by a slower, sustained release which can last for over 4 h[2,67,86,199]. The initial increase in ACTH release is extremely rapid, detectable within 60 s of exposure to CRH in culture[199], within 6–12 s in cell columns[116] and within 5 s in a microperfusion system[192].

Upon stimulation by CRH, corticotrophs undergo a 40–67% increase in cell area accompanied by a decrease in patches of ACTH immunostain within the cells[92,194], implying the incorporation of vesicles into the plasma membrane. This suggests that CRH increases secretion of ACTH by stimulating exocytosis. In addition to ACTH secretion, POMC biosynthesis in dispersed anterior pituitary cells is increased by CRH[53,102,169,185], cAMP analogs[53,101], forskolin[101], cholera toxin[101] and phosphodiesterase inhibitors[101]. Total ACTH content of anterior pituitary cultures (cellular stores plus that secreted into the medium) is increased 30% by a 24 h incubation with 100 nM CRH and 3-fold after 8 days[185]. Significant increases in POMC mRNA levels of between 20% and 50% have been reported after only 3–8 h of incubation with 1–5 nM CRH[102,169] and the rate of POMC gene transcription is increased 2–4-fold within only 5–15 min[53,58].

The mechanisms by which cAMP increases ACTH biosynthesis and secretion are uncertain. Since cAMP accumulation in anterior pituitary cells is correlated with a dose-related increase in A-kinase activity[7,8], protein phosphorylation may mediate these events[123]. Activation of A-kinase may be an essential step for increasing ACTH secretion and biosynthesis as elegantly demonstrated by Reisine and co-workers[141,142], who delivered an A-kinase inhibitor (Walsh inhibitor) into AtT-20 cells using liposomes. Inhibition of A-kinase decreased ACTH release stimulated by 100 nM CRH, 8-bromo-cAMP, isoproterenol and forskolin without altering the effects of K^+ and phorbol ester. Basal and stimulated POMC biosynthesis were also decreased by the A-kinase inhibitor[141].

The identity and function of the substrates of A-kinase within corticotrophs, and therefore, the precise mechanisms by which A-kinase modulates ACTH secretion and biosynthesis are undetermined. In homogenates of bovine anterior pituitaries[79] and in rat anterior pituitary cells[7], cAMP induces the phosphorylation of at least four different proteins, including at least one histone. A more detailed description of substrates of A-kinase has recently been obtained with AtT-20 cells[153]. In these cells, A-kinase phosphorylates two cytoplasmic proteins of 24 kilodaltons (kDa) and 40 kDa, two membrane-bound proteins of 32 and 60 kDa and one 30 kDa nuclear protein. The functions of these proteins remain to be elucidated, but it is possible that these proteins modulate POMC transcription, translation, processing and/or ACTH release (fig. 2).

Peptides which act through G_i, the G-protein which inhibits adenylate cyclase[33], in normal anterior pituitary corticotrophs have not been identified. An atrial natriuretic factor (ANF), α-ANF[1–28], inhibits CRH-stimulated ACTH secretion at concentrations of 0.01–1 nM[86], but the mechanism of action is unclear. It has been proposed that 100 nM α-ANF[1–28] decreases ACTH release by stimulating the production of cyclic guanosine 3',5'-monophosphate (cGMP)[11]. This is unlikely because α-ANF[5–28] does not inhibit ACTH release[86] at concentrations that stimulate cGMP production[5]. Delta sleep-inducing peptide (DSIP) also has been shown to inhibit CRH-stimulated ACTH secretion at 1–100 nM[128]. At 10 nM, DSIP reduces CRH-stimulated cAMP production by over 50%, but the involvement of G_i has not been demonstrated. In AtT-20 cells, somatostatin decreases cAMP accumulation through a pertussis toxin-sensitive G-protein, presumably G_i[143], but this does not occur in normal anterior pituitary corticotrophs[8].

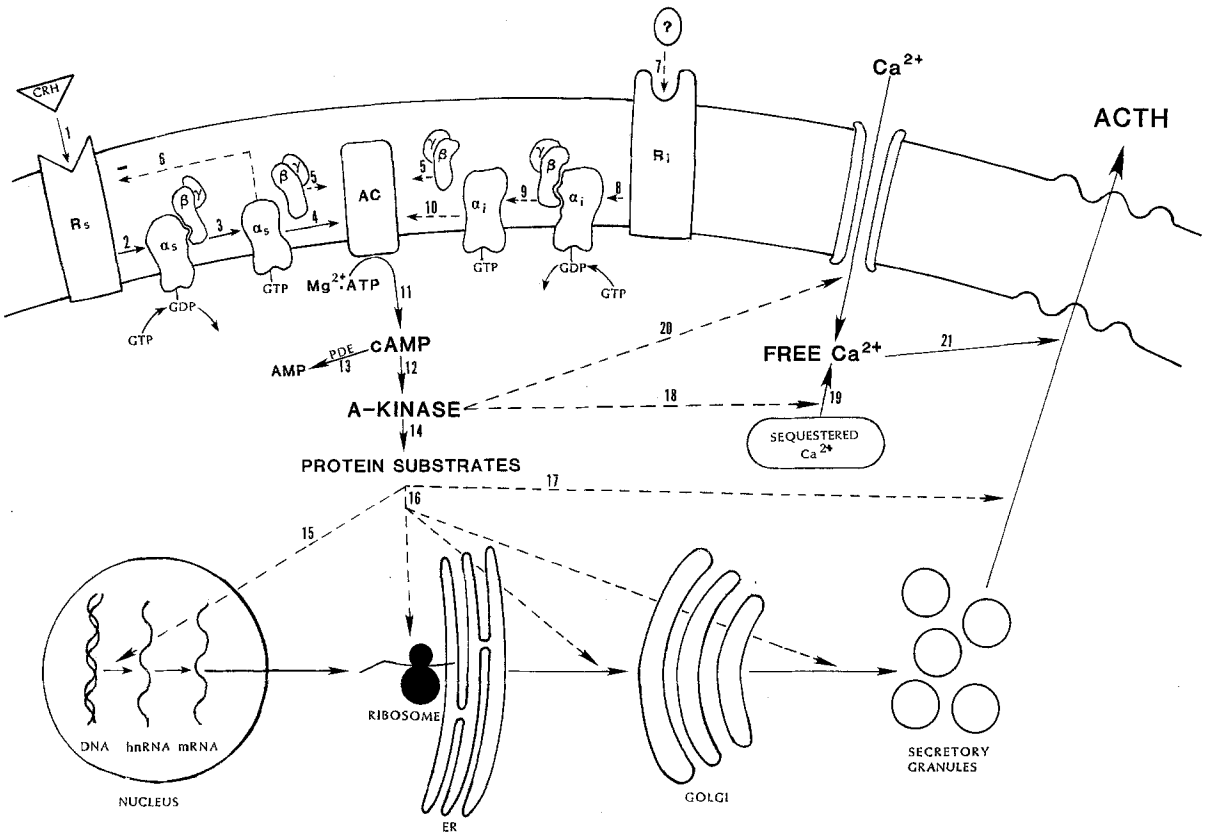

Figure 2. Schematic representation of the cAMP transduction pathway. Dashed lines indicate that the mechanism represented or its role in ACTH secretion is uncertain. Numbers in the figure refer to numbers in brackets cited below. The binding of CRH to its receptor [1][7,47,48,91,204], presumably stimulates the dissociation of the stimulatory guanine nucleotide-binding protein (G_s) [2] into α and $\beta \cdot \gamma$ subunits by catalyzing the exchange of GTP for GDP on the α subunit [2,3][7,33,93,134]. α_s-GTP stimulates adenylate cyclase (AC) [4] to convert Mg^{2+}. ATP to cAMP [11][33,35]. Guanine nucleotides inhibit CRH binding, presumably through interaction with G_s [6][7,134]. The $\beta \cdot \gamma$ subunits may also have physiological effects [5][33]. The role of an inhibitory receptor [7] and a G_i protein [8–10] in the modulation of ACTH secretion from normal anterior pituitary corticotrophs is unknown, although α-ANF[1–28][86] and delta sleep-inducing peptide[128] are potential inhibitory peptides. In normal corticotrophs, cAMP activates A-kinase [12][7,8] and is degraded by cAMP-dependent phosphodiesterase (PDE) [13][6]. A-kinase phosphorylates unidentified protein substrates [14][7,79,153], leading to increased biosynthesis and secretion of ACTH [15–17][53,58,101,102,164,169,182,184,185]. Activation of the cAMP pathway with CRH or forskolin increases cytosolic free $[Ca^{2+}]$[97], possibly by opening Ca^{2+} channels within the plasma membrane [20][105] or liberating internal sequestered Ca^{2+} [18]. These effects may be mediated by A-kinase[70]. Free internal Ca^{2+} increases ACTH secretion [21], as discussed under 'Role of calcium-dependent processes'.

In summary, accumulation of cAMP within corticotrophs initiates processes culminating in increased biosynthesis and secretion of ACTH. The activation of A-kinase is probably prerequisite for both of these effects, but the substrates of A-kinase responsible for manifesting these effects remain unknown. CRH, the most potent physiological stimulator of ACTH release, operates in part through the cAMP pathway (fig. 2).

Role of calcium-dependent processes

The calcium ion (Ca^{2+}) is important in the cellular function of nearly all cell types, playing the role of a classic intracellular messenger[137]. Indicating such a role for Ca^{2+} in ACTH secretion, the major secretagogues of ACTH, CRH and AVP, elevate cytosolic free $[Ca^{2+}]$ within individual corticotrophs, identified by the reverse hemolytic plaque assay[97], and in AtT-20 cells[70,105]. In normal corticotrophs, 100 nM CRH increases cytosolic $[Ca^{2+}]$, as measured with the flourescent dye fura-2, from basal levels between 50 nM and 100 nM to peaks of 300 nM, whereas 100 nM AVP causes a transient spike of cytosolic $[Ca^{2+}]$ reaching over 350 nM[97]. In general, the concentration of cytosolic free Ca^{2+} can be increased through the release of sequestered Ca^{2+} from internal stores[137], or through the activation of Ca^{2+}-specific channels in the plasma membrane[52,76,99,144]. While AVP probably elevates cytosolic free $[Ca^{2+}]$ by mobilizing internal Ca^{2+} stores through the action of a phospholipid metabolite (see 'Role of the PI pathway'), very little is understood about the mechanism by which CRH elevates cytosolic $[Ca^{2+}]$. The activation of A-kinase may be an essential step, since in AtT-20 cells, A-kinase inhibitor (Walsh inhibitor) decreases CRH and forskolin induced increases in cytosolic free $[Ca^{2+}]$ by 25% without altering the effect of K^+[70]. This suggests that A-kinase is involved in the mobilization of Ca^{2+}, but the source of Ca^{2+} is undetermined. Whole cell patch clamp

