Cortisol rapidly suppresses intracellular calcium and voltage-gated calcium channel activity in prolactin cells of the tilapia (*Oreochromis mossambicus*)

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Cortisol rapidly suppresses intracellular calcium and voltage-gated calcium channel activity in prolactin cells of the tilapia (*Oreochromis mossambicus*). Am J Physiol Endocrinol Metab 286: E626–E633, 2004. First published December 2, 2003; 10.1152/ajpendo.00088.2003. —Cortisol was previously shown to rapidly (10–20 min) reduce the release of prolactin (PRL) from pituitary glands of tilapia (*Oreochromis mossambicus*). This inhibition of PRL release by cortisol is accompanied by rapid reductions in $\Delta$Ca$^{2+}$ and cAMP accumulation. Cortisol’s early actions occur through a protein synthesis-independent pathway and are mimicked by a membrane-impermeable analog. The signaling pathway that mediates rapid, non-genomic membrane effects of glucocorticoids is poorly understood. Using the advantageous characteristics of the teleost pituitary gland from which a nearly pure population of PRL cells can be isolated and incubated in defined medium, we examined whether cortisol rapidly reduces intracellular free calcium (Cai$^{2+}$) and suppresses L-type voltage-gated ion channel activity in events that lead to reduced PRL release.

Microspectrofluorometry, used in combination with the Ca$^{2+}$-sensitive dye fura 2, revealed that cortisol reversibly reduces basal and associated mechanism similar to that observed with the fast effects of glucocorticoids rapidly modulate hormone secretion via a membrane-release. Microspectrofluorometry, used in combination with the Ca$^{2+}$-sensitive dye fura 2 revealed that cortisol reversibly reduces basal and hyposmotically induced Ca$^{2+}$ within seconds (P < 0.001) in dispersed pituitary cells. Somatostatin, a peptide known to inhibit PRL release through a membrane receptor-coupled mechanism, similarly reduces Ca$^{2+}$. Under depolarizing [K+]i, the L-type calcium channel agonist BAY K 8644, a factor known to delay the closing of L-type Ca$^{2+}$ channels, stimulates PRL release in a concentration-dependent fashion (P < 0.01). Cortisol (and somatostatin) blocks BAY K 8644-induced PRL release (P < 0.01; 30 min), within the time course over which its actions occur, independent of protein synthesis and at the level of the plasma membrane. Results indicate that cortisol inhibits tilapia PRL release through rapid reductions in Ca$^{2+}$ that likely involve an attenuation of Ca$^{2+}$ entry through L-type voltage-gated Ca$^{2+}$ channels. These results provide further evidence that glucocorticoids rapidly modulate hormone secretion via a membrane-associated mechanism similar to that observed with the fast effects of peptides and neurotransmitters.

BAY K 8644; nongenomic actions; glucocorticoids; osmoregulation; nifedipine; L-type voltage-gated ion channels; teleost

PROLACTIN (PRL) regulates a diverse array of physiological processes, including development, growth, metabolism, reproduction, and osmoregulation (for review see Refs. 14, 17). One central and possibly ancient function of PRL is the regulation of hydromineral balance in vertebrates. In euryhaline teleosts like the tilapia (*Oreochromis mossambicus*), PRL is critical to osmoregulation in freshwater environ-

ments, where it reduces salt and water fluxes (14). Consistent with this action, PRL release in vitro is inversely related to the osmolality of the surrounding medium (21, 56). In contrast with PRL, cortisol is an important seawater osmoregulatory hormone that promotes Na$^{+}$ excretion and thereby reduces the rise in blood osmolality that occurs when animals move to hyperosmotic environments (35). Cortisol may also inhibit the secretion of PRL, whose actions counteract seawater adaptation, to promote osmoregulation in seawater (8, 50).

