REVIEW ARTICLES

Multiple intracellular signallings involved in regulation of ion channels by GH releasing or inhibitory hormones in pituitary somatotropes*

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Abstract Influx of Ca^{2+} via Ca^{2+} channels is the major step triggering exocytosis of pituitary somatotropes to release growth hormone (GH). Voltage-gated Ca^{2+} and K^+ channels, the primary determinants of the influx of Ca^{2+} in somatotropes, are regulated by GH-releasing hormone (GHRH) and somatostatin (SRIF) through G protein-coupled signalling systems. Using whole-cell patch-clamp techniques, the changes of the Ca^{2+} and K^+ currents in primary cultured somatotropes were recorded and signalling systems were studied using pharmacological reagents and intracellular dialysis of non-permeable molecules including antibodies and antisense oligonucleotides. GHRH increased both L-and T-types Ca^{2+} currents and decreased transient (I_A) and delayed rectified (I_K) K^+ currents. The increase in Ca^{2+} currents by GHRH was mediated by cAMP/protein kinase A system but the decrease in K^+ currents required normal function of protein kinase C system. The GHRH-induced alteration of Ca^{2+} and K^+ currents augments the influx of Ca^{2+}, leading to an increase in the $[Ca^{2+}]i$ and the GH secretion. In contrary, a significant reduction in Ca^{2+} currents and increase in K^+ currents were obtained in response to SRIF. The ion channel response to SRIF was demonstrated as a membrane delimited pathway and can be recorded by classic whole-cell configuration. Intracellular dialysis of anti-αi_3 antibodies attenuated the increase in K^+ currents by SRIF whereas anti-αo antibodies blocked the reduction in the Ca^{2+} current by SRIF. Dialysis of antisense oligonucleotides specific for αo_2 sub-units also attenuated the inhibition of SRIF on the Ca^{2+} current. The Gi_3 protein mediated the increase in K^+ currents and the Go_2 protein mediated the reduction in the Ca^{2+} current by SRIF. The SRIF-induced alteration of Ca^{2+} and K^+ currents diminished the influx of Ca^{2+}, leading to a decrease in the $[Ca^{2+}]i$ and the GH secretion. It is therefore concluded that multiple signalling systems are employed in the ion channel response to GHRH or SRIF in somatotropes, which leads to an increase or decrease in the GH secretion.

Keywords: **GHRH, somatostatin, G protein, cAMP, protein kinases.**

Growth hormone-releasing hormone (GHRH) has been shown to stimulate GH secretion in several species since it was identified about two decades ago[1, 2]. It is generally accepted that exocytosis of growth hormone (GH) from somatotropes is regulated by intracellular free Ca^{2+} concentration ($[Ca^{2+}]i$). GHRH increases the $[Ca^{2+}]i$ in pituitary somatotropes, an effect that is totally abolished by using extracellular Ca^{2+} free solution or by the blockade of Ca^{2+} channels[3~5]. The large Ca^{2+} concentration gradient across the cell membrane allows a significant increase in the $[Ca^{2+}]i$ when membrane Ca^{2+} channels are opened. It has been shown that GHRH depolarises the membrane potential of pituitary somatotropes and increases the frequency of action potentials[6, 7]. The majority of Ca^{2+} channels on the membranes of somatotropes are voltage-gated[8] and the depolarisation or generation of action potential by GHRH would be expected to trigger an influx of Ca^{2+}. Since most of the

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ionic currents across the membrane at resting potential are carried by K^+, the transmembrane K^+ channels are thought to play an important role in GHRH-induced depolarization[7, 9~11]. GHRH indeed decreases membrane voltage-gated K^+ currents[12, 13]. The effect of GHRH on the membrane ion channels is most probably mediated by second messenger systems. This is because electrophysiological responses to GHRH cannot be observed with classical whole-cell recording (WCR) but do occur during microelectrode intracellular recording[6], on-cell patch-clamp recording[14] or nystatin-perforated recording[7, 9, 12], when intracellular macromolecular second messenger systems remain undisturbed. In order to clarify the signalling systems employed by GHRH on voltage-gated Ca^{2+} and K^+ channels, patch clamp techniques have been employed to record the channel responses to GHRH in primary cultured ovine and human somatotropes.