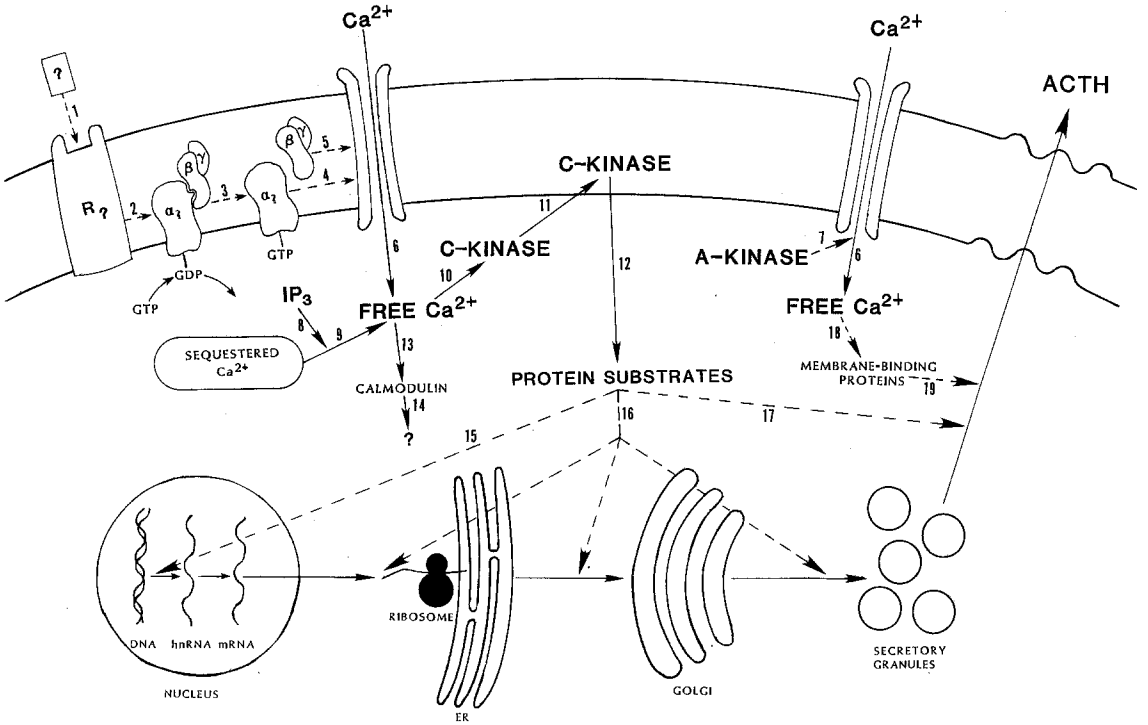

Figure 3. Schematic representation of the Ca^{2+} transduction pathway. Dashed lines indicate that the mechanism represented or its role in ACTH secretion is uncertain. Numbers in figure refer to numbers in brackets cited below. L- and T-type Ca^{2+} channels have been detected in the plasma membrane of anterior pituitary corticotrophs[38,108]. In general, Ca^{2+} channels in the plasma membrane can be activated by depolarization, receptor-activated G-proteins (G_s) [1–5] or phosphorylation [7][52,76,99,144]. For clarity of the figure, two Ca^{2+} channels are shown. In AtT-20 cells, activation of A-kinase increases Ca^{2+} channel opening [7][105], allowing external Ca^{2+} to enter the cell [6]. Sequestered internal Ca^{2+} can be released through the action of IP_3 [8] (fig. 4)[24,112,168,203], and possibly phosphorylation by A-kinase (fig. 2), elevating intracellular free [Ca^{2+}] [9]. Cytosolic free Ca^{2+} translocates C-kinase to the plasma membrane [10,11], where it is activated by phospholipids (fig. 4)[109,125,176]. C-kinase phosphorylates unidentified protein substrates [12][153] leading to increased secretion[3,44,162,182] and possibly biosynthesis[182,190] of ACTH [15–17]. Cytosolic free Ca^{2+} also activates calmodulin [13][36,111,137,191] and membrane-binding proteins [18][43,61], but their roles in ACTH secretion are undetermined [14,19].

recordings in AtT-20 cells indicate that A-kinase may act by increasing the opening of Ca^{2+} channels in the plasma membrane[105] (fig. 3).

Investigations of the role of external Ca^{2+} in ACTH secretion, by manipulation of the Ca^{2+} level in the medium, have been inconclusive. Some investigators report that removal of external Ca^{2+} does not alter spontaneous secretion from cultured anterior pituitary cells over a 1–3.5 h incubation[2,165,206], while others report up to a 60% decrease[38,46,68]. ACTH secretion stimulated by cAMP analogs[2,68,206], 10 mM theophylline[114], 10 nM AVP[2,114], phorbol esters[46,162] and 0.1–100 nM CRH[2,38,68,164,199] is attenuated in Ca^{2+}-free medium by between 21% and 100%. These results suggest that spontaneous ACTH release as well as that induced by secretagogues is, at least partially, dependent upon external Ca^{2+}. The effect on CRH- and AVP-stimulated release are only manifested after 45 min of incubation in the Ca^{2+}-free medium, leading to the proposal that there may be two phases of stimulated ACTH secretion: an initial, rapidly activated phase which relies on release of internal Ca^{2+} stores, and a later phase which is dependent upon external Ca^{2+}[2]. Experiments which directly test the role of internal Ca^{2+} in ACTH secretion, by pharmacologically blocking the release of Ca^{2+} from internal stores with a drug such as ryanodine[172], have not been conducted.

Supporting a role for external Ca^{2+} in ACTH secretion, two types of Ca^{2+} channels have been detected within the plasma membrane of corticotrophs[38,108]. One type of channel resembles the T-type channel, having a low threshold of activation and initiating a transient Ca^{2+} current, while the other has characteristics of L-type channels, a high threshold eliciting a prolonged response. Depolarization of corticotrophs, possibly by the opening of sodium channels[38], would activate these channels. Supporting a role for Ca^{2+} channels in ACTH release, maitotoxin[20] and Bay K 8644[2], drugs that activate L-type Ca^{2+} channels[42,127], stimulate ACTH release in culture.

To investigate the role of Ca^{2+} influx in ACTH secretion in vitro, Ca^{2+} channels can be pharmacologically blocked with drugs, such as $CoCl_2$, verapamil, diltiazem, nifedipine and nimodipine. If Ca^{2+} influx is essential for secretion, then blocking Ca^{2+} channels should abolish ACTH release. Vale and co-workers[184] reported that 2 mM $CoCl_2$, which may block both types of Ca^{2+} channels[180], abolishes ACTH secretion induced by CRH.

Other Ca^{2+} channel blockers, verapamil, diltiazem, nifedipine and nimodipine, which are effective blockers of L-type Ca^{2+} channels[21,42,180], either decrease spontaneous ACTH release in culture by 24–100%[2,38] or have no effect[68,119]. These blockers reduce ACTH secretion stimulated by 30 mM K^{+}[2], 10 nM AVP[2], phorbol esters[162] and 10 nM CRH[2,68,119,162], suggesting that stimulated release is partially dependent upon external Ca^{2+}. Thus, there is compelling evidence that an external Ca^{2+} source is required for the full action of CRH and AVP. There is, so far, only suggestive evidence for a role of internal Ca^{2+} stores, since pharmacological amounts (100 nM) of AVP were applied to elicit Ca^{2+} release from internal stores[97].

How Ca^{2+} affects the secretory process is not completely understood. In accordance with the stimulus-secretion coupling hypothesis, Ca^{2+} increases secretion by promoting exocytosis[50]. Influx of extracellular Ca^{2+}, induced by activation of Ca^{2+} channels with maitotoxin, stimulates ACTH release by 2-fold within 15 min[20], suggesting that stimulation of ACTH secretion occurs by increasing the efficacy of the release mechanism. Ca^{2+} may also regulate POMC biosynthesis. In cultured anterior pituitary cells, Ca^{2+} channel blockers decrease basal POMC mRNA levels by 50%[101], while Bay K 8644 increases POMC mRNA levels by 50% after 2 days[101]. Also, A23187, a Ca^{2+} ionophore, increases POMC gene transcription 7-fold in 15 min[53].

These effects of Ca^{2+} may occur through activation of Ca^{2+}-sensitive proteins which include Ca^{2+}-dependent protein kinase (C-kinase)[125,137], calmodulin[36,111,137,191] and membrane-binding proteins[43,61]. An increase in intracellular free [Ca^{2+}] results in the translocation of cytosolic C-kinase to the plasma membrane, where lipids, mainly diacylglycerol and phosphatidylserine, complete its activation[109,125,176]. Substrates of C-kinase have not been characterized in normal corticotrophs, however; in AtT-20 cells, activation of C-kinase leads to the phosphorylation of three cytoplasmic (25 kDa, 40 kDa and 49 kDa), two membrane (32 kDa and 60 kDa) and one nuclear protein (20 kDa) of unknown function(s)[153]. Similar to A-kinase, it is possible that these protein substrates modulate ACTH release by altering POMC transcription, translation, processing and/or release (fig. 3).

The Ca^{2+}-calmodulin complex directly activates some proteins including a cyclic nucleotide phosphodiesterase in bovine heart[177], the plasma membrane Ca^{2+} pump in red blood cell membranes[96], brain adenylate cyclase[29,37] and Ca^{2+}-calmodulin dependent protein kinases in several tissues[26,123]. Calmodulin inhibitors decrease CRH-stimulated ACTH release from cultured anterior pituitary cells by approximately 50%[118,165], suggesting a role for calmodulin in ACTH secretion. Since the calmodulin inhibitors may also have nonspecific effects including inhibition of C-kinase[201], which could account for the inhibition of ACTH release, these data are inconclusive.

A group of proteins, identified largely because of their ability to promote the fusion of chromaffin cell granules in the presence of Ca^{2+} and phospholipids[43,61], may also be responsible for Ca^{2+} effects on cell function. Since the release of hormones or neurotransmitters from vesicles may involve membrane fusion events, these proteins may provide a means by which Ca^{2+} regulates exocytosis. The Ca^{2+} sensitivity of these proteins varies and can be altered by phosphorylation[43], proposing intriguing possibilities for their role in secretion. A role for these proteins in ACTH release from corticotrophs has not yet been investigated.

In summary, three findings support a role for Ca^{2+} in ACTH secretion (fig. 3). First, CRH and AVP increase cytosolic free [Ca^{2+}] within normal corticotrophs from basal levels of approximately 100 nM to over 300 nM. Second, activation of Ca^{2+} channels with maitotoxin or Bay K 8644 stimulates ACTH release. Third, blockade of Ca^{2+} influx and removal of Ca^{2+} from the external medium can significantly attenuate the sustained phase of ACTH secretion. In spite of these findings, the role of Ca^{2+} in ACTH release is still unclear. The relative importance of internal and external sources of Ca^{2+} in the stimulation of ACTH secretion is controversial. The most coherent conclusion is that both external and internal Ca^{2+} sources are involved in ACTH secretion to some extent. Internal stores may be required for immediate ACTH release, while Ca^{2+} influx is necessary to sustain secretion. The mechanisms by which Ca^{2+} mediates the stimulation of ACTH secretion and enhances POMC biosynthesis are undetermined.

Role of the PI pathway

Hormone-receptor binding can also influence cellular processes by promoting the hydrolysis of inositol phospholipids, a minor lipid constituent of the plasma membrane[24,113,131,136]. Activated receptors may be coupled through G-proteins (G_p) to phospholipase C, which cleaves phosphatidylinositol 4,5-bisphosphate into inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG)[24,33,100,136] (fig. 4). Since IP_3 is highly charged and water soluble, it readily moves into the cytoplasm where it mobilizes internal Ca^{2+} stores[24,203]. The exact mechanisms by which IP_3 acts are unknown, but there is evidence indicating that IP_3 binds to portions of the endoplasmic reticulum, opening Ca^{2+} channels[112,168]. The activity of IP_3 is terminated by a phosphatase which dephosphorylates IP_3 to IP_2 or by additional phosphorylation to IP_4[136].