Studies in our laboratory have shown that cortisol rapidly reduces PRL release (<20 min) through a Ca$^{2+}$-dependent mechanism that involves comparably rapid reductions in $\Delta$Ca$^{2+}$ accumulation (<15 min) (8). The rapid effect of cortisol is not mediated by changes in gene expression, since the protein synthesis blocker cycloheximide was unable to overcome PRL release inhibited by cortisol (10). A membrane-impermeant glucocorticoid (cortisol 21-hemisuccinate-BSA) reduces PRL release with equal potency as the natural steroid, suggesting that cortisol exerts its early effect at the level of the plasma membrane (10). These studies provide compelling evidence for a rapid, nongenomic, membrane-associated action of glucocorticoids on PRL release.

Although there is a growing body of evidence that documents rapid, nongenomic actions of glucocorticoids, less is known about the signal transduction mechanisms or Ca$^{2+}$ pathways involved, particularly with regard to pituitary hormone secretion and responses derived from homogenous populations of normal cells (6, 34). Glucocorticoids or their synthetic agonist inhibit insulin release and Ca$^{2+}$ influx in rat pancreatic islets (4), Ca$^{2+}$ influx in PC12 and adrenal medullary chromaffin cells (43), and N- and L-type Ca$^{2+}$ currents in guinea pig hippocampal CA1 cells (16). In the pituitary, corticosterone elicits a biphasic inhibition of corticotropin-releasing factor-stimulated ACTH secretion. The early rapid phase (<30 min) is thought to be insensitive to inhibitors of protein synthesis, whereas the more delayed response (>30 min) requires the induction of new proteins (1, 15). Although studies in the corticotroph AtT cell line show that dexamethasone reduces cAMP and inhibits the late phase of ACTH secretion through a nongenomic action (27), the mechanism that mediates the rapid effect of corticosterone on ACTH release from anterior pituitary cells remains unknown. Despite its potent inhibition of PRL cell

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CORTISOL RAPIDLY SUPPRESSES INTRACELLULAR CALCIUM

function (26, 32), there are no reports to date that demonstrate nongenomic effects of glucocorticoids in lactotrophs other than the effect shown for tilapia (6, 9).

The present study was undertaken to determine whether cortisol reduces intracellular Ca²⁺ (Ca²⁺ᵢ) and voltage-gated Ca²⁺ channel activity in events that lead to reduced PRL release in tilapia. Microspectrofluorometry and the calcium-sensitive dye fura 2 were employed to measure real-time changes in Ca²⁺ᵢ in isolated PRL cells. This method, unlike ⁴⁰Ca²⁺ influx studies (8), provides measures of Ca²⁺ᵢ that reflect mobilization from both extracellular and intracellular pools and the temporal resolution necessary for refining the rapid nature by which cortisol alters Ca²⁺ᵢ metabolism. Investigations were also designed to examine whether cortisol blocks PRL release induced by depolarizing [K⁺] and the L-type Ca²⁺ channel agonist BAY K 8644 (BAYK), which, when used in conjunction, increase the probability that Ca²⁺ᵢ channels remain open (38). The actions of cortisol were compared with those of somatostatin (SRIF), a peptide known to work through membrane-associated mechanisms to rapidly alter PRL release (30, 41).

A principal impediment to the study of the function of a particular cell is the difficulty in isolating it within a morphologically complex tissue. To circumvent this problem, cell lines are often used to study stimulus-secretion coupling, but they may differ functionally from normal cells in their Ca²⁺ᵢ requirements, response to secretagogues, membrane receptor composition, and G-protein regulation (see Refs. 9 and 20). Our studies utilize the unique arrangement of the teleost pituitary, wherein a nearly pure population of PRL cells (95% mature) is segregated into a distinct region of the anterior pituitary, the rostral pars distalis (RPD) (20, 37). The RPD is easily isolated and can be studied in a completely defined, serum-free medium.

