Together with a transient accumulation of intracellular cAMP, thyrotropin (TSH) stimulation of the FRTL-5 thyroid cell induces phosphorylation and activation of a cAMP-specific phosphodiesterase (PDE4D3). Here we have investigated the impact of PDE4D3 activation on hormone responsiveness. Stimulation of FRTL-5 cells with TSH caused an increase in PDE activity within 3 min, with a maximal stimulation reached after 5 min. Preincubation with the protein kinase A (PKA) inhibitor H89 or (R)35-cAMPS, but not with the inactive isomer H85, blocked this activation. Preincubation with PKA inhibitors also blocked the shift in mobility of the PDE4D3 protein. Under these conditions, H89, but not H85, potentiated the cAMP accumulation induced by TSH. Incubation of FRTL-5 cells with the PKA activator 8-(4-chlorophenylthio)adenosine-cAMP caused an increase in PDE activity and a decrease in the endogenous cAMP, confirming the presence of a PKA-PDE feedback loop. MA-10 Leydig tumor cells stably transfected with either a wild type PDE4D3 or a PDE4D3 with mutations in the PKA phosphorylation sites showed an increase in PDE activity when compared with control cells. Human choriogonadotropin or Bt2cAMP treatment induced a PDE activity when compared with control cells. Human in the PKA phosphorylation sites showed an increase in either a wild type PDE4D3 or a PDE4D3 with mutations in the PKA phosphorylation sites.

Together with a transient accumulation of intracellular cAMP, thyrotropin (TSH) stimulation of the FRTL-5 thyroid cell induces phosphorylation and activation of a cAMP-specific phosphodiesterase (PDE4D3). Here we have investigated the impact of PDE4D3 activation on hormone responsiveness. Stimulation of FRTL-5 cells with TSH caused an increase in PDE activity within 3 min, with a maximal stimulation reached after 5 min. Preincubation with the protein kinase A (PKA) inhibitor H89 or (R)35-cAMPS, but not with the inactive isomer H85, blocked this activation. Preincubation with PKA inhibitors also blocked the shift in mobility of the PDE4D3 protein. Under these conditions, H89, but not H85, potentiated the cAMP accumulation induced by TSH. Incubation of FRTL-5 cells with the PKA activator 8-(4-chlorophenylthio)adenosine-cAMP caused an increase in PDE activity and a decrease in the endogenous cAMP, confirming the presence of a PKA-PDE feedback loop. MA-10 Leydig tumor cells stably transfected with either a wild type PDE4D3 or a PDE4D3 with mutations in the PKA phosphorylation sites showed an increase in PDE activity when compared with control cells. Human choriogonadotropin or Bt2cAMP treatment induced a stimulation of PDE activity in cells transfected with wild type PDE4D3, whereas the activation was absent in mutant- and control-transfected cells. The increase in cAMP accumulation elicited by human choriogonadotropin was reduced in cells transfected with the wild type PDE4D3, but not in cells transfected with the mutant PDE. Rolipram, a specific inhibitor of PDE4, restored the cAMP accumulation in the PDE4D3-transfected cells. These data provide evidence that a rapid activation of PDE4D3 is one of the mechanisms determining the intensity of the cAMP signal.

Hormone or neurotransmitter signaling is mediated by transient fluctuations in intracellular cAMP within a very narrow range of concentrations (1). Maximal biological effects are elicited with only 2–3-fold changes in intracellular cAMP levels, while the cell potential for cAMP production is usually much greater. In addition, the increase in intracellular cAMP is transient despite the continuous presence of the extracellular stimulus (2, 3). This limited and short-lived nature of the activating signal is an essential feature of hormone or neurotransmitter action. This is necessary to decrease the intrinsic “noise” in the signaling mechanism, to allow iterative signaling, and to prevent excessive stimulation.

