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# HYPOTHALAMIC HORMONES MODULATE G PROTEIN LEVELS AND SECOND MESSENGER RESPONSIVENESS IN $GH_3$ RAT PITUITARY TUMOUR CELLS

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**Abstract**—Thyroliberin (TRH), vasoactive intestinal peptide (VIP) and somatostatin (SRIF) act through receptors that are coupled to guanine nucleotide-binding regulatory proteins (G proteins). Regulation of hormone action may occur at the level of G protein coupling to the receptor or effector systems. In this study we demonstrate that prolonged exposure (for up to 48 hr) of cultured rat pituitary adenoma $GH_3$ cells to these hormones caused homologous and to some extent heterologous attenuation of the adenylyl cyclase (AC) (EC 4.6.1.1) responsiveness. In addition, TRH and SRIF diminished both TRH- and guanosine 5′-[$\beta\gamma$-imido]-triphosphate-enhanced phospholipase C (PLC) (EC 3.1.4.3) activity within the same time-course. Measurements of cells membrane levels of $G_s$ protein $\alpha$-subunit ($G_s\alpha$), $G_{i\text{-}1}\alpha$/$G_{i\text{-}2}\alpha$, $G_{i\text{-}3}\alpha$, $G_o\alpha$ and $G\beta$ by immunoblotting were performed. TRH and VIP upregulated levels of all G proteins except $G_o\alpha$ and $G\beta$. In contrast, SRIF caused a marked reduction of $G\beta$ levels. Thus, TRH and VIP, both acting through $G_s$, both modulated the $\alpha$-subunit levels of this signal transducer, whereas SRIF, which possibly acts through $G_{i\text{-}2}$, did not change the steady state level of $G_{i\text{-}2}\alpha$. The actions of TRH, VIP and SRIF are multifaceted at the G protein level, where modulations of subtypes not directly involved in their actions may occur. These findings emphasize the complexity expected to be found in the *in vivo* situation.

$GH_3$ cells are clonal rat pituitary tumour cells that produce and spontaneously secrete into the culture medium prolactin (PRL†) and growth hormone [1]. Hormone synthesis and secretion is subject to regulation by peptide hormones, such as thyroliberin (TRH), vasoactive intestinal peptide (VIP) and somatostatin (SRIF), that exert their effect by binding to membrane receptors [2].

Guanine nucleotide-binding regulatory proteins (G proteins), which are $\alpha\beta\gamma$ heterotrimers, mediate the transmembrane signalling from peptide hormone receptors to effectors such as adenylyl cyclase (AC) (EC 4.6.1.1), phospholipase C (PLC) (EC 3.1.4.3), phospholipase $A_2$, cGMP-dependent phosphodiesterase, $K^+$-channels and $Ca^{2+}$-channels (for reviews, see [3, 4]). Thus, modulation of the coupling of G proteins to receptors and effectors may be an important step in the regulation of transmembrane signalling. In addition to *in situ* modifications such as myristoylation [5], phosphorylation [6] and ADP-ribosylation by endogenous transferases [7], alterations in the quantity of G protein subunits have been shown to correlate to altered second messenger activity [8, 9].

In $GH_3$ cells, VIP conveys its action through AC activation and the formation of cellular cAMP [10], whereas TRH is bifunctional as it also activates the PLC system [11–13]. Both TRH and VIP stimulate PRL secretion and synthesis [2], whereas SRIF, which diminishes AC activity, has an inhibitory action [14]. Earlier work has shown that the effects of VIP and TRH on AC are mediated through $G_s$ [15, 16]. The inhibitory effect of SRIF occurs through activation of $G_i$ [17], possibly $G_{i\text{-}2}$ [16], which is shown to transduce $\alpha_2$-adrenergic inhibition of AC in platelets [18] and opioid inhibition of AC in NG108-15 hybrid cells [19]. The action of SRIF has also been shown to involve the modulation of ion channels [20, 21].

