Volume 336, number 3, 433–439 FEBS 13461 December 1993

Protein phosphatase 2A reverses inhibiton of inward rectifying K^+ currents by thyrotropin-releasing hormone in GH_3 pituitary cells

Francisco Barros[a,*], Gottfried Mieskes[b], Donato del Camino[a], Pilar de la Peña[a]

[a]*Departamento de Biología Funcional, Area de Bioquímica, Facultad de Medicina, Universidad de Oviedo, 33006 Oviedo, Spain*
[b]*Georg-August-Universität, Göttingen, Abt. Klinische Biochemie, Robert-Koch-Str. 40, D-3400 Göttingen, Germany*

Received 24 September 1993; revised version received 15 November 1993

Thyrotropin-releasing hormone (TRH) reduces an inwardly rectifying K^+ current in whole-cell voltage-clamped GH_3 rat anterior pituitary cells. The TRH effect depends on the maintenance of a background level of Ca^{2+} in the pipette buffer, and is rapidly minimized by the intracellular dialysis produced under whole-cell conditions. Introduction of ADP-NH-P, a non-hydrolizable ATP analog, in the pipettes, nearly abolishes the TRH-evoked inhibition. The TRH-induced reduction of the inwardly rectifying current is significantly enhanced by incubation of cells 2–4 h with cholera toxin, but not by inclusion of 1 mM cyclic AMP in the pipettes. Under control whole-cell conditions, the reduction caused by TRH is not reversed upon washout of the neuropeptide. However, this effect is readily reversed by addition of purified catalytic subunits of protein phosphatase 2A ($PP\text{-}2A_c$) but not $PP\text{-}1_c$ to the buffer used to fill the patch pipettes. Among previous results with PP inhibitors, these data indicate that PP2A is involved in the phosphorylation/dephosphorylation mechanism(s) that regulate the delayed TRH effects on GH_3 cell excitability.

Thyrotropin-releasing hormone; Inwardly rectifying K^+ current; GH_3 cell; Protein phosphatase; Anterior pituitary

1. INTRODUCTION

Thyrotropin-releasing hormone (TRH) produces a biphasic effect on clonal rat anterior pituitary GH cells excitability, consisting of an initial phase of transient hyperplarization followed by a second phase in which the membrane conductance is decreased and the rate of production and the length of action potentials are increased [1–5]. It is believed that the increased influx of extracellular Ca^{2+} through voltage-dependent Ca^{2+} channels as a consequence of the elevation in action potential frequency is the major cause of a sustained plateau of elevated intracellular Ca^{2+} [5–8], paralleled by a sustained increase on prolactin secretion [1,7,9,10]. We have recently shown that TRH-induced reductions on the inwardly rectifying K^+ current initially described by Bauer et al. [11] play a major role in maintenance of resting membrane conductance and regulation of GH_3 cells electrical activity by TRH [4,5,12]. In these earlier reports, perforated-patch conditions were used in order to correlate voltage-clamp studies on the currents and current-clamp analysis of firing frequency, since hormonal responses and membrane currents which are rapidly diminished using the whole-cell configuration, are fully preserved when perforated patches are used [2–5,12,13]. Based on the combined use of okadaic acid and calyculin A, two potent and selective inhibitors of protein phosphatases, we have shown that the delayed effects of TRH on inward rectifying K^+ currents and hence on cell excitability, are regulated by a phosphorylation/dephosphorylation mechanism [4,12]. The concentration dependencies of okadaic acid and calyculin A effects also suggested that a type 2A protein phosphatase was the enzyme specifically involved in reversion of the TRH effects [4]. In this report, the whole-cell configuration of the patch-clamp technique is used in order to obtain a more direct control of cell contents. This approach and the use of purified catalytic subunits of protein phosphatases strengthen the evidence that the effects of TRH on inward rectifying K^+ currents are controlled by phosphorylation. Our results also indicate that $PP\text{-}2A_c$ can specifically and effectively replace the intracellular factor(s) lost by dyalisis of the intracellular medium and which cause the reversion of the TRH effects. Furthermore, the possibility that a cholera toxin-sensitive G-protein couples the TRH receptor to activation of a protein kinase involved in regulation of inward rectifying K^+ currents is discussed.

2. MATERIALS AND METHODS

TRH, ATP, ADP-NH-P, C.T. and EGTA were purchased from Sigma (St. Louis, MO, USA). Maintenance of GH_3 cells (ATTCC-CCL 82.1) has been described previously [3,12]. Whole-cell recordings were made at room temperature (20–25°C) in a recording chamber of 0.2–0.3 ml continuously perfused at 1 ml/min, using a List EPC-7 (List

*Corresponding author. Fax: (34) (8) 5103534.