MATERIALS AND METHODS

Static incubations. Adult male tilapia (15–20 cm) were maintained in freshwater at a constant photoperiod (12:12-h light-dark) for ≥3 wk before all experiments. Fish were decapitated, and their pituitaries were removed and placed in hypotonic (360 mosmol/kgH₂O) medium. Medium consisted of a Krebs-Ringer bicarbonate solution (in mM: 2.35 KCl, 1.25 KH₂PO₄, 25 NaHCO₃, 1.4 MgSO₄, 2.1 CaCl₂, 140 NaCl) supplemented with glucose (0.5 mg/ml), L-glutamine (0.29 mg/ml), and a 2 ml/100 ml medium of 50× MEM essential amino acids (7). Medium with depolarizing [K⁺] was prepared by the addition of KCl to a final concentration of 50 mM. Osmotic pressure of the incubation medium was measured with a vapor pressure osmometer (Wescor, Logan, UT) and adjusted to 290 mOsm/kgH₂O with NaCl. Tissues were incubated in an airtight chamber at 28 °C. The chamber was continuously agitated at 60 rpm on a gyratory platform. After a 2-h preincubation to allow PRL release to stabilize to baseline levels (19), hyperosmotic medium (in mM: 2.35 KCl, 1.25 KH₂PO₄, 25 NaHCO₃, 1.4 MgSO₄, 2.1 CaCl₂, 140 NaCl) supplemented with glucose (0.5 mg/ml), L-glutamine (0.29 mg/ml), and a 2 ml/100 ml medium of 50× MEM essential amino acids (7). Medium with depolarizing [K⁺] was prepared by the addition of KCl to a final concentration of 50 mM. Osmotic pressure of the incubation medium was measured with a vapor pressure osmometer (Wescor, Logan, UT) and adjusted to 290 mOsm/kgH₂O with NaCl. Tissues were incubated in an airtight chamber at 28 ± 1°C in a humidified environment of 95% O₂:5% CO₂. The chamber was continuously agitated at 60 rpm on a gyratory platform. After a 2-h preincubation to allow PRL release to stabilize to baseline levels (19), hypotonic medium was removed and replaced with treatment medium. After 4- or 18-h incubations, the experiment was terminated, and medium and tissue were collected separately and ultrasonically disrupted in dodecyl sulfate-2-mercaptoethanol buffer.

PRL release was measured according to previously described and validated procedures (7, 18). Tissues and medium were subjected separately to SDS-PAGE. Gels were stained with Coomassie brilliant blue (R-250) and then destained until the PRL bands were discernible. The tilapia pituitary secretes two distinct PRL molecules (tPRL177 and tPRL188) that are encoded by separate genes and are not the products of differential processing of the same transcript (57). Both PRLs are clearly distinguishable from one another on the basis of their size differences, and representative bands constitute the predominant proteins seen on gels (51). They are also the only detectable proteins released by the tilapia RPD (51). PRL bands were quantified by laser densitometry (E.C. Apparatus, St. Petersburg, FL), and the peak area was measured using an electronic digitizer (Hewlett-Packard, Avondale, PA). The optical densities of stained PRL bands were linearly related over a range extending from 0.1 to 4 times the amount of hormone typically loaded on the gels (18). Data were calculated as the percentage of total hormone released or the amount of hormone released in medium divided by total hormone (medium + tissue) in the incubation. Both PRLs are released at similar rates by those secretagogues examined to date, including osmotic pressure, cortisol, and SRIF (10). In research presented here, only the release of tPRL188 is shown, although both hormones responded similarly to experimental treatments.

For short-term static incubation studies, the RPDs of sexually immature male tilapia (10–12 cm long) were incubated under conditions identical to those described above, except that medium was warmed to 30, 90, and 180-min intervals. Tissues were sonicated in radioimmunoassay (RIA) buffer (0.01 M phosphate buffer, pH 7.3, containing 0.14 M NaCl, 0.1% BSA, 0.01% Na₂SO₄, and 0.1% Triton X-100) and kept frozen at −20°C. Medium and tissue PRL were quantified using a homologous RIA as previously described (3, 56), and hormone release is expressed as a percentage of the total amount of hormone in the incubations.