On the other hand, the highly lipophilic DAG remains within the plasma membrane[203], where it activates C-kinase which has been translocated from the cytoplasm to the plasma membrane by increased cytosolic free [Ca^{2+}][109,125,176]. C-kinase continues the transduction process by phosphorylating several unidentified proteins within the cell[125]. In addition, C-kinase feeds back to decrease ligand-induced generation of IP_3 and to enhance

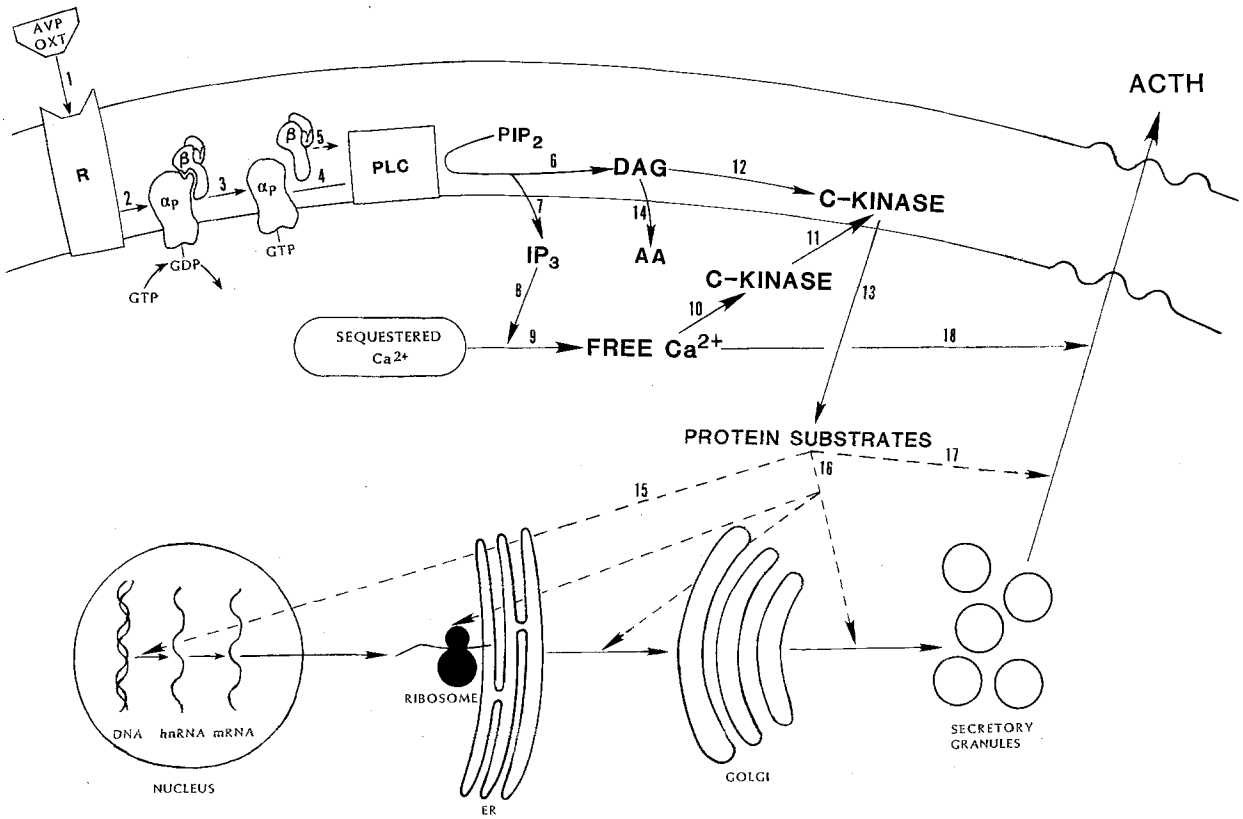

Figure 4. Schematic representation of the PI transduction pathway. Dashed lines indicate that the mechanism represented or its role in ACTH secretion is uncertain. Numbers in figure refer to numbers in brackets cited below. AVP and OXT bind to a receptor on anterior pituitary cells [1][39, 87, 90, 166] probably promoting the dissociation of a G-protein (G_p) [2,3][24, 33, 100, 136]. The αp-GTP molecule is presumed to stimulate phospholipase C (PLC) [4][24, 33, 100, 136] to hydrolyze phosphatidylinositol 4,5 bisphosphate (PIP_2) into inositol 1,4,5 trisphosphate (IP_3) [7] and diacylglycerol (DAG) [6][24, 100, 113, 131, 136]. The $\beta \cdot \gamma$ subunit may also have physiological effects [5][33]. IP_3 is known to enter the cytosol [7] and liberate sequestered Ca^{2+} [8,9][24, 112, 168, 203], increasing cytosolic free Ca^{2+} and leading to the activation of Ca^{2+}-dependent processes (fig 3). DAG remains in the plasma membrane [6] and activates C-kinase [12][109, 125, 176, 203], which had been translocated to the plasma membrane by Ca^{2+} [10,11][109, 125, 176]. C-kinase phosphorylates unidentified protein substrates [13][153] leading to increased secretion[3, 44, 46, 162, 182] and possibly biosynthesis of ACTH [15–17][182, 190]. DAG also serves as a source of arachidonic acid (AA) [14] and can be cleaved from DAG by DAG-lipase[26, 125, 202] to produce leukotrienes from the lipoxygenase pathway and prostaglandins and thromboxanes from the cyclooxygenase pathway (not shown)[202]. Through unknown mechanisms, these metabolites may stimulate and inhibit ACTH release, respectively[4, 73, 89, 163, 188].

IP_3 metabolism, thus attenuating the rise in cytosolic $[Ca^{2+}]$[81].

Phorbol esters, which directly activate C-kinase[34], stimulate ACTH release from anterior pituitary cells[3, 6, 44, 46, 162, 182], demonstrating that C-kinase regulates ACTH secretion. In culture, phorbol myristate acetate (PMA) increases ACTH secretion 4.5-fold within 15 min[44] and elicits a maximum of a 13-fold increase over 2 h[3]. Phorbol esters not only stimulate ACTH release, but also potentiate CRH-stimulated ACTH secretion[3, 6, 44, 162] through increased accumulation of cAMP[6, 44]. In culture, PMA increases CRH-stimulated cAMP accumulation by 4-fold after 15 min[6]. Phorbol esters may also stimulate ACTH biosynthesis[182], but these effects are weak compared to CRH[190].

In addition to activating C-kinase, DAG may affect cell function by giving rise to arachidonic acid, another intracellular messenger[26, 125, 202]. Arachidonic acid is hydrolyzed from DAG by phospholipase A_2 (fig. 4), and is then metabolized through the cyclooxygenase pathway into prostaglandins and thromboxanes, or through the lipoxygenase pathway into leukotrienes[202]. A role for these metabolites of arachidonic acid in normal corticotrophs has been postulated, with the cyclooxygenase pathway forming an inhibitor and the lipoxygenase pathway producing a stimulator of ACTH secretion[4, 73, 89, 163, 188].

AVP and OXT regulate ACTH secretion through stimulation of PI turnover, as suggested by measurements of the incorporation of ^{32}Pi[138] or ^{3}H-inositol[71, 179] into membrane phospholipids in cultured anterior pituitary cells. This effect is significant after only 4 min of application and persists for over 2 h[138]. AVP, at 5–100 nM, causes a 2–4-fold increase in ACTH secretion from cultured anterior pituitary cells over 1–4 h of incubation[7, 17, 18, 69, 94, 118, 119, 183]. AVP-induced ACTH release is biphasic, with an initial phase of rapid secretion which lasts about 45 min, followed by a slower constant phase[2]. The action of AVP may rely almost entirely upon C-kinase activation, because a 6-h pre-incubation with phorbol ester, which depletes 90% of cellular C-kinase, abolishes ACTH secretion stimulated by AVP, while CRH effects are normal[32]. Since the prolonged treatment with phorbol ester may have alternative effects

which alter ACTH secretion, these results should be evaluated with caution. AVP and OXT do not affect ACTH biosynthesis, even after 14 days in culture[189].

The most prominent role, however, of AVP and OXT in ACTH secretion is to potentiate the stimulatory effects of CRH[3,6,17,22,64,66,69,94,185]. This effect of AVP is very potent, since even maximal doses of CRH (5–100 nM) elicit more ACTH secretion when AVP is co-applied[17,22,66]. Also, low doses of AVP and OXT (0.05 nM), which weakly stimulate ACTH secretion alone, significantly potentiate CRH stimulation[17,22]. Synergism of CRH and AVP results in an amplified CRH-like ACTH response[6,192], further indicating that AVP potentiates CRH-induced intracellular pathways. Suggesting that AVP increases exocytosis, co-application of CRH and AVP produces a 15% larger increase in cell area and a more pronounced reduction in the number of secretory granules than CRH alone[92]. The synergistic effects of AVP may be restricted to facilitating release, because 100 nM AVP does not increase ACTH biosynthesis stimulated by CRH over 14 days[189]. Possible interactions between CRH- and AVP-initiated processes, which may account for the synergistic effects on release, are discussed in more detail below.

In summary, neurohypophysial hormones and phorbol esters stimulate ACTH release and potentiate CRH-induced ACTH secretion. Activation of the PI pathway most probably mediates these effects of AVP, OXT and phorbol esters since, AVP and OXT stimulate PI turnover giving rise to DAG, phorbol esters and DAG are known to activate C-kinase, and depletion of C-kinase by long-term treatment with phorbol esters abolishes AVP-stimulated ACTH release. There is little evidence that activation of the PI pathway stimulates ACTH biosynthesis.

Effects of glucocorticoids

Glucocorticoids can act at the pituitary level to decrease ACTH release[1,45,158]. In vivo, inhibition of ACTH secretion occurs by both fast and slow mechanisms[85]. Glucocorticoids suppress POMC gene transcription in vivo within 30 min[58], and inhibition of ACTH secretion can occur within 10 min of glucocorticoid application[84]. This suggests that ACTH release is blocked before ACTH production is reduced. Supporting this contention, 16 h of glucocorticoid treatment in vivo increases the number of secretory granules along the plasma membrane of anterior pituitary corticotrophs[161]. The mechanisms by which glucocorticoids act in these experiments remain unknown.