Abbreviations: TRH, thyrotropin-releasing hormone; PP, protein phosphatase; PP_3, catalytic subunit of PP; PK, protein kinase; ADP-NH-P, adenosine 5′-(β, γ-imino)triphosphate; C.T., cholera toxin

Electronics, Darmstadt, Germany) amplifier. Electrodes of 2–5 MΩ were fabricated from Boralex disposable micropipettes (Rochester Scientific, Rochester, NY, USA), and filled with a solution containing (in mM): 140 KCl, 2 $MgCl_2$, 0.7 $CaCl_2$, 1.1 EGTA and 10 HEPES titrated to pH 7.4 with KOH. The amount of free Ca^{2+} in this solution amounts 200–250 nM [11]. For experiments in which a Ca^{2+}-free internal solution was used, no $CaCl_2$ was added and EGTA was raised to 10 mM. Seals were obtained in an extracellular-like solution containing (in mM): 140 NaCl, 1 KCl, 1 $MgCl_2$, 10 glucose, 10 $CaCl_2$ and 10 HEPES titrated to pH 7.4 with NaOH [12]. Immediately after breaking the patch, this solution was changed to high-K^+,Ca^{2+}-free medium in order to maximize currents through inwardly rectifying K^+ channels. This medium contained (in mM): 140 KCl, 4 $MgCl_2$, 10 EGTA and 10 HEPES titrated to pH 7.4 with KOH. In experiments designed to check the effects of PPs, the KCl concentration was reduced to 115 mM in both internal and external solution to maintain osmolarity and symmetrical K^+ concentrations. This adjustment was performed in order to compenstate the nearly 50 mM glycerol added from the PPs stocks. In these cases, extracellular and control pipette solutions also received the indicated amounts of glycerol. Cell stimulation and acquisition of membrane currents under voltage-clamp were performed using an Atari computer and commercial software (Instrutech Corp., Elmont, NY, USA). Current records were sampled every 0.5 ms and digitally filtered at 500 Hz. Data are shown without correction for leakage. Uncompensated capacitive transients were subtracted using voltage pulses of the same magnitude as those employed to generate the currents. These pulses were applied to the cell in extracellular-like solution from holding potentials between −90 and −60 mV. Series resistance compensation was deliberately not employed along the experiments. This allowed us to detect any small change in input resistance due to plugging of the pipette which could interfere with free difussion through the pipette tip. Such a circumstance, manifested by a change in kinetics and/or magnitude of the currents, would be especially relevant given the subtractive procedure used to quantify the magnitude of the inward rectifying K^+ current (see below). On the other hand, it would limit the rapid exchange of pipette and/or cellular components along the time span used in the experiments (see below). In fact, plugging of the pipette tip seemed to be propitiated by transient Ca^{2+} liberations from intracellular stores at the beginning of the application of TRH. Therefore, only experiments in which amplitude and kinetics of the voltage-dependent K^+ current tail (see below) remained stable along the whole time course of recordings were used to construct the averaged graphs shown. Data are expressed as means ± S.E. for the number of cells indicated in parentheses. Preparations of purified PP-$2A_c$ and PP-1_c were obtained as described [14–16] and stored at −20°C in a buffer containing 50% (v/v) glycerol, 0.5 mM benzamidine, 0.1 mM EGTA, 0.5 mM dithioerythritol and 20 mM Tris- HCl at pH 7.4. These preparations were diluted 1:100 (v/v) in the pipette buffer with KCl concentration reduced to 115 mM. Enzymatic activity of the stored preparations was checked with a photometric assay with *p*-nitrophenyl phosphate as substrate as described in [14]. One unit of phosphatase was defined as the enzyme activity which hydrolyzes 1 μmol of *p*-nitrophenyl phosphate in 1 min under assay conditions. The specific enzymatic activities of the stocks were 0.2 U/mg for PP-1 and 25 U/mg for PP-2A with this substrate. Given the relative enzymatic activities uing *p*-nitrophenyl phosphate and phosphorylated myosin light chains as substrates [15], this would yield a similar activity for both enzyme preparations with the phosphorylated protein. The concentrations of PPs inside the patch pipette were 0.53 and 0.28 μM for PP-1_c and PP-$2A_c$, respectively. Alkaline phosphatase from calf intestine (1×10^3 U/ml, 3.4 mU/mg) was obtained from Boehringer Mannheim (Mannheim, Germany) and diluted 1:1,000 (v/v) in the pipette buffer.