We have recently quantified mRNA levels and membrane contents of the G protein subunits $G_s\alpha$, $G_o\alpha$ and $G_i\alpha$'s in various clonal strains of rat pituitary adenoma cells, i.e. $GH_12C_1$, $GH_3$ and $GH_4C_1$ cells [22], and demonstrated the presence of $G_x\alpha/G_z\alpha$ mRNA. We have also shown that TRH, VIP and SRIF alter G protein $\alpha$- and $\beta$-subunit mRNA expression in $GH_3$ cells [23].

The aim of this study was to investigate the change in responsiveness of AC and PLC to TRH, VIP and SRIF in $GH_3$ cells that were grown in the presence of these hormones. We also wished to observe the effects of these hormones on the levels of G protein $\alpha$- and $\beta$-subunits, and the possible implications of alterations of G protein subunit levels on second messenger responsiveness.

## MATERIALS AND METHODS

*Cell culture.* $GH_3$ cells [1] were grown for 5 days subsequent to subcultivation in plastic tissue culture

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† Abbreviations: PRL, prolactin; TRH, thyroliberin; VIP, vasoactive intestinal peptide; SRIF, somatostatin; Gpp(NH)p, guanosine 5′-[$\beta\gamma$-imido]-triphosphate; G protein, guanine nucleotide-binding regulatory protein; $G_s\alpha$ etc., $G_s$ protein $\alpha$-subunit; PLC, phospholipase C (EC 3.1.4.3.); AC, adenylyl cyclase (EC 4.6.1.1).

flasks containing Ham's F-10 medium (Flow Laboratories, Irvine, U.K.) [24] supplemented with 6.5% horse and 3% fetal calf serum at 37° in a humidified atmosphere of 95% air and 5% $CO_2$ [25]. Penicillin (50 IU/mL) and streptomycin (50 μg/mL) (Gibco, Glasgow, U.K.), and amphotericin B (2.5 μg/mL) (Flow Laboratories) were added to the culture medium. Culture medium was changed every 2–3 days.

*Experimental design.* The hormone treatment experiments were carried out as described previously [23]. In short, all cells were harvested simultaneously at the end of the experiment. Pharmacological doses of hormones, i.e. TRH (1 μM), VIP (1 μM) or SRIF (10 μM), were added 48, 24, 12 and 6 hr before cell harvest. Medium was changed at the start of the experiment and 24 hr before the cells were harvested. TRH and VIP exposure was always conducted in serum containing Ham's F-10 medium. SRIF treatment was performed in Serumless Medium (Neumann and Tytell) (Gibco) in order to amplify the putative inhibitory effect of SRIF on $GH_3$ cell physiology. Parallel controls (0-hr treatments) with both medium types were also run.

*Preparation of subcellular particulate fractions.* Culture medium was removed and the cells scraped in ice-cold 150 mM NaCl, 10 mM Tris–HCl, pH 7.5 and pelleted (700 *g*, 10 min, 4°) [25]. The cell pellet was washed once with the same buffer, resuspended in 20 vol. of 10 mM Tris–HCl, pH 7.5, 1 mM EDTA and homogenized on ice using an Ultra-Turrax (Janke Kunkel, F.R.G.) for 10 sec. The homogenate was subsequently filtered through nylon mesh and centrifuged at 27,000 *g* for 30 min at 4°. Finally, the pellet was resuspended in 10 vol. of Tris–EDTA buffer containing 0.1% bovine serum albumin, using the rotating knife, for 5 sec. This constituted the crude membrane fraction and contained about 5 mg membrane protein per mL.

*AC assay.* AC activity was measured in 20 μL aliquots of crude subcellular fractions which were diluted in homogenization buffer to obtain 40–55 μg of protein/assay tube [25]. The total incubation volume was 50 μL and contained 1 mM ATP [including $1.6 \times 10^6$ cpm of α-[$^{32}$P]-ATP (Amersham, U.K.)], 10 μM GTP, 2.8 mM $MgCl_2$, 1.4 mM EDTA, 1 mM cAMP [containing approx. $7 \times 10^3$ cpm of [8-$^3$H]cAMP (Amersham)], 20 mM creatine phosphate, 0.2 mg/mL creatine kinase, 0.02 mg/mL myokinase and 25 mM Tris–HCl, pH 7.4 in the absence or presence of TRH (1 μM), VIP (1 μM), SRIF (1 μM) or Gpp(NH)p (20 μM). Incubations were carried out at 35° for 20 min. Reactions were stopped with 0.1 mL of a solution comprising 10 mM cAMP, 40 mM ATP and 1% SDS. The [$^{32}$P]cAMP formed and the [$^3$H]cAMP added to monitor recovery (65–80%) were isolated as described previously using combined Dowex and aluminium oxide chromatography [26]. The enzyme activity was linear with time up to 60 min and protein concentration up to 150 μg protein (data not shown).