Ca²⁺ᵢ measurements. PRL cells were dissociated from Fura-buffered saline (PBS; 0.5 M NaH₂PO₄, 0.5 M Na₂HPO₄, pH 7.25, adjusted to 360 mosmol/kgH₂O with NaCl) containing 0.125% (wt/vol) trypsin for 45 min at room temperature. Cells were centrifuged (250 g, 5 min) and resuspended in 1 ml of PBS containing 0.125% (wt/vol) trypsin inhibitor for 10 min. Cells were washed, resuspended in PBS alone, and then dispersed by 5–10 gentle passages through a plastic Pasteur pipette. After an additional rinse, cells were centrifuged and then resuspended in hypotonic medium. Prolactin cells were placed onto poly-l-lysine (0.1 mg/ml)-coated glass coverslips. Cells were incubated in hypotonic medium for ≥12 h before determination of Ca²⁺ᵢ.

The experiments measuring Ca²⁺ᵢ were conducted at the Hawaii Institute of Marine Biology (Oahu, HI). PRL cells were loaded with 5 μM fura 2-AM, the membrane-permeable acetoxymethyl ester derivative of fura 2 (Molecular Probes, Eugene, OR), for 90 min at 28 ± 1°C. The fura 2-AM was solubilized in anhydrous dimethyl sulfoxide (Aldrich Chemical, Milwaukee, WI) to a concentration of 5 mM before its final dilution to 5 μM. The coverslip, plated with PRL cells, was then mounted onto a perfusion chamber. The perfusion chamber was placed on a microscope stage, and cells were continuously exposed to control medium before the start of the experiment.

Different experimental media were maintained in plastic 60-ml syringes connected to an eight-point manifold perfusate selector (Hamilton, Reno, NV) via one-way stopcocks and polyethylene tubing. The rate (0.5 ml/min) of perfusion through the chamber was maintained by keeping the height of the syringes and volume of all solutions in the syringes constant throughout the experiment. In experiments where a steroid hormone or BAYK was tested, all treatments contained the appropriate solvent (0.01% ethanol).

Simultaneous measurements of intensities of free fura 2 (380 nm) and fura 2 bound to Ca²⁺ (340 nm) were made with a dual-excitation spectrophotometer (ARCH-MIC-N; Spex Industries, Edison, NJ) equipped with a Nikon Microphot-FX microscope (×40 oil immersion fluoro objective, Nikon) and propriety software (Spex Industries). Excitation light alternated between 340 and 380 nm (narrow bandpass filters, SPEX) by a computer-controlled
chopper mirror. Fluorescent emission (500 nm) intensity of fura 2 was detected from PRL cells every 2 s by a photomultiplier tube. All data are expressed as relative intensity, the ratio of fura 2 fluorescence excited at 340 nm to that excited by 380 nm from which background was subtracted. Shifts in this ratio (340/380) result directly from changes in Ca$^{2+}$ (23).

Statistical analysis. Statistical differences in PRL release were analyzed using a one-way analysis of variance (ANOVA) test followed by the Fisher protected least significant difference test for predetermined comparisons of multiple treatments. All values for PRL release were expressed as means ± SE. Statistical differences in free Ca$^{2+}$ were analyzed using repeated-measures one-way ANOVA followed by Tukey's procedure for pairwise comparisons.

RESULTS

Before Ca$^{2+}$ measurements, initial experiments were conducted to verify that dispersed PRL cells were viable and continued to respond to osmotic pressure, cortisol, and SRIF in a manner similar to the cells of intact tissues. The Trypan blue exclusion test showed that the viability of individual cells exceeded 90%. PRL release from dispersed PRL cells was enhanced by 95% during exposure to reductions in medium osmolality. A concentration of 200 nM cortisol and 300 nM SRIF reduced PRL release under hyposmotic stimulation by 32 and 35%, respectively (P < 0.05; data not shown). The similar response to these PRL regulators between dispersed cells and cells within the intact RPD (7, 10, 46) indicates that receptors and other membrane components central to hormone secretion remain functional and that the dispersion process does not interfere with the mechanisms of action of either cortisol or SRIF.