3. RESULTS AND DISCUSSION

Fig. 1 shows the effect of TRH on inward rectifying K^+ currents in whole-cell voltage-clamped GH_3 cells. As shown in Figure 1A, hyperpolarization of the membrane from −20 to −100 mV in nearly symmetrical K^+ solutions elicited inward currents showing an initial maximum, followed by a period in which they inactivate

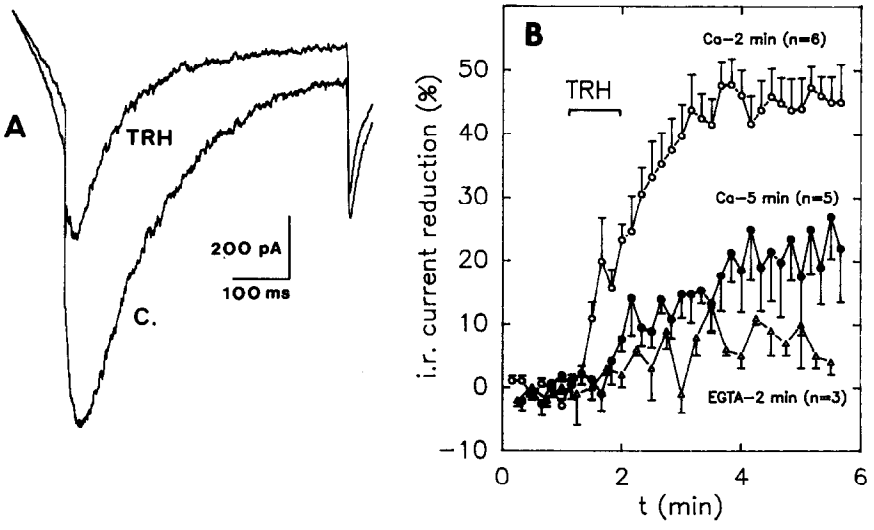

Fig. 1. Effect of TRH on inwardly rectifying K^+ currents under whole-cell clamp. (A) Representative current traces obtained just before (C) and 3 min after addition of 100 nM TRH for 40 s (TRH). 500 ms hyperpolarization pulses to −100 mV from a holding potential of −20 mV were preceded by a 100 ms voltage ramp from 0 to −50 mV. Note the inward 'tail' current elicited by returning to the holding potential after the hyperpolarization pulse (see text for explanations). (B) Time course of the TRH effect under different conditions. Whole-cell patch pipettes contained either a 200–250 nM Ca^{2+} buffer (circles, see Section 2) or a Ca^{2+}-free solution with 10 mM EGTA and no calcium added (triangles). Perfusion of the cells with 100 nM TRH is indicated by an horizontal bar. Entrance of the neuropeptide started either 2 min (open symbols) or 5 min (filled circles) after breaking the membrane patch to establish whole-cell conditions. The magnitude of the currents was obtained from the difference between peak and steady-state currents during hyperpolarization at −100 mV, and normalized to the averaged value of the data points before addition of TRH. Mean and S.E. values are indicated for every experimental time, with the number of averaged cells in parentheses.