*PLC assay.* This method was adapted from the one previously published by Jackowski *et al.* [27]. Aliquots (20 μL) of diluted crude membrane suspensions (40–55 μg protein) in 10 mM Tris–HCl, pH 7.4, 1 mM EDTA were mixed with 10 μL incubation mixture [100 mM Tris–HCl, pH 6.5, 400 μM GTP, 2.2 mM $CaCl_2$, 1 mM $MgCL_2$ and 10 μl of TRH (10 μM) or Gpp(NH)p (400 μM)] in 2.4-mL microfuge tubes on ice. Three microliters (42,000 cpm) of a [$^3$H]$PIP_2$ (New England Nuclear, Boston, MA, U.S.A.) stock solution in 2% sodium cholate were added to each tube, and incubation was carried out at 35° for 5 min. The reaction was stopped by adding in succession 150 μL $CHCl_3$:$CH_3OH$:HCl (1:2:0.02), 50 μL $CHCl_3$ and 50 μL 2 M KCl. After vortexing and phase separation at 5000 *g* in a microfuge, 10-μL aliquots of the aqueous layers were counted in a liquid scintillation counter. In the controls, approx. 2% of the radioactivity was retained in the aqueous phase. The enzyme activity was linear with time up to 20 min and protein concentration up to 100 μg protein (data not shown).

*Western blotting and immunostaining.* Membrane proteins were prepared from crude membrane fractions and analysed by electrophoresis on SDS-containing 10% polyacrylamide gels prior to transfer onto polyvinylidene difluoride membranes (Millipore, Bedford, MA, U.S.A.) and immunostaining as described previously [22]. The quality and quantity of the electrophoresis and transfer was asserted by Coomassie blue staining of parallel filters (data not shown).

Antisera (denominated RM, AS, EC, GO and MS) have previously been described [18, 28] and have been used at 1:200–1:400 final dilution. The specificity of the antisera is reported to be as follows [18, 28]: $G_s\alpha$ antiserum (RM) is specific for both known α-subunits of $G_s$ (52 and 45 kDa). $G_{i\text{-}1}\alpha$/$G_{i\text{-}2}\alpha$ antiserum (AS) detects both $G_{i\text{-}1}$ and $G_{i\text{-}2}$ α-subunits, of which the former is not found in GH

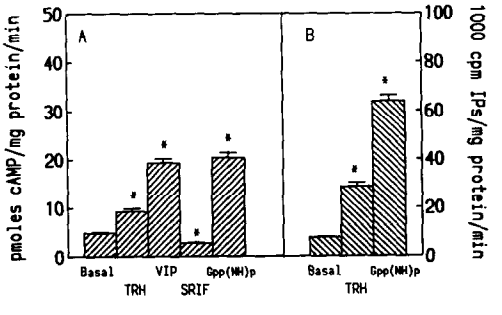


Fig. 1. Basal and modified AC and PLC activities in previously untreated $GH_3$ cells. Crude membrane fractions (40–50 μg) from $GH_3$ cells grown without addition of hormone were analysed for basal effector enzyme activity and modulatory effect of TRH (1 μM), VIP (1 μM), SRIF (10 μM) or Gpp(NH)p (20 μM) as described in Materials and Methods. Panel A, AC: results of basal and Gpp(NH)p- or hormone (TRH, VIP or SRIF)-modulated enzyme activity measurements are given as pmol cAMP/mg protein/min. Panel B, PLC: results of Gpp(NH)p- or TRH-modulated enzyme activity measurements are given as $10^3$ cpm $IP_3$/mg protein/min. All results are shown as means ± SD of triplicates of several experiments. *Indicates $P = \alpha < 0.05$ (significant difference from control; Wilcoxon rank test).

cells [22]. $G_{i\text{-}3}\alpha$ antiserum (EC) and $G_o\alpha$ antiserum (GO) display some reciprocal cross-reactivity. $G\beta$ antiserum (MS) detects both $G\beta_{35}$- and $G\beta_{36}$-subunits. Quantification of protein levels was carried out by densitometric scanning of the autoradiograms.