The rapid and transient changes in cAMP concentrations are the result of changes in both synthesis and degradation of the second messenger cAMP, involving steps at the receptor as well as at a postreceptor level (4, 5). Following the activation of $G_s$ and adenylyl cyclase, receptor phosphorylation causes an uncoupling from $G_s$, and therefore a decrease in cAMP synthesis. For the $\beta_2$-adrenergic receptor, $\beta_2$-adrenergic receptor kinases phosphorylate only agonist-occupied active receptors and enhance the affinity of the receptors for the inhibitor protein $\beta$-arrestin (6). Binding of $\beta$-arrestin to the phosphorylated receptors inhibits the receptor-$G_s$ interaction, thereby inducing the uncoupled or desensitized state of the receptors (7). Several kinases have also been implicated in the phosphorylation of the glycoprotein receptors including the GRK kinases, PKA (4), and PKC. While PKA efficiently phosphorylates and uncouples the $\beta$-adrenergic receptor (5), the involvement of this kinase in the glycoprotein receptor phosphorylation is less clear (8).

In addition to receptor uncoupling from $G$ protein and cyclic AMP-modulated phosphodiesterases and of cAMP degradation plays an essential role in the transient accumulation of cyclic nucleotides (9). This concept was initially inferred by the use of xanthine inhibitors of PDEs and by measuring the decay of the cAMP signal in intact cells (3). The use of cAMP analogs has confirmed that cAMP-dependent PKA activation in the cell causes an activation of cAMP degradation (10–12). The impact and physiological significance of this rapid feedback regulation is unclear.

Of the many PDEs expressed in the cell, two isoforms are activated by an increase in cAMP. In platelets (13, 14) and adipocytes (15, 16) a type 3 PDE is activated by a PKA-dependent phosphorylation lowering cAMP levels. Recently, a distinct PDE isoenzyme, a member of the PDE4 family, has been implicated in this feedback regulation (17). In thyroid cells, TSH causes a PKA-mediated phosphorylation and activation of a PDE4D3 variant. This conclusion is supported by studies involving PDE4-specific inhibitors and immunoprecipitation with PDE4-selective antibodies (17), and is consistent with cell-free phosphorylation and activation of the recombinant PDE4D3 enzymes (18). The site of PKA phosphorylation of PDE4D3 has been mapped to the amino terminus of PDE4D3 where Ser13 and Ser54 are the predominant phosphorylation sites. Only Ser54 phosphorylation, however, is essential for...
activation of cAMP hydrolysis (18). Similar PDE4 activation has been described in myoblasts (19), aortic smooth muscle cells (20), and osteoclasts (21), indicating the ubiquitous nature of this feedback regulation.

While the impact of overall PDE4 activity on cAMP levels during cell stimulation can be assessed using PDE4-selective inhibitors, the exact role of PDE4D activation during hormonal stimulation has not been explored. Here, we have taken advantage of different strategies to distinguish between the effect of basal and activated PDE on cAMP accumulation and determine how the increase in PDE4D activity affects the TSH-dependent responses of FRTL-5 cells. We demonstrate that PDE4D3 activation has a major impact on the CAMP response by controlling the intensity of the CAMP signal.

**EXPERIMENTAL PROCEDURES**

**Materials—**Coon’s modified Ham’s F-12 medium (Coon’s F-12), bovine TSH, bovine insulin, human transferrin for culture, and *Crotalus atrox* snake venom were purchased from Sigma; bovine TSH, bovine FSH, and hCG for stimulation were obtained from the National Hormone and Pituitary Agency of the NIDDK, National Institutes of Health; Pansorbin cells from Calbiochem (La Jolla, CA); Immobilon membrane from Millipore Corp. (Bedford, MA); radioactive compounds from NEN Life Science Products Inc. (Boston, MA); AG 1-X8 resin from Bio-Rad; CAMP analogs from BioLog Life Science Institute (Bremen, Germany); ECL Western blot detection kit from Amersham Pharma Biotech. Rolipram (4-(3-butoxy-4-methoxybenzyl)imidazol-2-one) was provided by Shering AG (Berlin, Germany). Unless otherwise designated, all other chemicals were the purest grade available from Sigma.