In vitro, glucocorticoids decrease stimulated ACTH secretion from anterior pituitary fragments[199] and acutely dispersed[158] or cultured anterior pituitary cells[1,164,183]. Inhibition is significant within 10–15 min of glucocorticoid application[1,199], reaching a maximum of up to 70% after 3–4 h[158]. Glucocorticoids inhibit ACTH secretion in vitro by decreasing both POMC biosynthesis and the efficacy of the release process[1]. As measured by a nuclear transcription run-on assay (a measurement of POMC gene activity in permeabilized cells), glucocorticoids reduce POMC transcription in cultured anterior pituitary cells to 20% of control values within 15–30 min of application[53,58]. Glucocorticoids may suppress the POMC gene promoter by activating cytosolic receptors which enter the nucleus and interact with segments of DNA upstream from the POMC gene[51]. It is unlikely that a reduction in the rate of POMC transcription could account for the rapid inhibition of ACTH release, because it may take up to 15 h of treatment with glucocorticoids to significantly decrease the total amount of POMC mRNA stored within cultured cells[169]. It has been proposed that glucocorticoids decrease CRH-stimulated cAMP production by up to 50%[25,164,185], possibly accounting for the rapid inhibition of ACTH secretion in vitro. Glucocorticoids may also act at a step subsequent to cAMP production, and interact with multiple intracellular pathways, because they attenuate ACTH release stimulated by cAMP analogs[1,56,67,185], methylxanthines[185] and cAMP-independent stimulators[1,46,57,162].

Possible interactions between transduction pathways

Interaction between the cAMP, Ca^{2+} and PI pathways provides sophisticated control of ACTH secretion, eliciting responses which integrate the various types of input that the corticotroph receives. The importance of interaction between these intracellular messengers is particularly evident when the PI and cAMP pathways are co-stimulated, since maximal responses to CRH are potentiated several-fold by activators of the PI system[3,6,22,44,66,69]. At least three possible points of interaction exist between these two pathways, and may be responsible for the synergistic effects of co-stimulation (fig. 5).

First, since activation of the PI pathway potentiates CRH-induced accumulation of cAMP in anterior pituitary cells[6,44,66,94], a component of the PI system must affect a process which regulates cAMP levels. This interaction does not occur in a crude membrane preparation[59], suggesting that the PI pathway modulates cAMP accumulation through a cytosolic mediator. C-kinase depletion abolishes the facilitation of cAMP accumulation induced by phorbol esters or AVP[32], indicating that C-kinase may be responsible for potentiation. Theoretically, C-kinase may increase cAMP accumulation by phosphorylating the CRH receptor, G_s, adenylate cyclase or cAMP-dependent phosphodiesterase. None of these possibilities have been completely explored to date. Since in anterior pituitary homogenates AVP decreases phosphodiesterase activity by 30%[6], modulation of phosphodiesterase activity by C-kinase may be partly responsible for AVP potentiation of stimulated cAMP accumulation. C-kinase may also interact with the cAMP

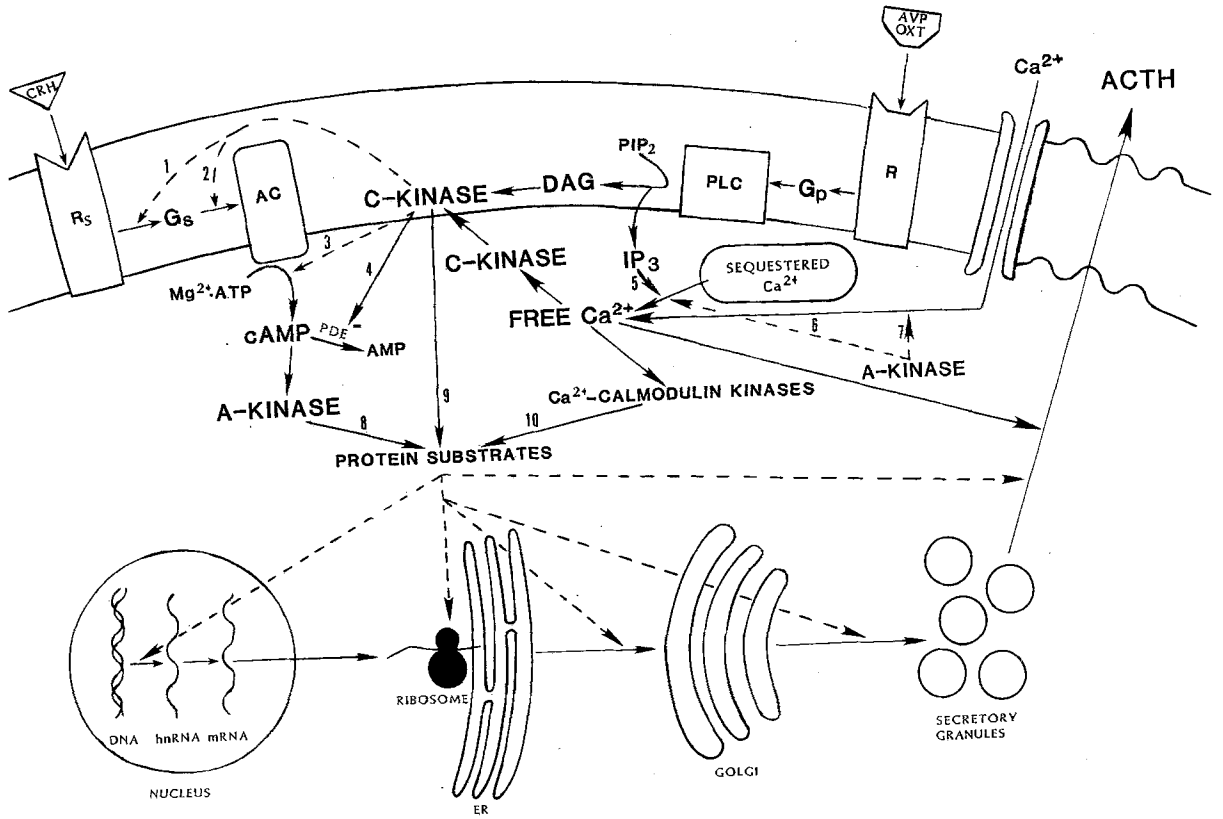

Figure 5. Schematic representation of the possible interactions between the cAMP, Ca^{2+} and PI pathways. Dashed lines indicate that the mechanism represented or its role in ACTH secretion is uncertain. Numbers in the figure refer to possible points of interaction cited in brackets. See preceeding figures for details on each pathway. C-kinase increases cAMP accumulation stimulated by CRH [6, 44, 66, 94], possibly by phosphorylating CRH receptors [1], G_s [2], adenylate cyclase (AC) [3] or phosphodiesterase (PDE) [4] [6]. Since both CRH and AVP increase cytosolic free Ca^{2+} within corticotrophs [5–7] [97], Ca^{2+}-dependent processes may link the cAMP and PI pathways. In addition, A-kinase [8], C-kinase [9] and Ca^{2+}-calmodulin kinases [10] may phosphorylate common substrates or different substrates involved in a single cellular process [123, 125, 137, 153]. Each or all of these interactions may lead to the synergistic effects when the cAMP and PI pathways are co-stimulated.

system at a step subsequent to or distinct from cAMP formation, because phorbol esters facilitate the stimulatory effects of 8-bromo-cAMP by 2-fold as well [3].

Second, since both the cAMP and PI systems increase cytosolic $[Ca^{2+}]$ [97], these two pathways may converge at the point of Ca^{2+} influx or mobilization to enhance ACTH release. Synergism may result from increased activation of C-kinase due to elevated $[Ca^{2+}]$, when both the cAMP and PI pathways are stimulated. It has been proposed that C-kinase then may act to increase cAMP accumulation, forming a positive feedback loop; the cytosolic free $[Ca^{2+}]$, and therefore probably secretion, would continue to rise until the cycle is terminated [97]. Alternatively, Ca^{2+} liberated by IP_3 may activate Ca^{2+}-calmodulin-dependent protein kinases which may interact with the cAMP system. Ca^{2+}-calmodulin has been postulated to both increase and decrease porcine, bovine and rat brain adenylate cyclase [29, 37] and to activate bovine heart phosphodiesterase [177], but this has not been shown to occur in pituitary. Cytosolic Ca^{2+} may be required for increases in cAMP levels in corticotrophs, as demonstrated by a decrease in cAMP accumulation stimulated by CRH in EGTA-treated cells [2]. Ca^{2+} liberated by IP_3 could also potentiate CRH-stimulated ACTH secretion by acting on membrane-binding proteins to increase exocytosis. Since Ca^{2+} mobilization induced by AVP is transient [97], the activation of calmodulin and/or membrane-binding proteins by IP_3 liberated Ca^{2+} probably can not account for the synergism between CRH and AVP, but nevertheless these processes may be key points of interaction between the cAMP and PI transduction pathways.

Third, it is possible that A-kinase, C-kinase and Ca^{2+}-calmodulin kinases interact by phosphorylating the same substrates, or different substrates which are involved in a single cellular process [123, 125, 137] (fig. 5.). Unfortunately, the substrates of the kinases within normal corticotrophs are not well known, so definite points of interaction can not be identified. However, recent data in AtT-20 cells suggest that A-kinase and C-kinase both phosphorylate a 40 kDa cytoplasmic protein and two membrane proteins of 60 kDa and 32 kDa [153]. These proteins may provide the link between the cAMP and PI pathways responsible for the synergistic effects of co-activation.

Future research

In spite of the recent advances in the understanding of corticotroph function outlined in this review, several challenging problems remain to be solved. Multiple stimuli cause the release of ACTH, but the relative role of CRH, neurohypophysial peptides and other mediators has not been clearly defined. Moreover, we do not know the type of hypothalamic neurotransmitters that modulate the release of these mediators under various physiological conditions. At the cell level, the peculiar arrangement of clusters of possibly interconnected corticotrophs has not yet been investigated in detail, and the physiological significance of this arrangement in signal transduction remains to be explored.

At the level of the corticotroph cell membrane, there is abundant functional evidence for separate receptors for CRH and AVP, and for the coupling between these receptors and second messenger systems through G-proteins. However, specific G-proteins have not yet been visualized by immunocytochemistry in corticotrophs, or demonstrated by ribosylation and gel-filtration. Of particular interest would be the identification of a G_i α subunit as a possible mediator of the inhibitory effects of α-ANF[1–28] on ACTH secretion.

Within corticotrophs, activation of the cAMP, Ca^{2+} and PI transduction pathways all contribute to ACTH secretion; however; critical experiments concerning the role of internal Ca^{2+} stores have not yet been performed. The involvement of C-kinase relies heavily on circumstantial evidence, such as the presumed activation and depletion of C-kinase by phorbol esters. The points of interaction between the cAMP, Ca^{2+} and PI systems are still not clear, probably because the events subsequent to kinase activation and those due to Ca^{2+}-activated proteins are not understood. The identification of protein substrates of A-kinase, C-kinase and Ca^{2+}-calmodulin kinases within normal anterior pituitary corticotrophs as well as the characterization of their function is essential to further our knowledge of the mechanisms by which signal transduction pathways regulate ACTH biosynthesis and secretion.