## RESULTS

### *Hormonal modulation of second messenger enzyme activity in untreated $GH_3$ cells*

For comparison, Fig. 1 demonstrates the effects of TRH, VIP, SRIF and the non-hydrolysable GTP analogue Gpp(NH)p on AC and PLC activity in membrane preparations from previously untreated $GH_3$ cells. Panel A shows the results of AC response to TRH (approx. 2-fold), VIP (4-fold) and Gpp(NH)p (4-fold). SRIF brought about a 35% inhibition of AC activity. Panel B depicts the effect of TRH and Gpp(NH)p on PLC, amounting to a 4- and 7-fold increase in enzyme activity, respectively. VIP and SRIF had no effect on PLC activity (data not shown).

### *Hormonal modulation of second messenger systems*

The time-dependent effect of continuous hormone exposure on the responsiveness of membrane signalling systems was measured in $GH_3$ cells that were incubated for up to 48 hr with TRH, VIP or SRIF. Membrane preparations were analysed for AC and PLC activity as described in Materials and Methods, and the results are shown in Figs 2 and 3 as net modulation of effector activity [actual activity minus basal, in per cent of controls (=100%)]. Levels of AC catalytic subunit, as measured by forskolin activation, were unchanged by any of the hormone treatment schemes (data not shown). Basal and modulated AC or PLC activity in untreated cells did also not alter significantly throughout the 48-hr period (data not shown).

Figure 2 shows the effect of prolonged hormone treatment on AC responsiveness. TRH treatment homologously abolished its own ability to activate AC, but did not significantly alter the effect of the other modulators (panel A). VIP treatment more slowly down-regulated stimulatory effects on AC by TRH and VIP (panel B), but transiently enhanced Gpp(NH)p-stimulated AC activity by some 60% after 24 hr of treatment. The inhibitory effect of SRIF on AC activity was simultaneously potentiated by approximately 60%.

SRIF treatment of the $GH_3$ cells rapidly and almost completely blocked TRH activation of AC (panel C), whereas the response to VIP was reduced to approximately 40%. Inhibition by SRIF of AC activity was homologously attenuated, while Gpp(NH)p-mediated activation of AC was transiently reduced by some 50% after 6 hr of exposure.

Experiments were also carried out to determine time-dependent alterations in PLC sensitivity to TRH and Gpp(NH)p during hormonal exposure of $GH_3$ cells. The results are shown in Fig. 3 as net enzyme response relative to control (=100%). TRH (panel A) and SRIF (panel C) treatment gradually reduced the response of PLC to both TRH and Gpp(NH)p. In contrast, exposure of $GH_3$ cells to VIP (panel B) had no significant effect on the action of TRH and Gpp(NH)p on PLC.