**Cell Culture—**FRTL-5 cells (ATCC number CRL8305), a line of rat thyroid follicular cells developed by Dr. F. S. Ambesi-Impimbiotta et al. (22), were generously provided by Dr. Leonard Kohn (Section of Cell Regulation, NIDDK, National Institutes of Health) and the Interthyroid Research Foundation (Baltimore, MD). Cells were routinely cultured in Coon’s F-12 medium supplemented with 10% FCS, 1% serum from NIDDK, 0.5% antibiotics, and 0.5% fungizone (Waymouth MB752/1 modified to contain 1.12 g/liter of sodium pyrophosphate, 1 mM pyrophosphate, 1 mM ethanol, 1 mM microcystin, 50 mM benzamidine, 0.5 mM leupeptin, 0.7 mM pepstatin, 4 mM aprotinin, 10 mM soybean trypsin inhibitor, and 2 mM phenylmethylsulfonyl fluoride. PDE activators were used as substrates according to the method of Thompson and Appleman (26) as detailed previously (24).

Samples were assayed in a total volume of 200 μl of reaction mixture containing 40 mM Tris-HCl, pH 8.0, 1 mM MgCl₂, 1.25 mM 2-mercaptoethanol, 1 μM cAMP, 0.1 mg/ml bovine serum albumin, and [³H]cAMP (0.1 μCi/tube). In some experiments, serial dilutions (1 μl to 10 μl) of Rolipram were added to the reaction mixture. After incubation at 34 °C for 60 min, the reaction was terminated by adding an equal volume of 40% methanol, and the cell extracts were dried and assayed by Radiiodination using 35S-labeled cAMP as substrate according to the method of Thompson and Appleman (26) as detailed previously (24).

**Immunoassay—**FRTL-5 cell extracts in RIPA buffer were immunoprecipitated using fixed *Staphylococcus aureus* cells (Pansorbin) according to the method of Macphie with minor modifications (28). Samples were incubated with Pansorbin and an anti-CAMP-PDE antiserum (K116) (29). The bound PDE was eluted from the pellet by incubation with 1% SDS in PBS at room temperature. Eluted samples were diluted in 4× sample buffer (62.5 mM Tris-HCl, pH 6.8, 10% glycerol, 2% (w/v) SDS, 0.7 M 2-mercaptoethanol, and 0.0025% (w/v) bromophen blue). The samples were boiled for 5 min and proteins were separated on an 8% SDS-polyacrylamide gel. The proteins were transferred to an Immobilon membrane, and non-specific binding was blocked by incubating the membrane overnight in 5% bovine serum albumin (w/v) dissolved in TBS-T solution (0.2% Tween 20, 20 mM Tris-HCl, 14 mM NaCl, pH 7.6). The following day, the membrane was incubated in a monoclonal antibody (M351) raised against the carboxyl-terminal region of rat PDE4D3 (29), diluted 1:100 (v/v) in TBS-T for 1 h, washed extensively for 1 h with multiple changes of TBS-T, and incubated for 1 h with peroxidase-conjugated secondary antibody (Amersham Pharmacia Biotech) diluted 1:7500 in TBS-T. After multiple washes in TBS-T for at least 1 h, bound antibodies were detected using a luminescence method (ECL, Amersham Pharmacia Biotech) and recorded after exposure to XAR-5 x-ray film (Eastman Kodak, Rochester, NY).

**cAMP Level Determination—**FRTL-5 cells were seeded in 35-mm dishes in Coon’s F-12 medium containing 5% serum and three hormones. After 5–7 days, cell monolayers were rinsed twice with Hanks’ balanced salt solution, and cultures were continued for an additional 24 h with Coon’s F-12 medium containing 0.1% bovine serum albumin to induce cell quiescence. The medium was changed 1 h before the experiment. MA-10 cells were plated in growth medium (see above). The medium was replaced on day 2 and the experiments were performed on day 3. On the day of the experiment, cells were washed 4 times with warm assay medium (Waymouth MB752/1 modified to contain 1.12 g/liter of NaHCO₃, 20 mM Hepes, 1 mg/ml bovine serum albumin, pH 7.4). At the end of the experiment, the cell monolayers were rinsed once with PBS solution, then homogenized in ice-cold buffer containing 20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 0.2 mM EGTA, 50 mM sodium fluoride, 10 mM sodium pyrophosphate, 1 μg/ml microcin, 50 μM benzamidine, 0.5 μM leupeptin, 0.7 μM pepstatin, 4 μM aprotinin, 10 μM soybean trypsin inhibitor, and 2 mM phenylmethylsulfonyl fluoride. PDE activity was measured using 1 μM cAMP as substrate according to the method of Thompson and Appleman (26) as detailed previously (24).