to reach an steady state. The hyperpolarization pulse was preceded by a 100 ms voltage ramp from 0 to −50 mV, which would yield an estimation of the membrane conductance within this voltage range (see [5,12]). Furthermore, a transient inward tail current is obtained after returning to the holding potential, due to activation of the transient voltage-dependent K^+ current present in these cells. As indicated above (see Section 2) the magnitude and kinetics of this current can be used as an additional control for specificity of the effects observed on the inward rectifier, and also as a detector of input resistance changes produced along the time course of the experiments. The magnitude of the specific inwardly rectifying K^+ current can be estimated in the hyperpolarization pulse from the difference between peak and steady state currents once complete inactivation is achieved. Perfusion of the cells for nearly 1 min with a solution containing 100 nM TRH produced a reduction of the inwardly rectifying K^+ current (Fig. 1A,B). The magnitude of this reduction was strongly dependent on the technical conditions used. Maximal reductions were obtained using Ca^{2+}-containing pipettes and TRH additions 2–3 min after breaking the membrane patch to establish whole-cell conditions (see Section 2). The use of Ca^{2+}-free EGTA-containing electrodes prevented the TRH effect. Furthermore, the effect of the neuropeptide was minimized when TRH was added 5 min after breaking the membrane patch (Fig. 1B). It is important to emphasize that the reductions showed in Fig. 1B probably represent a minimum of which readily happens when time in whole-cell is prolonged. Thus, only cells showing a detectable reduction of the current have been averaged in the graph. This condition is only fulfilled by 40% of the cells after 5 min of whole-cell, which contrasts with the nearly 70% of cells showing current reductions bigger than 20% after 2 min of whole-cell. Nevertheless, only a delayed 20% reduction is detected adding TRH after 5 min of whole-cell, even although only the aforementioned 40% population of cells showing some current reduction is considered. This demonstrates that the small response to TRH once 5 min of whole-cell had elapsed is not due to choice of TRH-unresponsive cells, but is due to the longer maintenance of the cells under the intracellular dialysis condition inherent to the whole-cell configuration. It is also interesting to indicate that addition of TRH 3.5 min after establishing whole-cell did not significantly alter the initial kinetics of TRH-induced current inhibition, but reduced its magnitude to a value intermediate between those obtained after 2 and 5 min of whole-cell. Furthermore, the run-down of the hormonal effect was not prevented by addition of adenine and guanine nucleotides to the pipette buffer (data not shown). The technical limitations imposed by the need to switch the perfusion to high-K^+ Ca^{2+}-free medium (necessary to measure the inward rectifying currents) followed by recording of several control pulses at a rate (0.03 to 0.1 Hz) which does not cause accumulation of current inactivation, did not allow us to test hormonal effects at times shorter than 2 min. However, the magnitude of the current reduction at 2 min of whole-cell parallels that obtained under perforated-patch conditions [5,12]. Altogether, these data indicate that a period of 2–3 min of whole-cell before TRH addition does not greatly impair the ability of the neuropeptide to trigger the cascade of events leading to reduction of the inward rectifying K^+ currents. Therefore, this time period was used in the rest of the experiments included in this report.

Although the magnitude of the TRH-evoked reductions on the inward rectifying K^+ currents is similar both under whole-cell and perforated-patch conditions (Fig. 1 and [5,12]), it has been previously shown that the use of the whole-cell configuration makes such an inhibition irreversible even several minutes after withdrawal of the hormone [11]. The quite 'extreme' conditions (i.e. use of high-K^+ Ca^{2+}-free extracellular solutions and effective dialysis of intracellular medium) chosen for our experiments, made it very difficult to extend the recordings longer than 5–10 min. However, we confirmed previous results showing irreversibility of the current inhibition, even in cells in which washout of TRH could be performed by a period of up to 10 min (Fig. 1B and data not shown). These data indicate that although a brief dialysis of 2–3 min is not enough to eliminate from the cell the component(s) necessary to produce the inhibitory effect, the subsequent recovery of such an effect is abolished at the longer whole-cell times at which the recovery takes place.

We have concluded from our previous studies using perforated-patch recording and PP inhibitors that a phosphorylation is involved in the TRH-induced reduction of GH_3 cells inward rectifying K^+ current causing enhanced cell excitability. Furthermore, a dephosphorylation mechanism is necessary for subsequent recuperation of the TRH-evoked effects [4,12]. however, a direct evidence for participation of a protein kinase on the TRH effects has been lacking. We took now advantage of the fact that the internal medium can be influenced by the content of the pipette solution, in order to demonstrate that ADP-NH-P (a nucleotide which can not donate its gamma phosphate in phosphorylation reactions) can effectively compete with endogenous ATP and reduce the inhibition of the inward rectifying K^+ current by TRH. Introduction of 1 mM of the ATP analog in the pipette buffer did not cause any consistent effect on the TRH-evoked inhibition of the current (not shown). However, the response to TRH was largely hampered by raising the ADP-NH-P concentration up to 10 mM (Fig. 2B,C). These experiments were performed filling the pipette tip with nucleotide-free solution, followed by back-filling with nucleotide-containing buffer, since no seals were obtained after filling the whole content of the electrodes with 10 mM nucleotide-containing buffers. In order to exclude the possibility