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1 Abou-Samra, A.-B., Catt, K. J., and Aguilera, G., Biphasic inhibition of adrenocorticotropin release by corticosterone in cultured anterior pituitary cells. Endocrinology *119* (1986) 972–977.
2 Abou-Samra, A.-B., Catt, K. J., and Aguilera, G., Calcium-dependent control of corticotropin release in rat anterior pituitary cell cultures. Endocrinology *121* (1987) 965–971.
3 Abou-Samra, A.-B., Catt, K. J., and Aguilera, G., Involvement of protein kinase C in the regulation of adrenocorticotropin release from rat anterior pituitary cells. Endocrinology *118* (1986) 212–217.
4 Abou-Samra, A.-B., Catt, K. J., and Aguilera, G., Role of arachidonic acid in the regulation of adrenocorticotropin release from rat anterior pituitary cell cultures. Endocrinology *119* (1986) 1427–1431.
5 Abou-Samra, A.-B., Catt, K. J., and Aguilera, G., Synthetic atrial natriuretic factors (ANFs) stimulate guanine 3',5'-monophosphate production but not hormone release in rat pituitary cells: peptide contamination with a gonadotropin-releasing hormone agonist explains luteinizing hormone-releasing activity of certain ANFs. Endocrinology *120* (1987) 18–24.
6 Abou-Samra, A.-B., Harwood, J. P., Manganiello, V. C., Catt, K. J., and Aguilera, G., Phorbol 12-myristate 13-acetate and vasopressin potentiate the effect of corticotropin-releasing factor on cyclic AMP production in rat anterior pituitary cells. J. biol. Chem. *262* (1987) 1129–1136.
7 Aguilera, G., Harwood, J. P., Wilson, J. X., Morell, J., Brown, J. H., and Catt, K. J., Mechanisms of action of corticotropin-releasing factor and other regulators of corticotropin release in rat pituitary cells. J. biol. Chem. *258* (1983) 8039–8045.
8 Aguilera, G., Wynn, P. C., Harwood, J. P., Hauger, R. L., Millan, M. A., Grewe, C., and Catt, K. J., Receptor-mediated actions of corticotropin-releasing factor in pituitary gland and nervous system. Neuroendocrinology *43* (1986) 79–88.
9 Allen, R. G., Herbert, E., Hinman, M., Shibuya, H., Pert., C. B., Coordinate control of corticotropin, β-lipotropin, and β-endorphin release in mouse pituitary cell cultures. Proc. natl Acad. Sci. *75* (1978) 4972–4976.
10 Antoni, F. A., Hypothalamic control on adrenocorticotropin secretion: advances since the discovery of 41-residue corticotropin-releasing factor. Endocr. Rev. *7* (1986) 351–378.
11 Antoni, F., and Dayanithi, G., Guanosine 3':5' cyclic monophosphate and activators of guanylate cyclase inhibit secretagogue-induced corticotropin release by anterior pituitary cells. Biochem. biophys. Res. Commun. *158* (1989) 824–830.
12 Antoni, F. A., Holmes, M. C., and Jones, M. T., Oxytocin as well as vasopressin potentiate ovine CRF in vitro. Peptides *4* (1983) 411–415.
13 Antoni, F. A., Holmes, M. C., Makara, G. B., Karteszi, M., and Laszlo, F. A., Evidence that the effects of arginine-8-vasopressin (AVP) on pituitary corticotropin (ACTH) release are mediated by a novel type of receptor. Peptides *5* (1984) 519–522.
14 Antoni, F. A., Palkovitz, M., Makara, G. B., Linton, E. A., Lowry, P. J., and Kiss, J. Z., Immunoreactive corticotropin-releasing hormone in the hypothalamoinfundicular tract. Neuroendocrinology *36* (1983) 415–423.
15 Axelrod, J., and Reisine, T. R., Stress hormones: their interaction and regulation. Science *224* (1984) 452–459.
16 Baertschi, A. J., Portal vascular route from hypophysial stalk/neural lobe to adenohypophysis. Am. J. Physiol. *239* (1980) R463–R469.
17 Baertschi, A. J., and Friedli, M., A novel type of vasopressin receptor on anterior pituitary corticotrophs? Endocrinology *116* (1985) 499–502.
18 Baertschi, A. J., Gähwiler, B., Antoni, F. A., Holmes, M. C., and Makara, G. B., No role of vasopressin in stress-induced ACTH secretion? Nature *108* (1984) 85–86.
19 Baertschi, A. J., Vallet, P., Baumann, J. B., and Girard, J., Neural lobe of pituitary modulates corticotropin release in the rat. Endocrinology *106* (1980) 878–882.
20 Baertschi, A. J., Yasumoto, T., Pence, R., and Cronin, M. J., ACTH release in response to CRF, forskolin, maitotoxin, phorbol ester and atrial natriuretic factor (ANF). 1st Int. Congr. Neuroendocr. (1986) Abst. No. 45.
21 Bean, B. P., Two kinds of calcium channel in canine atrial cells. J. gen. Physiol. *86* (1985) 1–30.
22 Beny, J.-L., and Baertschi, A. J., Synthetic corticoliberin needs arginine vasopressin for full corticotropin releasing activity. Experientia *38* (1982) 1078–1079.
23 Beny, J.-L., and Baertschi, A. J., Oxytocin: major corticotropin-releasing factor secreted from diabetes insipidus rat posterior pituitary in vitro. Neuroendocrinology *31* (1980) 261–264.
24 Berridge, M. J., and Irvine, R. F., Inositol trisphosphate, a novel second messenger in cellular signal transduction. Nature *312* (1984) 315–321.
25 Bilezikjian, L. M., and Vale, W. W., Glucocorticoids inhibit corticotropin-releasing factor-induced production of adenosine 3',5'-monophosphate in cultured anterior pituitary cells. Endocrinology *113* (1983) 657–662.
26 Blackshear, P. J., Nairn, A. C., and Kuo, J. F., Protein kinases 1988: a current perspective. FASEB J. *2* (1988) 2957–2969.
27 Blalock, J. E., Smith, E. M., and Meyer III, W. J., The pituitary-adrenocortical axis and the immune system. Clin. Endocr. Metab. *14* (1985) 1021–1038.

28 Bloom, F. E., Battenberg, E. L., Rivier, J., and Vale, W., Corticotropin-releasing factor (CRF) immunoreactive neurons and fibers in rat hypothalamus. Reg. Pept. *4* (1982) 43–48.

29 Brostrom, C. O., Huang, Y.-C., Breckenridge, B. M., Wolff, D. J., Identification of a calcium-binding protein as a calcium-dependent regulator of brain adenylate cyclase. Proc. natl Acad. Sci. USA *72* (1975) 64–68.

30 Brownstein, M. J., Russell, J. H., and Gainer, H., Synthesis, transport, and release of posterior pituitary hormones. Science *207* (1980) 373–378.

31 Burnstock, G., A basis for distinguishing two types of purinergic receptor, in: Cell Membrane Receptors for Drugs and Hormones: a Multidisciplinary Approach, pp. 107–116. Eds R. W. Straub and L. Bolis. Raven Press, New York 1978.

32 Carvallo, P., and Aguilera, G., Protein kinase C mediates the effect of vasopressin in pituitary corticotrophs. Soc. Neurosci. *14* (1988) Abst. No. 442.

33 Casey, P. J., and Gilman, A. G., G protein involvement in receptor-effector coupling. J. biol. Chem. *263* (1988) 2577–2580.

34 Castagna, M., Takai, Y., Kaibuchi, K., Sano, K., Kikkawa, U., and Nishizuka, Y., Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. J. biol. Chem. *257* (1982) 7847–7851.

35 Cerione, R. A., Codina, J., Benovic, J. L., Lefkowitz, R. J., Birnbaumer, L., and Caron, M. G., The mammalian β_2-adrenergic receptor: reconstitution of functional interactions between pure receptor and pure stimulatory nucleotide binding protein of the adenylate cyclase system. Biochemistry *23* (1984) 4519–4525.

36 Cheung, W. Y., Calmodulin plays a pivotal role in cellular regulation. Science *200* (1980) 19–27.

37 Cheung, W. Y., Bradham, L. S., Lynch, T. J., Lin, Y. M., and Tallant, E. A., Protein activator of cyclic 3':5'-nucleotide phosphodiesterase of bovine or rat brain also activates its adenylate cyclase. Biochem. biophys. Res. Commun. *66* (1975) 1055–1062.

38 Childs, G. V., Marchetti, C., and Brown, A. M., Involvement of sodium and two types of calcium channels in the regulation of adrenocorticotropin release. Endocrinology *120* (1987) 2059–2069.

39 Childs, G. V., Unabia, G., Burke, J. A., and Marchetti, C., Secretion from corticotropes after avidin-fluorescein stains for biotinylated ligands (CRF or AVP). Am. J. Physiol. *252* (1987) E347–E356.

40 Chretien, M., and Seidah, N. G., Chemistry and biosynthesis of pro-opiomelanocortin: ACTH, MSH's, endorphins and their related peptides. Molec. cell. Biochem. *34* (1981) 101–127.

41 Cochet, M., Chang, A. C. Y., and Cohen, S. N., Characterization of the structural gene and putative 5'-regulatory sequences for human proopiomelanocortin. Nature *297* (1982) 335–339.

42 Cohen, C. J., and McCarthy, R. T., Differential effects of dihydropyridines on two populations of Ca^{2+} channel in anterior pituitary cells. Biophys. J. *47* (1985) 513a.

43 Creutz, C. E., Zaks, W. J., Hamman, H. C., and Martin, W. H., The roles of Ca^{2+}-dependent membrane-binding proteins in the regulation and mechanism of exocytosis, in: Cell Fusion, pp. 45–68. Ed. A. E. Sowers. Plenum Press, New York 1987.

44 Cronin, M. J., Zysk, J. R., and Baertschi, A. J., Protein kinase C potentiates corticotropin releasing factor stimulated cyclic AMP in pituitary. Peptides 7 (1986) 935–938.

45 Dallman, M. F., and Yates, F. E., Dynamic asymmetries in the corticosteroid feedback path and distribution-metabolism-binding elements of the adrenocortical system. Ann. N.Y. Acad. Sci. *156* (1969) 696–721.

46 Dartois, E., and Bouton, M. M., Role of calcium on TPA-induced secretion of ACTH and PGE_2 by pituitary cells: effect of dexamethasone. Biochem. biophys. Res. Commun. *138* (1986) 323–329.

47 De Souza, E. B., Perrin, M. H., Rivier, J., Vale, W., and Kuhar, M. J., Corticotropin-releasing factor receptors in rat pituitary gland: autoradiographic localization. Brain Res. *296* (1984) 202–207.

48 De Souza, E. B., Perrin, M. H., Whitehouse, P. J., Rivier, J., Vale, W., and Kuhar, M. J., Corticotropin-releasing factor receptors in human pituitary gland: autoradiographic localization. Neuroendocrinology *40* (1985) 419–422.

49 Dornhorst, A., Carlson, D. E., Seif, S. M., Robinson, A. G., Zimmerman, E. A., and Gann, D. S., Control of release of adrenocorticotropin and vasopressin by the supraoptic and paraventricular nuclei. Endocrinology *108* (1981) 1420–1424.

50 Douglas, W. W., Involvement of calcium in exocytosis and the exocytosis-vesiculation sequence. Biochem. Soc. Symp. *39* (1974) 1–28.

51 Drouin, J., Chamberland, M., Charron, J., Jeannotte, L., and Nemer, M., Structure of the rat pro-opiomelanocortin (POMC) gene. FEBS Lett. *193* (1985) 54–58.

52 Dunlap, K., Holz, G. G., and Rane, S. G., G proteins as regulators of ion channel function. TINS *10* (1987) 241–244.

53 Eberwine, J. H., Jonassen, J. A., Evinger, M. J. Q., and Roberts, J. L., Complex transcriptional regulation by glucocorticoids and corticotropin-releasing hormone of proopiomelanocortin gene expression in rat pituitary cultures. DNA *6* (1987) 483–492.