While the impact of overall PDE4 activity on cAMP levels during cell stimulation can be assessed using PDE4-selective inhibitors, the exact role of PDE4D activation during hormonal stimulation has not been explored. Here, we have taken advantage of different strategies to distinguish between the effect of basal and activated PDE on cAMP accumulation and determine how the increase in PDE4D activity affects the TSH-dependent responses of FRTL-5 cells. We demonstrate that PDE4D3 activation has a major impact on the CAMP response by controlling the intensity of the CAMP signal.
Time course of the TSH-dependent shift in the electrophoretic mobility of the PDE4D3. Cells were incubated for different times with 10 nM TSH. At the end of the incubation, cells were harvested in RIPA buffer and the PDE protein was immunoprecipitated with the PDE4D-specific monoclonal antibody. SDS-PAGE and blotting, the PDE proteins were detected with a PDE4D-specific monoclonal antibody. The film was digitized by scanning and the intensity of the immunoreactive bands was quantitated by the NIH Image analysis. The plot of the signal intensity for each lane is reported in panel B in arbitrary units.

Preparation of FRTL-5 Cell Extracts for Sephadex G-25 Purification of cAMP—8-CPT-cAMP used for cell stimulation was first repurified by gel filtration chromatography on a 1 × 17-cm Sephadex G-25 (superfine) column. Cells were stimulated with purified 8-CPT-cAMP for 30 min at 30 °C and incubation was terminated by the addition of trichloroacetic acid (0.1%) in 95% EtOH. Cell extracts were centrifuged at 3,000 rpm at 4 °C. EtOH in the supernatant was evaporated using vacuum centrifugation at room temperature and the material was reconstituted with 50 mM NH4HCO3, pH 7.8. The sample was applied at 4 °C to a 1 × 45-cm Sephadex G-25 (superfine) column equilibrated in 50 mM NH4HCO3, pH 7.8, and 0.5-ml fractions/min were collected (12). Fractions corresponding to cAMP were evaporated and reconstituted with PBS solution, pH 7.4, and cAMP content was measured by RIA. Recovery of [3H]cAMP from the columns was approximately 50%. Protein Assay—Protein was measured by the method of Bradford (32) or Lowry (40) using bovine serum albumin or γ-globulin as a standard, respectively.

RESULTS

Time Course of PDE Activation and Phosphorylation in FRTL-5 Cells—The PDE activity expressed in FRTL-5 cells is subject to feedback activation on cAMP levels in FRTL-5 cells. FRTL-5 cultures were treated with 10 μM H89 or 10 μM H85 for 1 h prior to and during the TSH stimulation and cAMP levels were determined. H89 treatment potentiated the TSH-dependent cAMP accumulation, but H85 had no effect (Fig. 4). A detailed time course showed that TSH stimulation was maximal within 5 min, and the activity remained elevated for at least 30 min (see below). We have previously reported that this PDE activation is associated with phosphorylation and a shift in electrophoretic mobility of the PDE4D3 protein (18). A detailed time course study showed that a polypeptide with an apparent Mr of 93 was present in unstimulated cells, and incubation with TSH caused the appearance of an additional band with Mr, of 96 (Fig. 1, left panel). The appearance of the 98-kDa species was associated with a decrease in the intensity of the 93-kDa band (Fig. 1, right panel). Thus, PDE4D3 remained phosphorylated for at least 30 min after stimulation and the shift in mobility was consistent with the increase in activity. Protein Kinase A Inhibition Blocks PDE4D3 Phosphorylation and Activation—To test the role of PKA on activation of PDE, quiescent FRTL-5 cells were preincubated for 1 h in the absence or presence of either 10 μM H89, a PKA-specific inhibitor, or 1 mM (R)-3-CAMPS, a PKA antagonist. At the end of this preincubation, cells were stimulated with 10 nM TSH for 5 min, and PDE activity was measured in the cell homogenate (Table I). TSH stimulation of PDE was blocked by either H89 or (R)-3-CAMPS. Treatment with H89 completely obliterated the TSH-stimulated PDE activity at all time points studied (Fig. 2).