As observed with PRL release (7), Ca$^{2+}$ remained low during exposure to hyperosmotic medium. Cortisol, at a concentration (200 nM) measured in plasma of tilapia (8, 47), reduced Ca$^{2+}$ within 60 s during exposure to hyperosmotic medium (P < 0.05). The effect remained highly significant throughout the duration of cortisol exposure (P < 0.001; Fig. 1 and Table 1). Removal of cortisol from the treatment medium resulted in an equally rapid and full recovery of Ca$^{2+}$, which returned to baseline hyperosmotic levels within 30 s (P < 0.001; Fig. 1 and Table 1). Reductions in medium osmotic pressure increase Ca$^{2+}$ (21) and PRL release (19). In the present study, exposure to hyposmotic medium increases Ca$^{2+}$, and, as seen under basal hyperosmotic conditions, cortisol caused an immediate decline in the hyposmotic-induced rise in Ca$^{2+}$, which became significant within 90 s (P < 0.001; Fig. 1 and Table 1). The recovery phase was also immediate and reached significance within 120 s (P < 0.05; Fig. 1 and Table 1).

SRIF, which inhibits 45Ca$^{2+}$ and cAMP accumulation in tilapia RPD (20, 24), also rapidly reduces Ca$^{2+}$ (Fig. 2 and Table 1). This reduction (P < 0.001 within 60 s) is sustained over the course of the treatment, followed by an immediate recovery (P < 0.001 within 90 s) of Ca$^{2+}$ levels upon removal of the hormone (Fig. 2 and Table 1).

To examine whether cortisol alters PRL release through regulation of L-type voltage-gated Ca$^{2+}$ channel activity, we first determined the concentrations of BAYK that augment PRL release from the tilapia RPD. BAYK in the presence of depolarizing [K+] increases PRL release in a dose-related manner (P < 0.001), reaching significance at 10 μM (P < 0.01; Fig. 3A). The level of PRL release achieved with BAYK and depolarizing [K+] equaled that induced by hyposmotic medium. To verify that BAYK specifically targets L-type Ca$^{2+}$ channels on tilapia PRL cells, we examined whether nifedipine, an L-type channel antagonist, blocked BAYK-induced PRL release. Nifedipine at concentrations of 10 and 50 μM completely overcame BAYK-evoked PRL release (P < 0.01; Fig. 3B).

Cortisol significantly lowered PRL release stimulated by the combined depolarizing [K+] and 10 μM BAYK treatment (Fig. 4; P < 0.05, P < 0.01) during static incubations. The degree of PRL release inhibition increased with a greater concentration of cortisol (Fig. 4B; P < 0.05). The degree to which cortisol inhibits PRL release is similar to that observed when tissues were exposed to the peptide SRIF (P < 0.01; Fig. 4A) and to nifedipine (Fig. 3).

Cortisol reduces BAYK-evoked PRL release in a manner that is similar to that produced by SRIF over sustained incubations. It remained to be determined whether the steroid was effective within the time frame (≤4 h) over which it has been shown to act on PRL release in a nongenomic fashion at the level of the membrane (10). Figure 5 shows that cortisol reduces BAYK-evoked PRL release after 30 min (P < 0.05), 90 min (P = 0.0553), and 240 min (P < 0.01) of exposure.
Table 1. *Period of onset and recovery of Ca\textsuperscript{2+} following exposure and withdrawal of cortisol (200 nM) and somatostatin (300 nM), respectively, during incubations in hyperosmotic (360 mOsmolal) and hyposmotic (300 mOsmolal) media*.