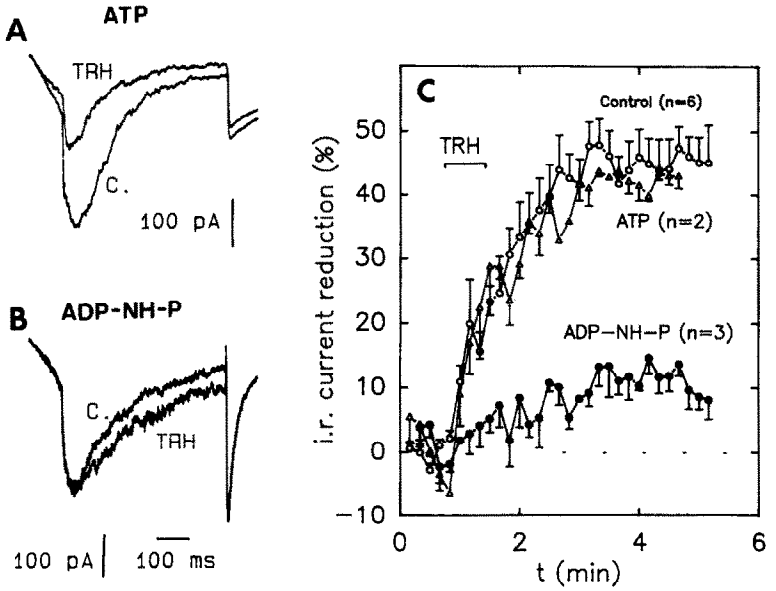

Fig. 2. Effect of adenine nucleotides on inhibition of inward rectifying K^+ currents by TRH. (A,B) Representative current traces obtained before (C) and 4 min after addition of 100 nM TRH (TRH). Same voltage protocol as that used in Fig. 1. Patch pipettes contained 10 mM of ATP (A) or ADP-NH-P (B) (see text for details). (C) Time course of the TRH effect in the absence of nucleotides (open circles) and in the presence of 10 mM ATP (triangles) or 10 mM ADP-NH-P (filled circles). Control cells as those averaged in Fig. 1 are shown for comparison.

that the ATP analog prevented the TRH effect by chelation of the submicromolar Ca^{2+} content of the pipette buffer, controls were run in the presence of 10 mM ATP. As shown in Fig. 2A,C, blockade of the TRH effect was specific for ADP-NH-P. These results demonstrate that the nucleotides are able to diffuse into the cell in the time span used in the experiments, even although the aforementioned back-filling of the patch pipettes is performed. On the other hand, they also indicate that at a concentration of 10 mM, the non-hydrolyzable ATP analog is able to effectively compete with and/or substitute intracellular ATP, adding further sup-

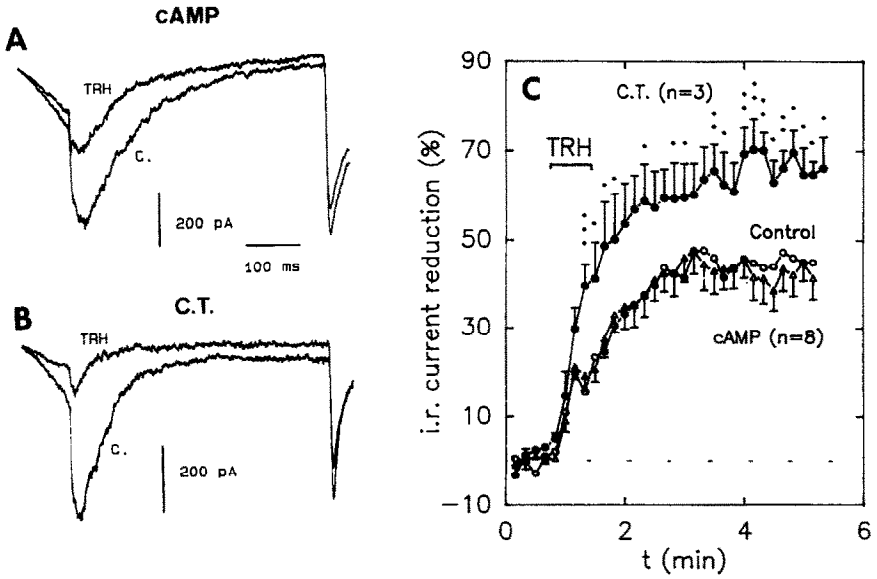

Fig. 3. Treatment with C.T., but not intracellular cAMP, enhances the TRH-induced inhibition of inward rectifying K^+ currents. (A,B) Representative current traces obtained before (C) and 4 min after addition of 100 nM TRH (TRH). Data from an untreated cell recorded with a pipette containing 1 μM cAMP (A) and a cell treated with 1 μg/ml C.T. for 3 h (B) are shown. (C) Time course of the TRH effect in cells treated 2–4 h with C.T. (filled circles) and in toxin-untreated cells recorded with pipettes containing 1 mM cAMP (triangles). Untreated cells recorded with standard pipette saline (open circles) are also shown for comparison. The triple dots near a symbol denote at least a $P < 0.001$ level of significance respect to control, a double dot $P < 0.02$ and a single dot $P < 0.05$. The values of inward rectifying currents normalized as in Fig. 1 at each time point were compared by a two-tailed Student's t-test.