Somatostatin (SRIF) is a well-defined inhibitor for the secretion of GH from pituitary somatotropes. A number of different ion channels in the somatotrope cell membrane have been demonstrated to be modified by somatostatin which leads to a reduction in the GH secretion[15]. One important action of SRIF is to increase K^+ currents leading to a hyperpolarisation of membrane potential and facilitate the repolarisation after action potential[16, 17]. These modifications of cell electrophysiological properties reduce the influx of Ca^{2+} via voltage-gated Ca^{2+} channels. The other important action of SRIF is to directly decrease transmembrane Ca^{2+} currents which has been observed in rat somatotropes[8, 18] and neurones[19, 20]. It has been suggested that G-proteins mediate both effects on the basis of blockade of the response by pertussis toxin treatment[8, 19, 20] and on the structure of a typical G-protein-coupled receptor[21]. Subtypes of SRIF receptors are thought to be coupled to different types of G-proteins[21] and the Gi protein is thought to mediate the effect of SRIF on K^+ channels[22]. Although Go protein has been suggested to play a role in the effect of SRIF to reduce Ca^{2+} currents and Gi protein has been suggested to increase K^+ currents in GH_3 tumour cell lines[22, 23], it is not clear as to which G-protein subtype mediates the effect of SRIF on membrane Ca^{2+} and K^+ currents in 'normal' primary cultured somatotropes. The present study aimed at resolving this issue by dialysis of antibodies against α sub-unit of G protein and antisense oligonucleotides against α sub-unit of G protein mRNA sequence into cells to block the function of certain sub-type of G proteins. In primary cultured ovine somatotropes, we found that αi_3 sub-unit of Gi_3 protein mediates the increase in the K^+ current and the αo_2 sub-unit of Go_2 protein mediates the reduction in the Ca^{2+} current by SRIF.

1 Effect of GHRH on voltage-gated Ca^{2+} and K+ currents

1.1 Ca^{2+} currents

The ovine somatotrope cultures were perfused with a bath solution containing 2.5 mmol/L Ca^{2+}, 130 mmol/L TEACl and 1 μmol/L TTX, to exclude K^+ and sodium currents. Ca^{2+} currents were recorded using nystatin-perforated Cs^+-internal whole-cell recording configuration.

Ovine somatotropes were found to have nifedipine-sensitive L-type Ca^{2+} currents and a low threshold transient T-type current. Recorded by nystatin perforated WCR, application of GHRH increased the amplitude of total Ca^{2+} currents to 146% of the control ($P < 0.05$) without modification of the activation properties (Fig. 1). The Ca^{2+} currents returned to control level about 20 min after removal of GHRH from bath solution (Fig. 1(b)). T-type Ca^{2+} currents were isolated by adding

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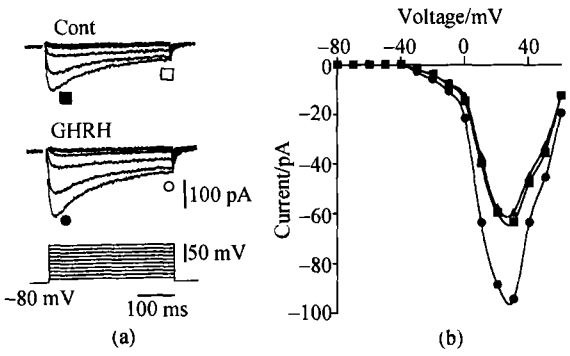

Fig. 1 Effect of GHRH on total Ca^{2+} currents. (a) From a holding potential of − 80 mV, depolarising pulses were applied as indicated at the bottom of the figure with a time interval of 3s between pulses. Upper current traces were recorded in control bath solution and middle current traces were recorded after the addition of GHRH (10 nmol/L) to the bath solution. (b) The current-voltage relationship obtained by plotting peak current against voltage of depolarising pulses in control (■) GHRH-containing (●) and 20 min after removal of GHRH from (▲) bath solution. Adapted from Ref. [7] with permission.