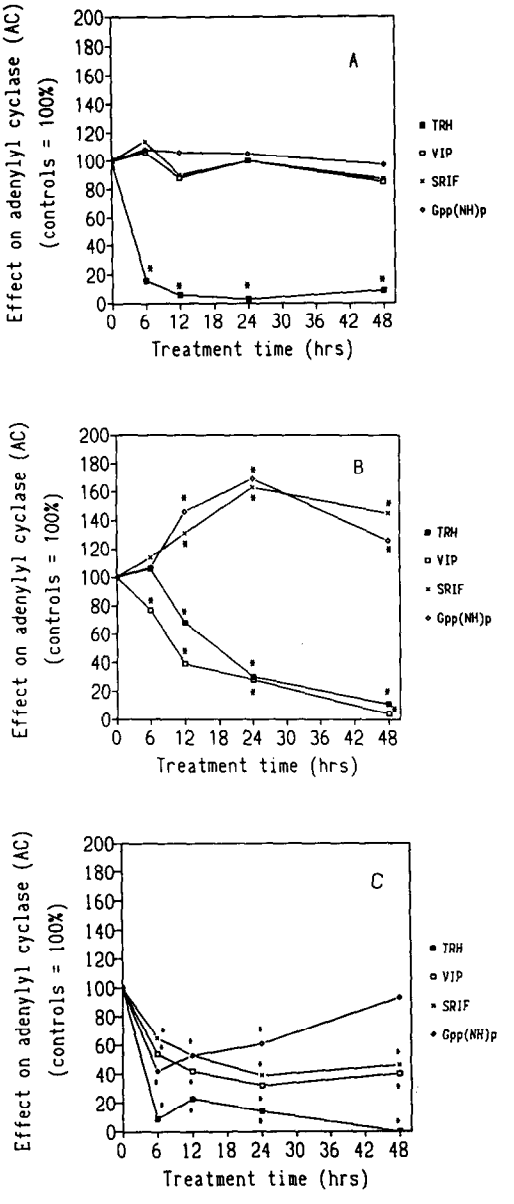


Fig. 2. The time-dependent effect of $GH_3$ cell exposure to TRH, VIP and SRIF on AC activity. AC activity in membranes from cells treated for up to 48 hr with TRH (panel A), VIP (panel B) or SRIF (panel C), as described in Materials and Methods, was measured as basal or in the presence of either TRH (1 $\mu$M), VIP (1 $\mu$M), SRIF (10 $\mu$M) or Gpp(NH)p (20 $\mu$M). The results are shown as per cent modulatory effect on AC (stimulation by TRH, VIP and Gpp(NH)p; inhibition by SRIF) or control values (= 100%) throughout the treatment period. Results are shown as means of triplicates. * Indicates $P = \alpha < 0.05$ (significant difference from controls; Wilcoxon rank test).

### *Hormonal regulation of G protein $\alpha$- and $\beta$-subunits in $GH_3$ cell membranes*

Western blot analyses were performed to quantify membrane levels of G protein $\alpha$- and $\beta$-subunits in $GH_3$ cells treated with 1 $\mu$M TRH, 1 $\mu$M VIP or