54 Eipper, B. A., and Mains, R. E., Phosphorylation of pro-adrenocorticotropin/endorphin-derived peptides. J. biol. Chem. *257* (1982) 4907–4915.

55 Emeric-Sauval, E., Corticotropin-releasing factor (CRF) – a review. Psychoneuroendocrinology *11* (1986) 277–294.

56 Fleischer, N., Donald, R. A., and Butcher, R. W., Involvement of adenosine 3',5'-monophosphate in release of ACTH. Am. J. Physiol. *217* (1969) 1287–1291.

57 Fleischer, N., and Vale, W., Inhibition of vasopressin-induced ACTH release from the pituitary by glucocorticoids in vitro. Endocrinology *83* (1968) 1232–1236.

58 Gagner, J-P., and Drouin, J., Opposite regulation of pro-opiomelanocortin gene transcription by glucocorticoids and CRH. Molec. cell. Endocr. *40* (1985) 25–32.

59 Gaillard, R. C., Schoenenberg, P., Favrod-Coune, C. A., Muller, A. F., Marie, J., Bockaert, J., and Jard, S., Properties of rat anterior pituitary vasopressin receptors: relation to adenylate cyclase and the effect of corticotropin-releasing factor. Proc. natl Acad. Sci. USA *81* (1984) 2907–2911.

60 Gann, D. S., Ward, D. G., Baertschi, A. J., Carlson, D. E., and Maran, J. W., Neural control of ACTH release in response to hemorrhage. Ann. N.Y. Acad. Sci. *297* (1977) 477–497.

61 Geisow, M. J., and Walker, J. H., New proteins involved in cell regulation by Ca^{2+} and phospholipids. TIBS *11* (1986) 420–423.

62 Gibbs, D. M., Measurement of hypothalamic corticotropin-releasing factors in hypophyseal portal blood. Fedn Proc. *44* (1985) 203–206.

63 Gibbs, D. M., and Vale, W., Presence of corticotropin releasing factor-like immunoreactivity in hypophysial portal blood. Endocrinology *111* (1982) 1418–1420.

64 Gibbs, D. M., Vale, W., Rivier, J., and Yen, S. S. C., Oxytocin potentiates the ACTH-releasing activity of CRF(41) but not vasopressin. Life Sci. *34* (1984) 2245–2249.

65 Giguere, V., and Labrie, F., Additive effects of epinephrine and corticotropin-releasing factor (CRF) on adrenocorticotropin release in rat anterior pituitary cells. Biochem. biophys. Res. Commun. *110* (1983) 456–462.

66 Giguere, V., and Labrie, F., Vasopressin potentiates cyclic AMP accumulation and ACTH release induced by corticotropin-releasing factor (CRF) in rat anterior pituitary cells in culture. Endocrinology *111* (1982) 1752–1754.

67 Giguere, V., Labrie, F., Cote, J., Coy, D. H., Sueiras-Diaz, J., and Schally, A. V., Stimulation of cyclic AMP accumulation and corticotropin release by synthetic ovine corticotropin-releasing factor in rat anterior pituitary cells: site of glucocorticoid action. Proc. natl Acad. Sci. *79* (1982) 3466–3469.

68 Giguere, V., Lefevre, G., and Labrie, F., Site of calcium requirement for stimulation of ACTH release in rat anterior pituitary cells in culture by synthetic ovine corticotropin-releasing factor. Life Sci. *31* (1982) 3057–3062.

69 Gillies, G. E., Linton, E. A., and Lowry, P. J., Corticotropin releasing activity of the new CRF is potentiated several times by vasopressin. Nature *299* (1982) 355–357.

70 Guild, S., and Reisine, T., Molecular mechanisms of corticotropin-releasing factor stimulation of calcium mobilization and adrenocorticotropin release from anterior pituitary cells. J. Pharmac. exp. Therap. *241* (1987) 125–130.

71 Guillon, G., Gaillard, R. C., Kehrer, P., Shoenenberg, P., Muller, A. F., and Jard, S., Vasopressin and angiotensin induce inositol lipid breakdown in rat adenohypophysial cells in primary culture. Reg. Pept. *18* (1987) 119–129.

72 Hashimoto, K., Ohno, N., Aoki, Y., Kageyama, J., Takahara, J., and Ofuji, T., Distribution and characterization of corticotropin-releasing factor and arginine vasopressin in rat hypothalamic nuclei. Neuroendocrinology *34* (1982) 32–37.

73 Hedge, G. A., Roles for the prostaglandins in the regulation of anterior pituitary secretion. Life Sci. *20* (1977) 17–33.

74 Herbert, E., Budarf, M., Phillips, M., Rosa, P., Policastro, P., Oates, E., Roberts, J. L., Seidah, N. G., and Chretien, M., Presence of a pre-sequence (signal sequence) in the common precursor to ACTH and endorphin and the role of glycosylation in processing of the precursor and secretion of ACTH and endorphin. Ann. N.Y. Acad. Sci. *343* (1980) 79–93.

75 Hinman, M. B., and Herbert, E., Processing of the precursor to adrenocorticotropic hormone and β-lipotropin in monolayer cultures of mouse anterior pituitary. Biochemistry *19* (1980) 5395–5402.

76 Hosey, M. M., and Lazdunski, M., Calcium channels: molecular pharmacology, structure and regulation. J. Membrane Biol. *104* (1988) 81–105.

77 Imura, H., Control of biosynthesis and secretion of ACTH: a review. Horm. Metab. Res. [Suppl.] *16* (1987) 1–6.

78 Jezova, D., Kvetnansky, R., Kovacs, K., Oprsalova, Z, Vigas, M., and Makara, G. B., Insulin-induced hypoglycemia activates the release of adrenocorticotropin predominantly via central and propranolol insensitive mechanisms. Endocrinology *120* (1987) 409–415.

79 Jolicoeur, P., and Labrie, F., Phosphorylation of nuclear proteins from bovine anterior pituitary gland induced by adenosine 3':5'-monophosphate. Eur. J. Biochem. *48* (1974) 1–9.

80 Jones, M. T., and Gillham, B., Factors involved in the regulation of adrenocorticotropic hormone/β-lipotropic hormone. Physiol. Rev. *68* (1988) 743–818.

81 Judd, A. M., Jarvis, W. D., and MacLeod, R. M., Attenuation of pituitary polyphosphoinositide metabolism by protein kinase C activation. Molec. cell. Endocr. *54* (1987) 107–114.

82 Kahn, D., Abrams, G. M., Zimmerman, E. A., Carraway, R., and Leeman, S. E., Neurotensin neurons in the rat hypothalamus: an immunocytochemical study. Endocrinology *107* (1980) 47–54.

83 Kaplan, N. M., The adrenal glands, in: Textbook of Endocrine Physiology, pp. 245–272. Eds J. E. Griffin and S. R. Ojeda. Oxford University Press, New York 1988.

84 Keller-Wood, M., and Bell, M. E., Evidence for rapid inhibition of ACTH by corticosteroids in dogs. Am. J. Physiol. *255* (1988) R344–R349.

85 Keller-Wood, M. E., and Dallman, M. F., Corticosteroid inhibition of ACTH secretion. Endocr. Rev. *5* (1984) 1–24.

86 King, M. S., and Baertschi, A. J., Physiological concentrations of atrial natriuretic factors with intact N-terminal sequences inhibit corticotropin-releasing factor-stimulated adrenocorticotropin secretion from cultured anterior pituitary cells. Endocrinology *124* (1989) 286–292.

87 Knepel, W., Gotz, D., and Fahrenholz, F., Interaction of rat adenohypophyseal vasopressin receptors with vasopressin analogues substituted at positions 7 and 1: dissimilarity from the V_1 vasopressin receptor. Neuroendocrinology *44* (1986) 390–396.

88 Knepel, W., Homolka, L., Vlaskovska, M., and Nutto, D., In vitro adrenocorticotropin/β-endorphin-releasing activity of vasopressin analogs is related neither to pressor nor to antidiuretic activity. Endocrinology *114* (1984) 1797–1804.

89 Knepel, W., and Meyen, G., Effect of blockers of arachidonic acid metabolism on release of beta-endorphin and adrenocorticotropin-like immunoreactivity induced by phospholipase A_2 from rat adenohypophysis in vitro. Neuroendocrinology *43* (1986) 44–48.

90 Koch, B., and Lutz-Butcher, B., Specific receptors for vasopressin in the pituitary gland: evidence for down-regulation and desensitization to adrenocorticotropin-releasing factors. Endocrinology *116* (1985) 671–676.

91 Koch, B., and Lutz-Butcher, B., Characterization and modulation of high affinity receptors for CRF in the pituitary gland. Neuroendocr. Lett. *5* (1983) 227–232.

92 Kubba, M. A., and McNicol, A. M., Stimulation of pituitary corticotrophs in the rat-ultrastructural studies. Virchows Arch. B *54* (1987) 119–126.

93 Labrie, F., Gagne, B., and Lefevre, G., Corticotropin-releasing factor stimulates adenylate cyclase activity in the anterior pituitary gland. Life Sci. *31* (1982) 1117–1121.

94 Labrie, F., Giguere, V., Proulx, L., and Lefevre, G., Interactions between CRF, epinephrine, vasopressin and glucocorticoids in the control of ACTH secretion. J. Steroid Biochem. *20* (1984) 153–160.

95 Labrie, F., Veilleux, R., Lefevre, G., Coy, D. H., Sueiras-Diaz, J., and Schally, A. V., Corticotropin-releasing factor stimulates accumulation of adenosine 3',5'-monophosphate in rat pituitary corticotrophs. Science *216* (1982) 1007–1008.

96 Larson, F. L., and Vincenzi, F. F., Calcium transport across the plasma membrane: stimulation by calmodulin. Science *204* (1979) 306–309.

97 Leong, D. A., A complex mechanism of facilitation in pituitary ACTH cells: recent single-cell studies. J. exp. Biol. *139* (1988) 151–168.

98 Leroux, P., and Pelletier, G., Radioautographic study of binding and internalization of corticotropin-releasing factor by rat anterior pituitary corticotrophs. Endocrinology *114* (1984) 14–21.

99 Levitan, I. B., Modulation of ion channels in neurons and other cells. A. Rev. Neurosci. *11* (1988) 119–136.

100 Litosch, I., and Fain, J. N., Regulation of phosphoinositide breakdown by guanine nucleotides. Life Sci. *39* (1986) 187–194.

101 Loeffler, J. P., Kley, N., Pittius, C. W., and Hollt, V., Calcium ion and cyclic adenosine 3',5'-monophosphate regulate proopiomelanocortin messenger ribonucleic acid levels in rat intermediate and anterior pituitary lobes. Endocrinology *119* (1986) 2840–2847.

102 Loeffler, J. P., Kley, N., Pittius, C. W., and Hollt, V., Corticotropin-releasing factor and forskolin increase proopiomelanocortin messenger RNA levels in rat anterior and intermediate cells in vitro. Neurosci. Lett. *62* (1985) 383–387.

103 Lowry, P. J., Silas, L., Mclean, C., Linton, E. A., and Estivariz, F. E., Pro-γ-melanocyte-stimulating hormone cleavage in adrenal gland undergoing compensatory growth. Nature *306* (1983) 70–73.

104 Lowry, P. J., Gillies, G., Hope, J., and Jackson, S., Structure and biosynthesis of peptides related to corticotrophin and lipotrophin. Hormone Res. *13* (1980) 201–210.