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<th>Time to Onset and Recovery of Cortisol’s Effects</th>
<th>Time to Onset and Recovery of SRIF’s Effects</th>
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Values are means ± SE. F, cortisol; SRIF, somatostatin. Intracellular Ca\textsuperscript{2+} (Ca\textsuperscript{2+}) is expressed as relative intensity of fura 2 fluorescence (340/380 nm) measured at 10-s increments for the 5 separate experiments displayed in Figs. 1B and 2. Ca\textsuperscript{2+} is presented as a percentage of control values represented by the original 300-s exposure to hyperosmotic medium (time 0 s). *P < 0.05, †P < 0.001 vs. baseline (time 0) at each respective 360 and 290 mosmol/kgH\textsubscript{2}O exposure. ‡P < 0.01 vs. last onset time point (300 s, equivalent to baseline for recovery) at each respective exposure to 360 and 290 mosmol/kgH\textsubscript{2}O with cortisol or SRIF.

**DISCUSSION**

These studies clearly show that cortisol, a steroid normally thought to act slowly on target cells through alterations in gene expression, reduces Ca\textsuperscript{2+} within seconds in isolated PRL cells. This fall in Ca\textsuperscript{2+} occurs well within the time resolution with which cortisol reduces PRL release during perfusion incubations (10–20 min) (8). This, along with prior evidence that the response of PRL release to cortisol is dependent on reductions in cytosolic Ca\textsuperscript{2+} (8), suggests that the ion is a central mediator of cortisol’s rapid inhibition of PRL release. Insofar as cholesterol (the precursor to all steroids) failed to alter Ca\textsuperscript{2+} (data not shown) or PRL release (8), the actions of cortisol on these parameters does not appear to result from a nonspecific perturbation or interaction of a steroid-like molecule with the plasma membrane. Rather, the PRL secretory response to cortisol appears specific to this seawater-adapting hormone. 17β-Estradiol and testosterone stimulate PRL release, whereas cortisone (cortisol’s conversion product; data not shown), aldosterone, 11-deoxycorticosterone, and the primary fish and mammalian progestins were without effect (8).

In the classical model of steroid action, steroids diffuse through the plasma membrane and bind cytosolic (glucocorticoid) or nuclear receptors. The activated steroid-receptor complex is then translocated to the DNA, where it binds palindromic sequences in conjunction with other transactivating proteins to alter gene transcription and subsequent protein synthesis (58). Through this pathway, steroids typically produce cell responses with latency periods ranging from hours to days (6, 34). Even after elimination of the steroid, a considerable lag period may exist before the cell response subsides. This lag period, which can last for hours, is needed to reduce the accumulation of newly synthesized proteins that was required for the initial cell response (49). In studies here, Ca\textsuperscript{2+} levels were reduced for as long as the cells were exposed to cortisol but promptly returned to the control level once the steroid was removed. The rapid onset and comparably rapid recovery of Ca\textsuperscript{2+} following the initial application and withdrawal of cortisol, respectively, support the idea that this steroid is not working through a genomically mediated process but may instead act via membrane-associated mechanisms. SRIF’s mode of action has been well characterized in mammalian pituitary cell systems (32, 41) but to a lesser degree in mammalian lactotrophs and tilapia PRL cells. Through the cAMP-independent pathway, SRIF binds one or more G protein-coupled membrane receptor(s) to increase K\textsuperscript{+} conductance, which secondarily reduces Ca\textsuperscript{2+} influx through voltage-sensitive channels. The subsequent decline in Ca\textsuperscript{2+} results in an inhibition of hormone release (11, 29). In agreement with studies on rat anterior pituitary cells, somatotrophs as well as PRL- and ACTH-secreting neoplastic cells (33, 36, 44, 48), we found that SRIF, like cortisol, reduces Ca\textsuperscript{2+} within seconds.
and that this effect is immediately reversible upon removal of the peptide (Fig. 2 and Table 1).