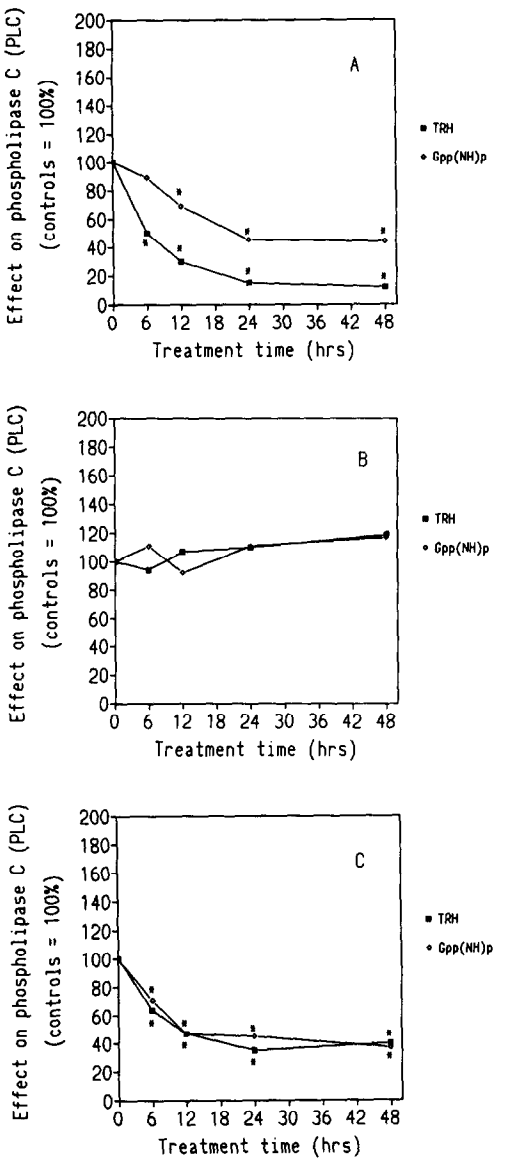


Fig. 3. The time-dependent effect of $GH_3$ cell exposure to TRH, VIP and SRIF on PLC activity in $GH_3$ cells. PLC activity in membranes from cells treated for up to 48 hr with TRH (panel A), VIP (panel B) or SRIF (panel C), as described in Materials and Methods, was measured as basal or in the presence of TRH (1 $\mu$M) or Gpp(NH)p (20 $\mu$M). The results are shown as per cent modulatory effect on PLC (stimulation by TRH and Gpp(NH)p) of control values (=100%) within the treatment period. Results are shown as means of triplicate determinations. * Indicates $P = \alpha < 0.05$ (significant difference from control; Wilcoxon rank test).

10 $\mu$M SRIF for up to 48 hr. Figure 4 shows the results of such an experiment. The effect of growing cells in Serumless Medium is also seen ($C_{sf}$).

Growth of $GH_3$ cells in the presence of either TRH or VIP altered the levels of $G_s\alpha$. We detected two forms of this $\alpha$-subunit, 46 and 42 kDa, of which the larger was the most abundant. For TRH, the increase in $G_s\alpha$ protein quantity peaked at 24 hr incubation, when the protein levels were 474 ± 12% compared to basal (C = 100%), as determined by densitometric scanning. VIP had increased $G_s\alpha$ subunit levels to steady-state (386 ± 9%) after only 6 hr. Serumless Medium itself caused a remarkable decrease in $G_s\alpha$ protein levels (non-detectable) compared with cells grown in medium containing serum. SRIF treatment gave a transient increase in $G_s\alpha$ protein levels after 12 hr compared to control ($C_{sf}$), but a complete reversal of the effect of Serumless Medium was not seen.

No significant alterations in $G_o\alpha$ protein levels (82–99%) were seen in cells treated with TRH or VIP. Growth in Serumless Medium gave a decrease in $G_o\alpha$ protein levels (62 ± 5% compared with C), whereas additional SRIF treatment caused only a small increase in $G_o\alpha$ protein levels (151 ± 6% compared with $C_{sf}$).

$G_{i\text{-}1}\alpha$ has previously been shown to be absent from $GH_3$ cells [22]; thus, the $G_{i\text{-}1}\alpha/G_{1\text{-}2}\alpha$ antiserum would detect only $G_{i\text{-}2}\alpha$ protein. TRH and SRIF had no influence on $G_{i\text{-}2}\alpha$ levels, while VIP treatment caused a rapid, lasting increase (up to 420 ± 5%) in the quantity of $G_{i\text{-}2}\alpha$ protein. $G_{i\text{-}3}\alpha$ protein levels increased after 6 hr (177 ± 6%) in the presence of TRH, with no further increase after 12–48 hr. VIP caused a similar increase in $G_{i\text{-}3}\alpha$ protein levels after 24–48 hr incubation (171 ± 5%). SRIF had no effect on $G_{i\text{-}3}\alpha$ protein levels.

Protein levels for the G$\beta$-subunits decreased to approximately 50% (53 ± 5%) after 12 hr incubation with TRH, while VIP treatment caused no significant changes in this protein. SRIF treatment resulted in a time-dependent decrease in the quantity of the G$\beta$-subunits, to almost non-detectable levels (11 ± 3%).

## DISCUSSION

Modulation of hormone-elicited second messenger responses by the hormones themselves may be due to alterations that influence receptor–G protein or G protein–effector coupling, e.g. changes in the quantity of G protein in the cell membrane, alterations in subcellular distribution or modification of the subunit *in situ*. The ability of ligands also to modify signal mechanisms not directly involved in their actions is well known. This interplay, or "cross-talk", between different membrane signalling systems play an important role in the regulation of hormone action [29–31].

Previous studies [32, 33] emphasize the use of high pharmacological doses ($10^{-7}$–$10^{-5}$) of hypothalamic hormones in cell culture systems to allow for detectable alterations in second messenger cascades leading to the synthesis and release of pituitary hormones. Such investigations may also help to predict unwanted effects on neurosecretion in patients with hormone-producing adenomas (e.g. VIPomas) or in subjects given these hormones for diagnostic or therapeutical purposes.