105 Luini, A., Lewis, D., Guild, S., Corda, D., and Axelrod, J., Hormone secretagogues increase cystolic calcium by increasing cAMP in corticotropin-secreting cells. Proc. natl Acad. Sci. *82* (1985) 8034–8038.

106 Lundblad, J. R., and Roberts, J. L., Regulation of proopiomelanocortin gene expression in pituitary. Endocr. Rev. *9* (1988) 135–158.

107 Mains, R. E., and Eipper, B. A., Synthesis and secretion of ACTH, β-endorphin, and related peptides, in: Neurosecretion and Brain Peptides, pp. 35–47. Eds J. B. Martin, S. Reichlin and K. L. Bick. Raven Press, New York 1981.

108 Marchetti, C., Childs, G. V., and Brown, A. M., Membrane currents of identified isolated rat corticotropes and gonadotropes. Am. J. Physiol. *252* (1987) E340–E346.

109 May Jr, W. S., Sahyoun, N., Wolf, M., and Cuatrecasas, P., Role of intracellular calcium mobilization in the regulation of protein kinase C-mediated membrane processes. Nature *317* (1985) 549–551.

110 McEwen, B. S., DeKloet, E. R., and Rostene, W., Adrenal steroid receptors and action in the nervous system. Physiol. Rev. *66* (1986) 1121–1188.

111 Means, A. R., and Dedman, J. R., Calmodulin – an intracellular calcium receptor. Nature *285* (1980) 73–77.

112 Meyer, T., Holowka, D., and Stryer, L., Highly cooperative opening of calcium channels by inositol 1,4,5-trisphosphate. Science *240* (1988) 653–656.

113 Michell, R. H., Inositol phospholipids in membrane function. TIBS *4* (1979) 128–131.

114 Milligan, J. V., and Kraicer, J., Physical characteristics of the Ca^{2+} compartments associated with in vitro ACTH release. Endocrinology *94* (1974) 435–443.

115 Moriarty, G. C., and Halmi, N. S., Electron microscopic study of the adrenocorticotropin-producing cell with the use of unlabeled antibody and the soluble peroxidase-antiperoxidase complex. J. Histochem. Cytochem. *20* (1972) 590–603.

116 Mulder, G. H., and Smelik, P. G., A superfusion system technique for the study of the sites of action of glucocorticoids in the rat hypothalamus-pituitary-adrenal system in vitro. I. Pituitary cell superfusion. Endocrinology *100* (1977) 1143–1152.

117 Munck, A., Guyre, P. M., and Holbrook, N. J., Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr. Rev. *5* (1984) 25–44.

118 Murakami, K., Hashimoto, K., and Ota, Z., Calmodulin inhibitors decrease CRF- and AVP-induced ACTH release in vitro: interaction of calcium-calmodulin and the cyclic AMP system. Neuroendocrinology *41* (1985) 7–12.

119 Murakami, K., Hashimoto, K., and Ota, Z., The effect of nifedipine on CRF-41 and AVP-induced ACTH release in vitro. Acta endocr. *109* (1985) 32–36.

120 Nakane, P. K., Classifications of anterior pituitary cell types with immunoenzyme histochemistry. H. Histochem. Cytochem. *18* (1970) 9–20.

121 Nakane, P. K., Setalo, G., and Mazurkiewicz, J. E., The origin of ACTH cells in rat anterior pituitary. Ann. N.Y. Acad. Sci. *297* (1977) 201–204.

122 Nakanishi, S., Teranishi, Y., Watanabe, Y., Notake, M., Noda, M., Kakidani, H., Jingami, H., and Numa, S., Isolation and characterization of the bovine corticotropin/β-lipotropin precursor gene. Eur. J. Biochem. *115* (1981) 429–438.

123 Nestler, E. J., and Greengard, P., Protein phosphorylation in the brain. Nature *305* (1983) 583–588.

124 Niimi, M., Takahara, J., Hashimoto, K., and Kawanishi, K., Immunohistochemical identification of corticotropin releasing factor-containing neurons projecting to the stalk-median eminence of the rat. Peptides *9* (1988) 589–593.

125 Nishizuka, Y., The role of protein kinase C in cell surface signal transduction and tumour promotion. Nature *308* (1984) 693–698.

126 Notake, M., Tobimatsu, T., Watanabe, Y., Takahashi, H., Mishina, M., and Numa, S., Isolation and characterization of the mouse corticotropin-β-lipotropin precursor gene and a related pseudogene. FEBS Lett. *156* (1983) 67–71.

127 Nowycky, M. C., Fox, A. P., and Tsien, R. W., Three types of neuronal calcium channel with different calcium agonist sensitivity. Nature *316* (1985) 440–443.

128 Okajima, T., and Hertting, G., Delta-sleep-inducing peptide (DSIP) inhibited CRF-induced ACTH secretion from rat anterior pituitary gland in vitro. Horm. Metab. Res. *18* (1986) 497–498.

129 Oliver, C., Mical, R. S., and Porter, J. C., Hypothalamic-pituitary vasculature: evidence for retrograde blood flow in the pituitary stalk. Endocrinology *101* (1977) 598–604.

130 Olschowka, J. A., O'Donohue, T. L., Mueller, G. P., and Jacobowitz, D. M., The distribution of corticotropin releasing factor-like immunoreactive neurons in rat brain. Peptides *3* (1982) 995–1015.

131 Osbourne, N. N., Tobin, A. B., and Ghazi, H., Role of inositol trisphosphate as a second messenger in signal transduction processes: an essay. Neurochem. Res. *13* (1988) 177–191.

132 Paglin, S., Stukenbrok, H., and Jamieson, J. D., Interaction of angiotensin II with dispersed cells from the anterior pituitary of the male rat. Endocrinology *114* (1984) 2284–2292.

133 Palade, G., Intracellular aspects of the process of protein synthesis. Science *189* (1975) 347–358.

134 Perrin, M. H., Haas, Y., Rivier, J., and Vale, W. W., Corticotropin-releasing factor binding to the anterior pituitary receptor is modulated by divalent cations and guanyl nucleotides. Endocrinology *118* (1986) 1171–1179.

135 Plotsky, P. M., Bruhn, T. O., and Vale, W., Hypophysiotropic regulation of adrenocorticotropin secretion in response to insulin-induced hypoglycemia. Endocrinology *117* (1985) 323–329.

136 Putney Jr, J. W., The role of phosphoinositide metabolism in signal transduction in secretory cells. J. exp. Biol. *139* (1988) 135–150.

137 Rasmussen, H., and Barrett, P. Q., Calcium messenger system: an integrated review. Physiol. Rev. *64* (1984) 938–984.

138 Raymond, V., Leung, P. C. K., Veilleux, R., and Labrie, F., Vasopressin rapidly stimulates phosphatidic acid-phosphatidylinositol turnover in rat anterior pituitary cells. FEBS Lett. *182* (1985) 196–200.

139 Reisine, T., Neurohumoral aspects of ACTH release. Hosp. Pract. *23* (1988) 77–96.

140 Reisine, T., Regulation of adrenocorticotropin release from the anterior pituitary. Psychopharmac. Bull. *21* (1985) 438–442.

141 Reisine, T., Rougon, G., and Barbet, J., Liposome delivery of cyclic AMP-dependent protein kinase inhibitor into intact cells: specific blockade of cyclic AMP-mediated adrenocorticotropin release from mouse anterior pituitary tumor cells. J. Cell Biol. *102* (1986) 1630–1637.

142 Reisine, T., Rougon, G., Barbet, J., and Affolter, H.-U., Corticotropin-releasing factor-induced adrenocorticotropin hormone release and synthesis is blocked by incorporation of the inhibitor of cyclic AMP-dependent protein kinase into anterior pituitary tumor cells by liposomes. Proc. natl Acad. Sci. USA *82* (1985) 8261–8265.

143 Reisine, T., Zhang, Y.-L., and Sekura, R. D., Pertussis toxin blocks the inhibition of somatostatin and increases the stimulation by forskolin of cyclic AMP accumulation and adrenocorticotropin secretion from mouse anterior pituitary tumor cells. J. Pharmac. exp. Ther. *232* (1985) 275–282.

144 Reuter, H., Calcium channel modulation by neurotransmitters, enzymes and drugs. Nature *301* (1983) 569–573.

145 Rhodes, C. H., Morrell, J. I., and Pfaff, D. W., Immunohistochemical analysis of magnocellular elements in rat hypothalamus: distribution and numbers of cells containing neurophysin, oxytocin, and vasopressin. J. comp. Neur. *198* (1981) 45–64.

146 Richardson, U. I., and Schonbrunn, A., Inhibition of adrenocorticotropin secretion by somatostatin in pituitary cells in culture. Endocrinology *108* (1981) 281–290.

147 Rivier, C., Rivier, J., and Vale, W., Inhibition of adrenocorticotropic hormone secretion in the rat by immunoneutralization of corticotropin-releasing factor. Science *218* (1982) 377–378.

148 Rivier, C., and Vale, W., Modulation of stress-induced ACTH release by corticotropin-releasing factor, catecholamines and vasopressin. Nature *305* (1983) 325–327.

149 Rivier, C. L., and Plotsky, P. M., Mediation by corticotropin releasing factor (CRF) of adenohypophysial hormone secretion. A. Rev. Physiol. *48* (1986) 475–494.

150 Roberts, J. L., and Herbert, E., Characterization of a common precursor to corticotropin and β-lipotropin: cell-free synthesis of the precursor and identification of corticotropin peptide in the molecule. Proc. natl Acad. Sci. *74* (1977) 4826–4830.

151 Roberts, J. L., Phillips, M., Rosa, P. A., and Herbert, E., Steps involved in the processing of common precursor forms of adrenocorticotropin and endorphin in cultures of mouse pituitary cells. Biochemistry *17* (1978) 3609–3618.

152 Rodbell, M., The role of hormone receptors and GTP-regulatory proteins in membrane transduction. Nature *284* (1980) 17–22.

153 Rougon, G., Barbet, J., and Reisine, T., Protein phosphorylation induced by phorbol esters and cyclic AMP in anterior pituitary cells: possible role in adrenocorticotropin release and synthesis. J. Neurochem. *52* (1989) 1270–1278.

154 Sawchenko, P. E., Adrenalectomy-induced enhancement of CRF and vasopressin immunoreactivity in parvocellular neurosecretory neurons: anatomic, peptide, and steroid specificity. J. Neurosci. *7* (1987) 1093–1096.

155 Sawchenko, P. E., Swanson, L. W., and Vale, W. W., Co-expression of corticotropin-releasing factor and vasopressin immunoreactivity in parvocellular neurosecretory neurons of the adrenalectomized rat. Proc. natl Acad. Sci. *81* (1984) 1883–1887.

156 Sawchenko, P. E., Swanson, L. W., and Vale, W. W., Corticotropin-releasing factor: co-expression within distinct subsets of oxytocin-, vasopressin-, and neurotensin-immunoreactive neurons in the hypothalamus of the male rat. J. Neurosci. *4* (1984) 1118–1129.

157 Sayers, G., Hanzman, E., and Bodanszky, M., Hypothalamic peptides influencing secretion of ACTH by isolated adenohypophysial cells. FEBS Lett. *116* (1980) 236–238.