To determine whether the PKA inhibition is associated with a change in mobility of the PDE4D3 expressed in FRTL-5 cells,
of cAMP/mg of protein). This latter finding suggests that the H89 effect was not due to changes in the rate of cAMP synthesis.

Sertoli and FRTL-5 cells often have been compared because they provide similar models with which to study peptide hormone receptor activation of target cells. While TSH produces a rapid activation of a PDE in FRTL-5 cells, FSH does not stimulate PDE activity in a short term fashion in immature Sertoli cells. This difference is due to the fact that PDE4D3, the target for PKA activation in FRTL-5 cells, is undetectable in immature Sertoli cells (34). Thus, Sertoli cells were used to determine whether H89 treatment potentiated the hormone-dependent cAMP accumulation in the absence of a short term PDE activation. Fig. 6 shows that H89 did not potentiate the FSH stimulation of cAMP in the Sertoli cells during this short term incubation. No change or a decrease in cAMP accumulation was observed with H89 after 30 and 60 min (Fig. 6). This finding indicates that H89 was ineffective in increasing intracellular cAMP when PDE4D3 activation was absent.

The cAMP Analog 8-CPT-cAMP Lowers Intracellular cAMP in FRTL-5 Cells—According to the feedback model, a direct activation of PKA bypassing the adenylyl cyclase step should produce a decrease in cAMP in the cell. This possibility was tested by incubating FRTL-5 cells with cAMP analogs that can be separated from the endogenous cAMP (35). Given its chromatographic properties, 8-CPT-cAMP can easily be separated from cAMP on a G-25 gel filtration column (10). In a preliminary experiment, FRTL-5 cells were incubated without or with different concentrations of 8-CPT-cAMP for 20
min, then PDE activity was measured. Consistent with data obtained with TSH and dibutyryl cAMP, 8-CPT-cAMP treatment caused an increase in PDE activity (control, 96.50 ± 2.1 pmol/min/mg; 0.85 μM 8-CPT-cAMP, 132.17 ± 4.55 pmol/min/mg; 8.5 μM 8-CPT-cAMP, 154.51 ± 3.33 pmol/min/mg). When endogenous cAMP was extracted and measured after 8-CPT treatment of the FRTL-5 cells, a significant decrease in cAMP concentration was observed (Fig. 7). The decrease was dependent on the concentration of the analog used. Thus, this observation further confirms the presence of a feedback loop in FRTL-5 cells regulating cAMP and involving PKA phosphorylation of PDE4D3.

Impact of PDE4D3 Phosphorylation on PDE Activity and cAMP Accumulation—To further investigate the impact of short-term stimulation of PDE4D3, a reconstitution system where PDE4D3 is expressed in a hormone responsive cell was used. A Leydig tumor cell line (MA-10, see Ref. 29) does not express PDE4D mRNAs or protein. These cells express trace amounts of PDE4A, which is not activated by LH/hCG either in a short term or long term fashion (36). Using an expression vector that codes for rat wild type PDE4D3 or PDE4D3 mutant (in which two PKA-phosphorylation sites, Ser13 and Ser54, are substituted with Ala (18), stable transfectant cell lines expressing these two cAMP-PDEs were generated. After clonal selection in the presence of G418, cells were stimulated with hCG or Bt2cAMP, and PDE activity was measured in the cell homogenates. As expected, mock-transfected cells did not show any changes in PDE activity after stimulation with either hCG or Bt2cAMP. Conversely, hCG or Bt2cAMP treatment increased PDE activity in cells transfected with PDE4D3 wild type cDNA (Fig. 8). Incubation with Rolipram prior to hCG stimulation potentiated the PDE activation. In cells transfected with mutant PDE4D3, the basal PDE activity was 2-fold higher than that in control cells but the stimulation by hCG or Bt2cAMP was absent. Western blot analysis demonstrated that similar amounts of an immunoreactive peptide of 93 kDa was expressed in the PDE4D3 WT and in the PDE4D3 mutant cell line, but not in the control cell line (Fig. 8, inset). Moreover, Bt2cAMP treatment caused a shift in the mobility of the PDE4D3 wild type protein but not of the mutant PDE4D3 (data not shown). Thus, the short term hormone-dependent PDE activation can be transferred to a heterologous cell by the transfection of PDE4D3.