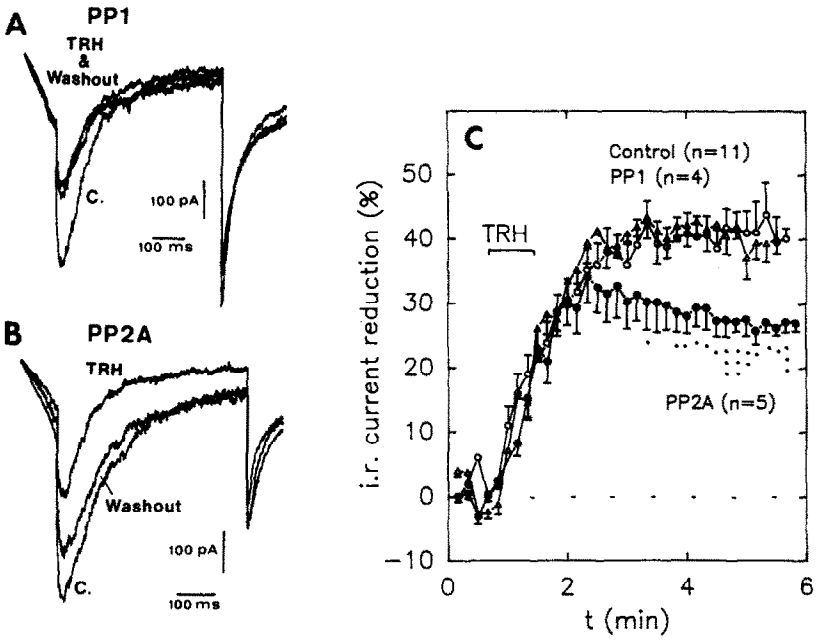

Fig. 4. Effect of different protein phosphatases on reductions of inward rectifying K^+ currents by TRH. (A,B). Representative current traces obtained before (C), 2 min after addition of 100 nM TRH (TRH) and several minutes after withdrawal of the neuropeptide (washout). Pipettes containing either PP-1 (A) or PP-2A (B) purified preparations were used. Washout times were 7 min and 10 min for (A) and (B) respectively. (C) Time course of the TRH effect in cells recorded with pipettes containing no protein phosphatase (open circles), PP-1 (triangles) or PP-2A (closed circles). Control pipettes and external buffers contained an amount of glycerol equivalent to that added with PPs. Subsequently, KCl concentrations were reduced to 115 mM. Note that this maneuver did not modify the TRH-induced inhibition. Significance levels vs. control are expressed by dots as in Fig. 3.

port to the conclusion that a phosphorylation reaction is involved in the reduction of the inward rectifying K^+ current by TRH.

The identity of the PK involved in the TRH effects on the inward rectifier is not known. It has been previously suggested that activation of PKC participates in the sustained effects of TRH [1,7,9,10] including the modulation of the K^+ channel activity, which determines the firing rate [1,9,10,17,18]. However, an effect of phosphorylation by PKC on a specific K^+ conductance has not been demonstrated in GH_3 cells. On the other hand, the reductions on inward rectifying current produced by TRH seem to be independent on PKC activation [11,12]. We have previously shown using perforated patches that the hormone-induced inhibition is potentiated by treatment of the cells with C.T. for 2–4 h [5]. The ability of C.T. to ADP-ribosylate the α subunit of G_s (the GTP-binding protein stimulatory of adenylyl cyclase) and to increase intracellular cAMP levels is well known [19]. However, neither treatment of the cells with inhibitors of PKs A and/or C, nor incubation with permeable cAMP analogs or cAMP phosphodiesterase inhibitors were able to modify the TRH-induced inhibition [5,12]. As a more direct way to check the possible participation of the cyclic nucleotide (and PKA) on TRH effects, we compared the influence of pretreatment of the cells with C.T. and that of introducing cAMP into the cell through the patch pipette. As shown in Fig. 3B,C, treatment of GH_3 cells with C.T. for 3 h significantly enhanced the inhibition caused by TRH on the inward rectifying current. However, the magnitude of the current and the inhibitory effect of TRH remained unaltered when cAMP-containing pipettes were used (Fig. 3A,C). It is important to indicate that these experiments were performed filling the whole content of the pipette with cAMP-containing solutions. The rapid diffusion of nucleotides demonstrated in the experiments shown in Figure 2, excludes that failure to detect any effect of cAMP is due to limited entrance of the nucleotide into the cell. Among previous results [5,11,12], this indicates that activation of PKA by cAMP seems not to play a major role on the TRH effects. Involvement of a receptor protein kinase such as that associated with the β-adrenergic receptor [20,21] is also not probable, since the effect of TRH remains unaltered when pipettes containing heparin are used (Barros F., unpublished). Clearly, the identity of the PK involved on regulation of inward rectifying currents remains to be established.