We have demonstrated previously the effect of TRH, VIP and SRIF on the regulation of G protein mRNA levels in $GH_3$ pituitary tumour cells [23].

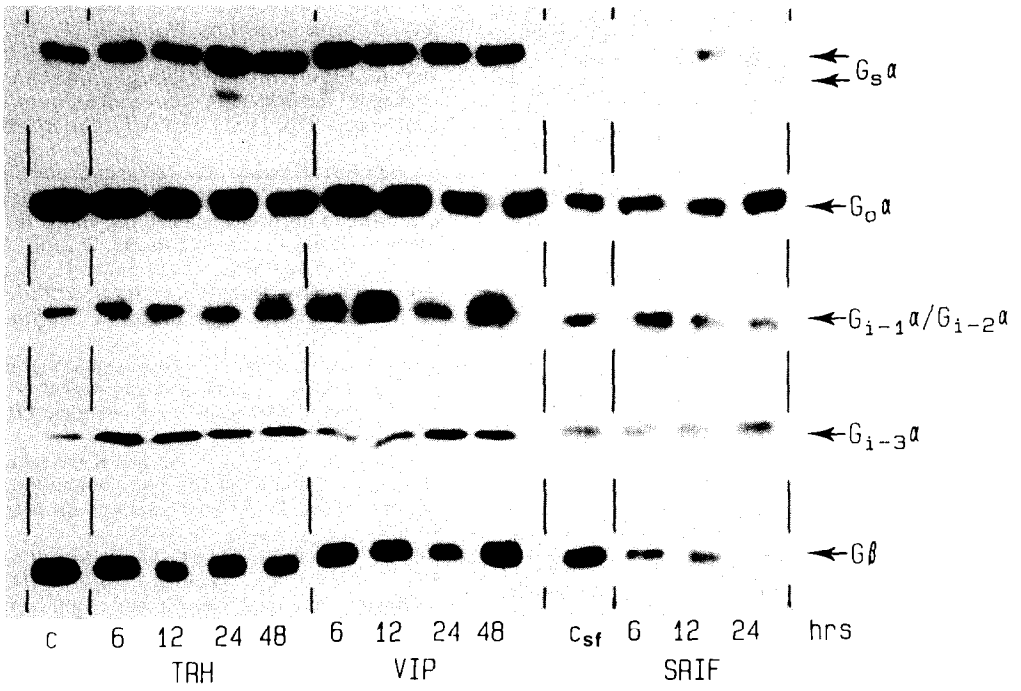


Fig. 4. Hormonal regulation by TRH, VIP and SRIF of G protein subunit levels in $GH_3$ cells. $GH_3$ cells were cultured in serum-supplemented medium without hormone (C) or in the presence of TRH (1 μM) or VIP (1 μM), or alternatively in Serumless Medium without hormone ($C_{sf}$) or with SRIF (10 μM) added. Incubation times (6–48 hr) are indicated. Crude membrane fractions (20 μg protein/lane) were separated on 10% SDS–PAGE gels and transferred to polyvinylidene difluoride membranes. The occurrence of G protein α-subunits was detected with antibodies against C-terminal decapeptides specific for $G_s\alpha$, $G_{i-1}\alpha/G_{i-2}\alpha$, $G_{i-3}\alpha$, $G_o\alpha$ and Gβ as indicated, followed by incubation with $^{125}$I-protein A and autoradiography. The results shown for each G protein are from individual experiments which were carried out for up to three times with similar results.

Thus, not surprisingly, these hormones also alter the quantity of G protein subunits themselves.