In order to determine the impact of PDE4D3 activation on cAMP accumulation, the transfected cells were incubated with hCG for 15 min and the intracellular cAMP levels were measured. The hCG-dependent cAMP accumulation was impaired in cells transfected with wild type PDE4D3 (Fig. 9), while cells transfected with PDE4D3 mutant retained the same responsiveness of control cells. Furthermore, treatment with Rolipram reverted the phenotype of the cells, and in the presence of the PDE inhibitors, all three cell lines were equally responsive to hCG (Fig. 9). These data further demonstrate that activation of PDE4D3 in a cell has a major impact on hormone-dependent
cAMP accumulation. Since the phenotype was reverted by Rolipram, we could also conclude that the reduction in response is due to the transfected PDE and not to unrelated adaptive mechanisms induced during the selection of the cell lines.

**DISCUSSION**

The studies reported herein demonstrate the presence of a feedback loop that controls cAMP levels in FRTL-5 thyroid cells and involves phosphorylation of a PDE4. Following TSH receptor occupancy and activation of adenylyl cyclase, an increase in intracellular cAMP activates PKA that in turn phosphorylates and activates PDE4D3. Activation of PDE4D3 and the consequent increase in the rate of degradation dampens the cAMP signal. The presence of this feedback loop is demonstrated by experiments activating or inhibiting PKA in FRTL-5 cells as well as by a reconstitution system where cAMP levels are greatly reduced when a PDE4D3 is expressed. In addition, this feedback regulation is not active in cells where PDE4D3 is not expressed or a phosphorylation deficient mutant is present. In all cases, it was possible to dissociate the effect of the PDE activation from the basal PDE activity present in the cell.

Although H89 has often been used to block PKA-dependent phosphorylations (27), it has been reported to also affect several signaling steps other than the PKA catalytic activity. Indeed, we have found that in a cell-free system, H89 inhibits PDE4D activity with an IC50 of approximately 50 μM. This effect on PDE activity, however, cannot be the cause of the increase in cAMP in FRTL-5 cells exposed to H89. At the concentration of 10 μM used, it produces only 10% inhibition of PDE activity in a cell-free system, and lower concentrations are most likely attained when the intact cell is exposed to this concentration. Furthermore, H85, which is more potent than H89 in inhibiting the PDE activity, had no effect on cAMP accumulation, thus lessening the possibility that the PDE inhibition is the cause of the observed effects.

Iwami et al. (37) have shown that PKA directly phosphorylates type V adenylyl cyclase, the major isof orm expressed in heart, and thereby inhibits catalytic activity. This inhibition is negated in the presence of a PKA inhibitor, suggesting that PKA-mediated phosphorylation and inactivation of adenylyl cyclase represent a mechanism for heterologous desensitization of the G protein-coupled receptor pathway. Whether this mechanism is operating in FRTL-5 cells is unclear. Our data showing that H89 has no effect in the presence of Rolipram indicates that inhibition of PKA affects the rate of cAMP degradation but not the rate of synthesis by adenylyl cyclases. It is also possible that H89 potentiates the TSH-dependent increase in cAMP by blocking a PKA-mediated phosphorylation of the TSH receptor itself. This is a mechanism described for the β-adrenergic receptor where a PKA-mediated phosphorylation causes uncoupling of the receptor from Gs. There is at present no clear evidence that PKA phosphorylates the TSH or other glycoprotein hormone receptors as shown for the β-adrenergic receptors. Several reports have shown that PKA-dependent phosphorylation does not play an important role in uncoupling glycoprotein hormone receptors (38). All these considerations render it unlikely that an increased cAMP accumulation is caused by a blockade of steps at the level of the membrane. Finally, the conclusion that H89 causes an increase in cAMP by blocking PDE activation is consistent with the observation in Sertoli cells. Although the FSH receptor is highly homologous to the TSH receptor, H89 does not potentiate cAMP accumulation in these cells where the short term feedback activation of a PDE is not functional. In some but not all experiments, treatment of Sertoli cells with H89 caused a decrease in cAMP accumulation. Since this inhibition was observed only after 30–60 min of incubation, this may be due to the prolonged PKA inhibition.