Regardless of the identity of the PK involved on the TRH effects, our previous results with perforated patches and PP inhibitors suggested that a dephosphorylation mechanism is necessary to reverse the action of TRH [4,12]. This and the irreversibility of the TRH effect under whole-cell conditions (this report and [11]) lead to the suggestion that a PP or a factor necessary for its activity is lost under whole-cell mode. The relative effects of different okadaic acid and caly-

culin A concentrations also opened the possibility that PP-2A was the enzyme specifically involved in reversion of the TRH effect [4]. In order to directly address these possibilities, different purified PPs were introduced into the cell through the patch pipettes. In this case, the KCl concentration of the solutions was adjusted at 115 mM to compensate the amount of glycerol (50 mM) added with the buffers used to store purified PPs in an active form. As shown in Fig. 4A,C the TRH-induced inhibition of the inward rectifying K^+ current was significantly reversed 2–3 min after retrieval of the hormone when PP-2A was included in the buffer used to fill the pipette. Although quantitatively not very large, the reversal effect was specific for PP-$2A_c$. Thus, neither PP-1_c (Fig. 4A,C), alkaline phosphatase from calf intestine, nor PP-$2A_c$ preparations heat inactivated at 100°C for 10 min (not shown) inside the pipette were able to modify the TRH effect at any time. It is also important to emphasize that the magnitude of the reversion closely resembles that obtained at the same times under perforated-patch conditions (e.g. see Fig. 1 in [5]). Although the need to perform the TRH additions after a delay no longer than 2–3 min (see above) imposes a strong limitation to these experiments, these results also demonstrate that PP-$2A_c$ can specifically and effectively replace the intracellular factor(s) lost after whole-cell dialysis of the intracellular medium.

The results obtained with ADP-NH-P, a non-hydrolyzable ATP analog, the reversion obtained in the presence of PP-$2A_c$, and our previous results with PP inhibitors [4,12], indicate that a phosphorylation is involved in the TRH-induced reduction of the inward rectifying K^+ currents. It is also clear that this effect is modified by C.T. in a cAMP-independent way. The modulation of the inward rectifying K^+ current by TRH is mediated by a G protein [11]. In addition to the well known coupling to cholera and pertussis toxin insensitive $G_{q/11}$ which activates phospholipase C [22,23], coupling of TRH receptors to a C.T.-sensitive GTP-binding protein has been reported [24]. Furthermore, although TRH acts as a poor activator of adenylate cyclase in GH_3 cells, a direct interaction of the GH_3 cell TRH-receptor with G_s-like protein has also been recently demonstrated [25]. The cAMP-independent C.T.-induced enhancement of the TRH effect could be explained by the ADP-ribosylation of a regulatory component (i.e. G_s or a G_s-like protein) of the cascade which couples the TRH receptor to inhibition of the inward rectifying K^+ current. In the absence of hormones, the covalent modification of G_s by C.T. can be much more rapid than dissociation of guanyl nucleotides [26,27]. If a similar situation takes place in vivo after a relatively short treatment of GH_3 cells with C.T., the acceleration of nucleotide exchange in the G-protein by addition of TRH without subsequent deactivation of the ADP-ribosylated transducer, could explain the enhanced inhibition of the current by the neuropeptide. It could also be expected that extension of the C.T. treatment to times at which spontaneous or C.T.-induced nucleotide exchange can take place would cause a permanent reduction of the basal G-protein-regulated current. However, we have observed that exposition of GH_3 cells to C.T. for 15–20 h abolishes the TRH-induced inhibition of the inward rectifier without a substantial reduction of the basal current (F. Barros, unpublished). As previously reported [28], it is possible that such a long treatment does not simply maintain an activated population of $G_s\alpha$ at the plasma membrane, but leads to elimination of most of the protein from the cell. Although other mechanisms of regulation can also be considered, the possibility that TRH modulates GH_3 cells inward rectifying K^+ currents, and hence cell excitability, by direct coupling of the TRH receptor to a PK via a C.T.-sensitive G-protein is currently under investigation.