Changes in Ca\textsuperscript{2+} may result from an increase or decrease in the release of Ca\textsuperscript{2+} from intracellular Ca\textsuperscript{2+} pools, or the influx of extracellular Ca\textsuperscript{2+} through various channel types, including L-type voltage-gated Ca\textsuperscript{2+} channels. Activation of these channels by depolarizing [K\textsuperscript{+] rapidly stimulates PRL release and \textsuperscript{45}Ca\textsuperscript{2+} entry into tilapia RPD (22, 45). By stimulating the opening of L-type channels with depolarizing [K\textsuperscript{+] and delaying their closing with BAYK, additional effects on PRL release were seen in this study: increased PRL release over that observed with depolarizing [K\textsuperscript{+] alone. The ability of nifedipine, a specific antagonist of L-type Ca\textsuperscript{2+} channels, to block BAYK-induced PRL release from tilapia RPD provides further evidence that L-type voltage-gated Ca\textsuperscript{2+} channels were effectively targeted in these studies. Collectively, these studies and others (54) suggest that the PRL cells of teleost fish, like those of mammals (5, 32), are excitable with spontaneous action potentials playing an integral role to the secretory process, one which likely involves an influx of Ca\textsuperscript{2+} through voltage-sensitive channels.

Cortisol reduced BAYK-stimulated PRL release from tilapia RPD in as little as 30 min. In a previous study, cortisol was also shown to reduce \textsuperscript{45}Ca\textsuperscript{2+} accumulation and PRL release (8) within a similar time frame. These results suggest that cortisol inhibits PRL release by rapidly reducing Ca\textsuperscript{2+} via an inhibition of L-type Ca\textsuperscript{2+} channels and thereby an influx of extracellular Ca\textsuperscript{2+}. This is supported indirectly through studies that show
Although evidence points to the presence of a putative membrane receptor on PRL cells, future studies are required to determine its identity and pharmacological signature. Evidence to date suggests that glucocorticoids may elicit rapid effects through various types of membrane-resident proteins. These include membrane corticosteroid-binding globulins (52), G protein-coupled receptor(s) distinct from the intracellular type (39), intracellular-like receptors (12), or perhaps other types (6, 31).

The concentration of cortisol used in this study falls within the physiological levels measured in tilapia and is below that typically found in stressed animals. During an initial 4-min nonstressed sampling period, plasma cortisol levels range from 20 to 50 nM in freshwater and 120 to 220 nM in seawater fish (8, 47). Over a more sustained sampling time course (5–9 min) that likely incorporates responses to stress (2), circulating cortisol levels rise two- to threefold and remain elevated in seawater (175–470 nM) compared with freshwater (100–150 nM) fish (47). It is well established that cortisol is an important seawater-osmoregulatory hormone and PRL is critical for freshwater adaptation in many euryhaline teleosts including the tilapia. Considering the opposing osmoregulatory actions of PRL and cortisol, we postulate that cortisol may serve the dual role of rapidly reducing the secretion of PRL, a seawater-antagonistic hormone, while itself activating osmoregulatory processes critical to seawater adaptation (53). It is also possible that cortisol may limit the rapid rise in circulating PRL that accompanies a stress response shown for a closely related tilapia species [O. niloticus (2)].