Kinetic models of cAMP accumulation predict that PDE activation causes a shortening of the time required for cAMP levels to reach a plateau (3). PDE inhibition should instead cause a delay in the time of maximal cAMP accumulation. With the caveat that it may be technically difficult to make accurate cAMP measurements 3–5 min after addition of hormone, in our studies with either H89 or Rolipram, we found that the time required to reach a maximum was not grossly affected after blocking PDE4D3 activation. Instead, the major consequence was a change in the maximum level of cAMP accumulating in the cell. Surprisingly, the subsequent phase in which cAMP decreases toward basal levels was not greatly affected, whereas it is generally believed that PDE activation contributes to terminating the cAMP signal. A major impact of cAMP extrusion from the cell is unlikely at these early time points, because cAMP accumulated in 30 min in the extracellular space is less than 10% of the intracellular cAMP. On the basis of these observations, we propose that the feedback mechanism involving PDE4D3 activation controls the intensity of the cAMP signal, while its impact on the time course of cAMP accumulation or the termination of the stimulus is less clear. Other PDEs and/or adenylyl cyclase desensitization may play a more prominent role in the return of cAMP to basal levels.

The effect of Rolipram on the TSH-stimulated cyclic AMP was 1 order of magnitude larger than the effect of blocking the PDE activation with H89. This is likely due to the fact that Rolipram blocks both the transient PDE4D activation as well as the basal activity of all PDE4s present in the cell. Even though PDE4D3 has substantial activity in a cell-free assay when mutated in the PKA phosphorylation sites (18), it is unclear whether a completely dephosphorylated enzyme is active in the intact cell. Further experiments are required to clarify this point.

In summary, we have demonstrated that PDE4D3 phosphorylation and activation is a mechanism regulating cAMP responses. This mechanism is activated by cAMP, and it occurs with the same time course as adenylyl cyclase activation. Our findings indicate that this PDE regulation counteracts the in-
creased rate of cAMP synthesis and that it affects the maximal cAMP accumulation rather than the rate of cAMP disappearance from the cell. Thus, this regulation represents a dampening system to maintain cAMP within a narrow range of concentrations. It may also be a mechanism necessary to reduce the noise in cAMP signaling and to limit the diffusion of cAMP from the site of its synthesis. Working on the manuscript.

Acknowledgment—We are indebted to Caren Spencer for editorial work on the manuscript.