Acknowledgements: This work was supported by Grants PB90–0789 from D.G.I.C.Y.T. of Spain and DFG Mi 293/1–3 of the Deutsche Forschungsgemeinschaft. D. del C. is recipient of a fellowship from F.I.C.Y.T. of Asturias (Spain).

REFERENCES

[1] Ozawa, S. and Sand, O. (1986) Physiol. Rev. 66, 887–952.
[2] Dufy, B., MacDermott, A. and Barker, J.L. (1986) Biochem. Biophys. Res. Commun. 137, 388–396.
[3] Barros, F., Delgado, L.M., Maciá, C. and de la Peña, P. (1991) FEBS Lett. 279, 33–37.
[4] Delgado, L.M., de La Peña, P., del Camino, D. and Barros, F. (1992) FEBS Lett. 311, 41–45.
[5] Barros, F., Villalobos, C., García-Sancho, J., del Camino, D. and de la Peña, P. (1993) Pflügers Arch., in press.
[6] Albert, P.R. and Tashjian Jr., A.H. (1985) J. Biol. Chem. 260, 8746–8759.
[7] Gershengorn, M.C. In: Secretion and its Control (G.S. Oxford and C.M. Armstrong, Eds.), Rockefeller University Press, NY, 1989, pp 1–15.
[8] Winiger, B.P. and Schlegel, W. (1988) Biochem. J. 255, 161–167.
[9] Drummond, A.H. In: Inositol Lipids in Cell Signalling (R.H. Michell, A.H. Drummond, M. Bushfield and C.H. MacPhee, Eds.), Academic Press, London, 1989, pp. 355–375.
[10] Bjoro, T., Sand, O., Ostberg, B.C., Gordeladze, J.O., Torjesen, P., Gautvik, K.M. and Haug, E. (1990) Biosci. Rep. 10, 189–199.
[11] Bauer, C.K., Meyerhof, W. and Schwarz, J.R. (1990) J. Physiol. 429, 169–189.
[12] Barros, F., Delgado, L.M., del Camino, D. and de la Peña, P. (1992) Pflügers Arch. 422, 31–39.
[13] Simasko, S.M. (1991) Endocrinology 128, 2015–2026.
[14] Takai, A., Murata, M., Torigoe, K., Isobe, M., Mieskes, G. and Yasumoto, T. (1992) Biochem. J. 284, 539–544.
[15] Takai, A. and Mieskes, G. (1991) Biochem. J. 275, 233–239.
[16] Braulke, T. and Mieskes, G. (1992) J. Biol. Chem. 267, 17347–17353.
[17] Gammon, C.M., Oxford, G.S., Allen, A.C., McCarthy, K.D. and Morell, P. (1989) Brain Res. 479, 217–224.
[18] Ostberg, B.C., Sand, O. Bjoro, T. and Haug, E. (1986) Acta Physiol. Scand. 126, 517–524.
[19] Moss, J. and Vaughan, M. In: G Proteins (R. Iyengar and L. Birnbaumer, Eds.), Academic Press, San Diego, CA, 1990, pp. 179–200.
[20] Benovic, J.L., Stone, W.C., Caron, M.G. and Lefkowitz, R.J. (1989) J. Biol. Chem. 264, 6707–6710.
[21] Palcewski, K. and Benovic, J.L. (1991) Trends Biochem. Sci. 16, 387–391.

[22] Hsieh, K.P. and Martin, T.F.J. (1992) Mol. Endocrinol. 6, 1673–1681.
[23] Aragay, A.M., Katz, A. and Simon, M.I. (1992) J. Biol. Chem. 267, 24983–24988.
[24] Yajima, Y., Akita, Y. and Saito, T. (1988) Mol. Pharmacol. 33. 592–597.
[25] Paulssen, R.H., Paulssen, E.J., Gautvik, K.M. and Gordelazde, J.O. (1992) Eur. J. Biochem. 204, 413–418.
[26] Burns, D.L., Moss, J. and Vaughan, M. (1982) J. Biol. Chem. 257, 32–34.
[27] Burns, D.L., Moss, J. and Vaughan, M. (1982) J. Biol. Chem. 258, 1116–1120.
[28] MacLeod, K.G. and Milligan, G. (1990) Cell. Signaling 2, 139–151.