158 Sayers, G., and Portanova, R., Secretion of ACTH by isolated anterior pituitary cells: kinetics of stimulation by corticotropin-releasing factor and of inhibition by corticosterone. Endocrinology *94* (1974) 1723–1730.

159 Schoenenberg, P., Kehrer, P., Muller, A. F., and Gaillard, R. C., Angiotensin II potentiates corticotropin-releasing activity of CRF_{41} in rat anterior pituitary cells: mechanism of action. Neuroendocrinology *45* (1987) 86–90.

160 Siperstein, E. R., and Miller, K. J., Further cytophysiologic evidence for the identity of the cells that produce adrenocorticotrophic hormone. Endocrinology *86* (1970) 451–486.

161 Siperstein, E. R., and Miller, K. J., Hypertrophy of the ACTH-producing cell following adrenalectomy: a quantitative electron microscopic study. Endocrinology *93* (1973) 1257–1268.

162 Sobel, D. O., Characterization of 12-O-tetradecanoyl-phorbol-13 acetate mediated ACTH release. Endocr. Res. *14* (1988) 149–163.

163 Sobel, D. O., Characterization of PGE_2 inhibition of corticotropin releasing factor-mediated ACTH release. Brain Res. *411* (1987) 102–107.

164 Sobel, D. O., Role of cyclic AMP in corticotropin releasing factor mediated ACTH release. Peptides *6* (1985) 591–595.

165 Sobel, D. O., The role of calcium in the mechanism of corticotropin releasing factor mediated ACTH release. Peptides *7* (1986) 443–448.

166 Spinedi, E., and Negro-Vilar, A., Arginine vasopressin and adrenocorticotropin release: correlation between binding characteristics and biological activity in anterior pituitary dispersed cells. Endocrinology *114* (1984) 2247–2251.

167 Stillman, M. A., Recht, L. D., Rosario, S. L., Seif, S. M., Robinson, A. G., and Zimmerman, E. A., The effects of adrenalectomy and glucocorticoid replacement on vasopressin and vasopressin-neurophysin in the zona externa of the median eminence of the rat. Endocrinology *101* (1977) 42–49.

168 Streb, H., Irvine, R. F., Berridge, M. J., and Shulz, I., Release of Ca^{2+} from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol-1,4,5-trisphosphate. Nature *306* (1983) 67–68.

169 Suda, T., Tozawa, F., Yamada, M., Ushiyama, T., Tomori, N., Sumitomo, T., Nakagami, Y., and Shizume, K., In vitro study on proopiomelanocortin messenger RNA levels in cultured rat anterior pituitary cells. Life Sci. *42* (1988) 1147–1152.

170 Sutherland, E. W., and Rall, T. W., Fractionation and characterization of a cyclic adenine ribonucleotide formed by tissue particles. J. biol. Chem. *232* (1958) 1077–1091.

171 Sutherland, E. W., Rall, T. W., and Menon, T., Adenyl cyclase: I. Distribution, preparation and properties. J. biol. Chem. *237* (1962) 1220–1227.

172 Sutko, J. L., Ito, K., and Kenyon, J. L., Ryanodine: a modifier of sarcoplasmic reticulum calcium release in striated muscle. Fedn. Proc. *44* (1985) 2984–2988.
173 Swanson, L. W., Sawchenko, P. E., and Vale, W. W., Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. Neuroendocrinology *36* (1983) 165–186.
174 Syndor, K. L., and Sayers, G., Blood and pituitary ACTH in intact and adrenalectomized rats after stress. Endocrinology *55* (1954) 621–636.
175 Takahashi, H., Hakamata, Y., Watanabe, Y., Kikuno, R., Miyata, T., and Numa, S., Complete nucleotide sequence of the human corticotropin-β-lipotropin precursor gene. Nucleic Acids Res. *11* (1983) 6847–6858.
176 Takai, Y., Kishimoto, A., Iwasa, Y., Kawahara, Y., Mori, T., and Nishizuka, Y., Calcium-dependent activation of a multifunctional protein kinase by membrane phospholipids. J. biol. Chem. *254* (1979) 3692–3695.
177 Teo, T. S., and Wang, J. H., Mechanisms of activation of a cyclic adenosine 3':5'-monophosphate phosphodiesterase from bovine heart by calcium ions. J. biol. Chem. *248* (1973) 5950–5955.
178 Tilders, F. J. H., Berkenbosch, F., Vermes, I., Linton, E. A., and Smelick, P. G., Role of epinephrine and vasopressin in the control of the pituitary-adrenal response to stress. Fedn Proc. *44* (1985) 155–160.
179 Todd, K., and Lightman, S. L., Vasopressin activation of phosphatidylinositol metabolism in rat anterior pituitary in vitro and its modification by changes in the hypothalamo-pituitary-adrenal axis. Neuroendocrinology *45* (1987) 212–218.
180 Tsunoo, A., Yoshii, M., and Narahashi, T., Differential block of two types of calcium channels in neuroblastoma cells. Biophys. J. *47* (1985) 433a.
181 Uhler, M., and Herbert, E., Complete amino acid sequence of mouse pro-opiomelanocortin derived from the nucleotide sequence of pro-opiomelanocortin cDNA. J. biol. Chem. *258* (1983) 257–261.
182 Vale, W., and Rivier, C., Substances modulating the secretion of ACTH by cultured anterior pituitary cells. Fedn Proc. *36* (1977) 2094–2099.
183 Vale, W., Rivier, C., Yang, L., Minick, S., and Guillemin, R., Effects of purified hypothalamic corticotropin-releasing factor and other substances on the secretion of adrenocorticotropin and β-endorphin-like immunoreactivities in vitro. Endocrinology *103* (1978) 1910–1915.
184 Vale, W., Spiess, J., Rivier, C., and Rivier, J., Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and β-endorphin. Science *213* (1981) 1394–1397.
185 Vale, W., Vaughan, J., Smith, M., Yamamoto, G., Rivier, J., and Rivier, C., Effects of synthetic ovine corticotropin-releasing factor, glucocorticoids, catecholamines, neurohypophysial peptides, and other substances on cultured corticotropic cells. Endocrinology *113* (1983) 1121–1130.
186 Vandesande, F., De Mey, J., and Dierickx, K., Identification of neurophysin producing cells. I. The origin of the neurophysin-like substance-containing nerve fibers of the external region of the median eminence of the rat. Cell Tiss. Res. *151* (1974) 187–200.
187 Vandersande, F., Dierickx, K., and De Mey, J., The origin of vasopressinergic and oxytocinergic fibers of the external region of the median eminence of the rat hypophysis. Cell Tiss. Res. *180* (1977) 443–452.
188 Vlaskovska, M., and Knepel, W., Beta-endorphin and adrenocorticotropin release from rat adenohypophysis in vitro: evidence for local modulation by aracidonic acid metabolites of the cyclooxygenase and lipoxygenase pathway. Neuroendocrinology *39* (1984) 334–342.
189 Wand, G. S., and Eipper, B. A., Effect of chronic secretagogue exposure on pro-adrenocorticotropin/endorphin production and secretion in primary cultures of rat anterior pituitary. Endocrinology *120* (1987) 953–961.
190 Wand, G. S., May, V., and Eipper, B. A., Comparison of acute and chronic secretagogue regulation of proadrenocorticotropin/endorphin synthesis, secretion, and messenger ribonucleic acid production in primary cultures of rat anterior pituitary. Endocrinology *123* (1988) 1153–1161.
191 Wang, J. H., and Waisman, D. M., Calmodulin and its role in the second-messenger system. Cur. Topics cell. Regul. *15* (1979) 47–107.
192 Watanabe, T., and Orth, D. N., Detailed kinetic analysis of adrenocorticotropin secretion by dispersed rat anterior pituitary cells in a microperifusion system: effects of corticotropin-releasing factor and arginine vasopressin. Endocrinology *121* (1987) 1133–1145.
193 Westendorf, J. M., and Schonbrunn, A., Peptide specificity for stimulation of corticotropin secretion: activation of overlapping pathways by the vasoactive intestinal peptide family and corticotropin-releasing factor. Endocrinology *116* (1985) 2528–2535.
194 Westlund, K. N., Aguilera, G., and Childs, G. V., Quantification of morphological changes in pituitary corticotropes produced by in vitro corticotropin-releasing factor stimulation and adrenalectomy. Endocrinology *116* (1985) 439–445.
195 Westlund, K. N., Wynn, P. C., Chmielowiec, S., Collins, T. J., and Childs, G. V., Characterization of a potent biotinconjugated CRF analog and the responses of anterior pituitary corticotropes. Peptides *5* (1984) 627–634.
196 Whitnall, M. H., and Gainer, H., Major pro-vasopressin-expressing and pro-vasopressin-deficient subpopulations of corticotropin-releasing hormone neurons in normal rats. Neuroendocrinology *47* (1988) 176–180.
197 Whitnall, M. H., Mezey, E., and Gainer, H., Co-localization of corticotropin-releasing factor and vasopressin in median eminence neurosecretory vesicles. Nature *317* (1985) 248–250.
198 Whitnall, M. H., Smyth, D., and Gainer, H., Vasopressin coexists in half of the corticotropin-releasing factor axons in the external zone of the median eminence in normal rats. Neuroendocrinology *45* (1987) 420–424.
199 Widmaier, E. P., and Dallman, M. F., The effects of corticotropin-releasing factor on adrenocorticotropin secretion from perifused pituitaries in vitro: rapid inhibition by glucocorticoids. Endocrinology *115* (1984) 2368–2374.
200 Widmaier, E. P., Lim, A. T., and Vale, W., Secretion of corticotropin-releasing factor from cultured rat hypothalamic cells: effects of catecholamines. Endocrinology *124* (1989) 583–590.
201 Wise, B. C., Glass, D. B., Chou, C.-H. J., Raynor, R. L., Katoh, N., Schatzman, R. C., Turner, R. S., Kibler, R. F., and Kuo, J. F., Phospholipid-sensitive Ca^{2+}-dependent protein kinase from heart. II. substrate specificity and inhibition by various agents. J. biol. Chem. *257* (1982) 8489–8495.
202 Wolfe, L. S., Eicosanoids: prostaglandins, thromboxanes, leukotrienes, and other derivatives of carbon-20 unsaturated fatty acids. J. Neurochem. *38* (1982) 1–14.
203 Worley, P. F., Baraban, J. M., and Snyder, S. H., Beyond receptors: multiple second-messenger systems in brain. Ann. Neurol. *21* (1987) 217–229.
204 Wynn, P. C., Aguilera, G., Morell, J., and Catt, K. J., Properties and regulation of high affinity pituitary receptors for corticotropin-releasing factor. Biochem. biophys. Res. Commun. *110* (1983) 602–608.
205 Zimmerman, E. A., Stillman, M. A., Recht, L. D., Antunes, J. L., and Carmel, P. W., Vasopressin and corticotropin-releasing factor: an axonal pathway to portal capillaries in the zona externa of the median eminence containing vasopressin and its interaction with adrenal corticoids. Ann. N.Y. Acad. Sci. *297* (1977) 405–419.
206 Zimmerman, G., and Fleischer, N., Role of calcium ions in the release of ACTH from rat pituitary tissue in vitro. Endocrinology *87* (1970) 426–429.

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