Plasma glucocorticoid levels can rise dramatically within minutes following a stress event or osmotic perturbation. Therefore, it is not surprising that this class of steroids may exert rapid as well as delayed effects on target tissues. We show that cortisol rapidly attenuates PRL release through a nongenomic mechanism that involves interactions with the plasma membrane and modulation of the Ca\textsuperscript{2+} messenger systems. Evidence is provided that suggests that the inhibition of PRL release by cortisol, like that of the fast-acting peptide hormones (SRIF), is mediated through a reduction of Cai\textsuperscript{2+} (20) and our demonstration that it exerts a similar inhibitory effect on Ca\textsuperscript{2+} and BAYK-evoked PRL release as cortisol (Figs. 3 and 5). In neuronal tissue or cell lines, glucocorticoids rapidly inhibit N- and L-type calcium currents and nicotine-induced calcium influx through a putative membrane receptor-mediated, pertussis toxin-sensitive, G protein-linked pathway (16, 43). Cortisol also rapidly hyperpolarizes the membrane potential of guinea pig neurons (25). Whether cortisol rapidly reduces PRL release in a manner similar to SRIF through an inhibition of voltage-sensitive Ca\textsuperscript{2+} channels directly or via conductance of other ions in the tilapia PRL cell remains to be determined (29, 36). Nevertheless, the similarities in the ability of cortisol to reduce voltage-gated Ca\textsuperscript{2+} channel activity and Ca\textsuperscript{2+} in tilapia PRL cells and mammalian neuronal preparations suggest that the mechanisms that mediate rapid inhibitory glucocorticoid effects may be well conserved among vertebrates and possibly different cell systems.

Glucocorticoids are implicated in attenuating plasma PRL in humans and abrogating PRL hypersecretion in rodents (26, 42). They also suppress PRL release elicited by various stimuli in GH\textsubscript{3} and rat pituitary cells (32, 55). Although the genome may be involved in mediating some of these responses, and the sustained effects observed here, more detailed studies of acute glucocorticoid actions on PRL cell function are warranted. Glucocorticoids have been shown to bind with high affinity to rat pituitary plasma membranes (28) and suppress ACTH secretion from mouse AtT cells through a nongenomic mechanism of action over a 3-h period (27). With respect to PRL regulation, there is new evidence correlating rapid estrogenic induction of PRL release to specific membrane steroid receptors in GH\textsubscript{3}/B\textsubscript{6} cells and normal pituitary fragments (13, 40). Several lines of evidence suggest that the early responses in Ca\textsuperscript{2+} and PRL release elicited by cortisol in tilapia are nongenomic and likely occur at the membrane. First, the time course over which cortisol alters BAYK-evoked PRL release (minutes) and Ca\textsuperscript{2+} (seconds) in particular is too short to encompass all steps in the intracellular signaling pathway leading to the synthesis of new proteins essential to a genomically mediated, classical steroid response (6, 34). Second, to a large degree, cortisol mimics the actions of SRIF and rapidly modulates the same signaling molecules (e.g., Ca\textsuperscript{2+} and cAMP) that transduce membrane receptor-coupled responses (9). Third, cycloheximide at concentrations that completely block de novo protein synthesis in tilapia RPDs is ineffective during static incubations in hyperosmotic (360 mosmol/kg H\textsubscript{2}O) medium. RPD were incubated for 240 min, and medium samples were taken at 30, 90, and 240 min (n = 6–8 wells/group with 2 RPD/well). ***P < 0.001 vs. hyperosmotic medium alone; *P < 0.05, **P < 0.01 vs. BAYK-depolarizing [K\textsuperscript{+}] treatment group; (P = 0.0553 for BAYK/50 nM KCl alone vs. that containing cortisol at 90 min).

Fig. 5. Time course effect of 200 nM cortisol (F) on BAYK-induced PRL release from tilapia RPD during static incubations in hyperosmotic (360 mosmol/kg H\textsubscript{2}O) medium. RPD were incubated for 240 min, and medium samples were taken at 30, 90, and 240 min (n = 6–8 wells/group with 2 RPD/well). ***P < 0.001 vs. hyperosmotic medium alone; *P < 0.05, **P < 0.01 vs. BAYK-depolarizing [K\textsuperscript{+}] treatment group; (P = 0.0553 for BAYK/50 nM KCl alone vs. that containing cortisol at 90 min).
known to influence and possibly impair several physiological processes, including behavior, reproduction, immune function, and osmoregulation.

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