VIP more markedly and rapidly enhanced the expression of $G_s\alpha$ protein than did TRH, which may be related to its more potent and selective activation of AC in $GH_3$ cells. This increase is in accordance with the situation in thyroid follicle cells, where AC activation by TSH markedly increases $G_s$ levels [34]. TRH-enhanced AC activity was, however, homologously attenuated, in accordance with our previous findings [25], whereas VIP treatment of these cells caused a reduction in the response of AC to both VIP and TRH, although with different kinetics. It is reasonable to postulate that the rapid homologous and heterologous AC desensitization is due to receptor uncoupling as shown for other receptor–effector systems [35], such as a cAMP-mediated heterologous desensitization involving the phosphorylation of $G_s\alpha$-coupled receptors [36]. The concomitant substantial increase in $G_s\alpha$ and $G_{i\text{-}2}\alpha$ protein levels may thus be regarded as a compensatory reaction yielding enhanced Gpp(NH)p-elicited AC activity. However, we have shown previously that TRH and VIP, but not Gpp(NH)p activation of AC is attenuated by a decrease in $G_s$ [16], which calls for a more complex model in the understanding of Gpp(NH)p action.

VIP, but not TRH, caused a strong increase in $G_{i\text{-}2}\alpha$ levels that was parallelled by an enhanced inhibitory effect of SRIF on AC. This is in accordance with our assumption that SRIF solely mediates its effect on $GH_3$ cell AC through this G protein. Although conjectural, the VIP-induced reduction of AC responsiveness to TRH and VIP could be explained partially by the increase in the levels of $G_{i\text{-}2}\alpha$, which could override the concomitant rise in $G_s\alpha$.

The recently cloned TRH and VIP receptors strikingly resemble each other in parts of the third cytoplasmatic loop [37, 38], known to play a role in G protein coupling [36]. On the other hand, the size of the loop and N-terminal part of the cytoplasmic tail (C-4) differs [37, 38]. It is therefore possible that both TRH and VIP receptors couple to $G_s\alpha$, but with different AC activation potentials and uncoupling dynamics as demonstrated in this paper, as well as in our experiments employing antisense RNA inhibition of $G_s\alpha$ expression [16].

The alterations in hormone effects on PLC activation are less obviously explained by our results. The G protein involved in TRH activation of PLC (denominated $G_p$) has yet to be identified. PLC activity in HL60 cells is shown to be under the influence of a pertussis toxin-sensitive G protein [39], whereas TRH activation of the enzyme in GH cells [40] and rat 7315c cells [13] is insensitive to

pertussis toxin treatment. $G_p$ in GH cells may thus be identical to $G_z$ [41] or to $G_q/G_{11}$ [42] which has been shown to couple directly to PLC [13, 43]. TRH exposure down-regulated both the TRH- and Gpp(NH)p-enhanced PLC activity while VIP treatment did not, which indicates that cAMP is not involved in receptor–$G_p$ uncoupling in GH cells.

SRIF exposure had little effect on the quantity of G $\alpha$-subunits compared with untreated cells grown in Serumless Medium, but caused a marked reduction in the levels of G$\beta$. Contrastingly, there was a general decrease in the effect of all modulators of AC or PLC activity. A model for the interaction between $\alpha$-subunits and $\beta\gamma$-complexes includes the possibility that the latter generally promote receptor–$\alpha$-subunit interaction, and thereafter stabilize the activated G protein [3]. Accordingly, reduction of the membrane $\beta$-subunit content should lead to attenuation of all G protein-mediated signal transduction, which was also observed.

It should be noted that all experiments concerning $GH_3$ cell response to prolonged treatment with SRIF were conducted in a medium devoid of serum which enhanced prolactin mRNA levels and the rate of synthesis in $GH_3$ cells (data not shown). This growth condition was selected in order to amplify the inhibitory effect of SRIF on $GH_3$ cell physiology. We observed that growth in Serumless Medium altered the levels of certain G protein $\alpha$-subunits, most markedly those of $G_s\alpha$ and somewhat less $G_o\alpha$, as observed previously for the corresponding mRNA levels [23].

In this work we have shown that "cross-talk" between hormonal signalling systems in $GH_3$ cells partially involves changes in the levels of G protein $\alpha$- and $\beta$-subunits. The ability of hormones to activate G protein-coupled effectors is also altered parallel to a change in the ratio of G$\alpha$ to G$\beta$. We believe these events to be important in the mechanisms of peptide hormone action on $GH_3$ cells.

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