REFERENCES

1. Soderling, T. R., Corbin, J. D., and Park, C. R. (1973) J. Biol. Chem. 248, 1822–1829
2. Su, Y. F., Cubeddu, L., and Perkins, J. P. (1976) J. Cyclic Nucleotide Res. 2, 257–270
3. Barber, R., Clark, R. B., Kelly, L. A., and Butcher, R. W. (1978) Adv. Cyclic Nucleotide Res. 9, 507–516
4. Hausdorff, W. P., Caron, M. G., and Lefkowitz, R. J. (1990) FASEB J. 4, 2818–2889
5. Lohse, M. J. (1993) Biochim. Biophys. Acta 1179, 171–188
6. Lohse, M. J., Benovic, J. L., Codina, J., Caron, M. G., and Lefkowitz, R. J. (1990) Science 248, 1547–1550
7. Lohse, M. J., Andexinger, S., Pitcher, J., Trukawinski, S., Codina, J., Faure, J. P., Caron, M. G., and Lefkowitz, R. J. (1992) J. Biol. Chem. 267, 8558–8564
8. Ascoli, M. (1996) Biochem. Pharmacol. 52, 1647–1655
9. Conti, M., Nemoz, G., Selle, C., and Vicini, M. (1995) Endocrinology 16, 370–389
10. Corbin, J. D., Gettys, T. W., Blackmore, P. F., Beebe, S. J., Francis, S. H., Glass, D. B., Redmon, J. B., Sheoran, V. S., and Landiss, L. R. (1988) Methods Enzymol. 159, 74–82
11. Gettys, T. W., Blackmore, P. F., Redmon, J. B., Beebe, S. J., and Corbin, J. D. (1987) J. Biol. Chem. 262, 333–339
12. Corbin, J. D., Beebe, S. J., and Blackmore, P. F. (1985) J. Biol. Chem. 260, 8731–8735
13. Macphee, C. H., Reifsnyder, D. H., Moore, T. A., and Beavo, J. A. (1986) J. Cyclic Nucleotide Protein Phosphorylation Res. 11, 487–496
14. Grant, P. G., Mannarino, A. F., and Coleman, R. W. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 9071–9075
15. Gettys, T. W., Vine, A. J., Simonds, M. F., and Corbin, J. D. (1988) J. Biol. Chem. 263, 10359–10363
16. Manganiello, V. C., Smith, C. J., Newman, A. H., Rice, K., Degerman, E., and Belfrage, P. (1986) J. Cyclic Nucleotide Protein Phosphorylation Res. 11, 497–511
17. Sette, C., Iona, S., and Conti, M. (1994) J. Biol. Chem. 269, 9245–9252
18. Sette, C., and Conti, M. (1996) J. Biol. Chem. 271, 16526–16534
19. Ball, E. H., Seth, P. K., and Sanwal, B. D. (1980) J. Biol. Chem. 255, 2962–2968
20. Ekholm, D., Belfrage, P., Manganiello, V., and Degerman, E. (1997) Biochim. Biophys. Acta 1356, 64–70
21. Ahlstrom, M., and Lamberg-Allardt, C. (1997) J. Bone Miner. Res. 12, 172–178
22. Ambesi-Impiombato, F. S., Parks, L. A., and Coon, H. G. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 3455–3459
23. Ascoli, M. (1981) Endocrinology 108, 88–95
24. Conti, M., Toscano, M. V., Petrelli, L., Geremia, R., and Stefanini, M. (1982) Endocrinology 110, 1189–1196
25. Parker, B. A., and Stark, G. R. (1979) J. Virol. 31, 366–369
26. Thompson, W. J., and Appleman, M. M. (1971) Biochemistry 10, 311–316
27. Hidaka, H., Watanabe, M., and Kobayashi, R. (1991) Methods Enzymol. 201, 329–339
28. Macphee, C. H., Harrison, S. A., and Beavo, J. A. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 6660–6663
29. Iona, S., Cuomo, M., Bushnik, T., Naro, F., Sette, C., Hess, M., Shelton, E. R., and Conti, M. (1998) Mol. Pharmacol. 53, 23–32
30. Steiner, A. L., Pagliara, A. S., Chase, L. R., and Kipnis, D. M. (1972) J. Biol. Chem. 247, 1114–1120
31. Harper, J. F., and Brooker, G. (1975) J. Cyclic Nucleotide Res. 1, 207–218
32. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
33. Peterson, G. L. (1979) Anal. Biochem. 100, 201–220
34. Sette, C., Vicini, E., and Conti, M. (1994) J. Biol. Chem. 269, 18271–18274
35. Beebe, S. J., and Corbin, J. D. (1995) in The Enzymes (Boyer, P. D., ed) pp. 43–75, Academic Press, Orlando, FL
36. Swinnen, J. V., D’Souza, B., Conti, M., and Ascoli, M. (1991) J. Biol. Chem. 266, 14383–14389
37. Iwami, G., Kawabe, J., Ebina, T., Cannon, P. J., Homcy, C. J., and Ishikawa, Y. (1995) J. Biol. Chem. 270, 12481–12484
38. Ascoli, M. (1996) Biochem. Pharmacol. 52, 1647–1655
39. Jin, S. L., Richard, F. J., Kuo, W. P., D’Ercole, A. J., and Conti, M. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 1998–2003
40. Lowry, O. H., Rosebrough, N. J. Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
Short Term Feedback Regulation of cAMP in FRTL-5 Thyroid Cells: ROLE OF PDE4D3 PHOSPHODIESTERASE ACTIVATION
Noriko Oki, Shin-Ichiro Takahashi, Hiroyoshi Hidaka and Marco Conti

J. Biol. Chem. 2000, 275:10831-10837.
doi: 10.1074/jbc.275.15.10831

Access the most updated version of this article at http://www.jbc.org/content/275/15/10831

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 39 references, 19 of which can be accessed free at http://www.jbc.org/content/275/15/10831.full.html#ref-list-1