3 μmol/L nifedipine into the bath solution and were also increased by GHRH[7]. This effect on T-type currents was also reversible after removal of GHRH from bath solution for 20 min[7]. GHRH addition to the bath solution did not modify the voltage-dependent inactivation properties or time-dependent removal of inactivation properties. To evaluate the time taken for GHRH to increase Ca^{2+} currents, single step pulses from a holding potential of − 80 mV to + 10 mV were applied repeatedly at intervals of 10 s before and after GHRH application. The increase in Ca^{2+} current per unit membrane capacitance (pF) occurred about 30 s after GHRH application and became maximal by 2 ~ 3 minutes[7]. Incubation of cells with Rp-cAMP (100 μmol/L), a membrane permeable cAMP antagonist, for 10 min did not change the Ca^{2+} current but totally prevented its response to GHRH (Fig. 2(a)). H_{89}, a selective PKA inhibitor, at a concentration of 200 nmol/L did not modify the voltage-gated Ca^{2+} current but also prevented the Ca^{2+} current response to GHRH (Fig. 2(b)). PKC inhibitors, such as calphostin C or chelerythrine did not change the levels of voltage-gated Ca^{2+} current or the Ca^{2+} response to GHRH.

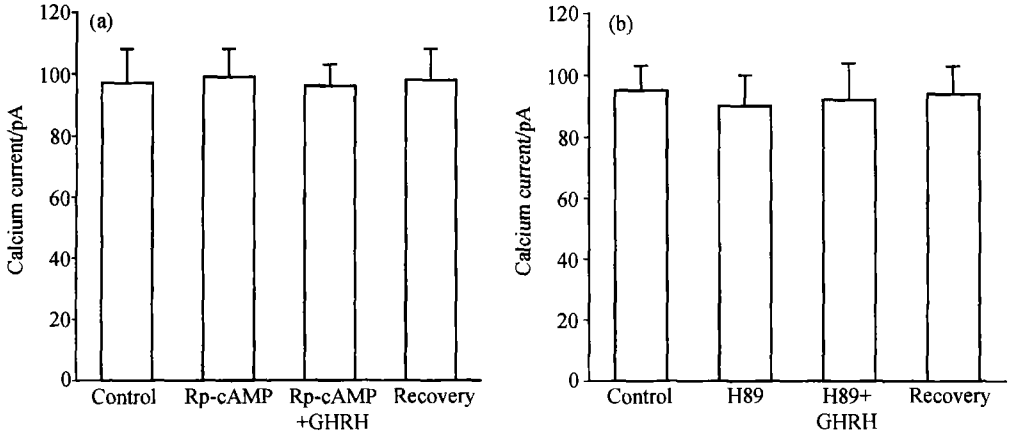

Fig. 2 Involvement of cAMP-PKA system in the Ca^{2+} current response to GHRH. Statistical data (mean ± s.e.m., $n = 5$) for the Ca^{2+} current evoked by depolarizing pulse from a hp of − 80 mV to + 10 mV. The application of (a) 100 μmol/L Rp-cAMP or (b) 1 μmol/L H_{89} did not significantly modify the recorded Ca^{2+} current. Both reagents, however, prevented GHRH from increasing the amplitude of the Ca^{2+} current. In the continued presence of Rp-cAMP or H_{89}, the amplitude of the Ca^{2+} current was unaffected by removal of GHRH.

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1.2 K^+ currents

The human somatotropes were perfused with a bath solution containing 0.5 mmol/L Ca^{2+}, 1 mmol/L Cd^{2+} and 1 μmol/L TTX, to exclude Ca^{2+} and Na^+ currents. The K^+ currents were recorded using nystatin-perforated, K^+-internal recording configuration.

When the K^+ currents were recorded from the cells held at − 80 mV and stepped to a range of test potentials (− 50 ~ + 60 mV at 10 mV increments over 400 ms), somatotropes were found to have a mixture of a voltage-gated transient K^+ current (I_A) and a delayed rectified K^+ current (I_K). The large, transient I_A current was present when the somatotropes were held at − 80 mV and stepped to potential above − 30 mV, but was absent when the cells were held at − 40 mV and stepped to the same test potential. Only the I_K current was recorded during the depolarising pulse when a holding potential of − 40 mV was used. Application of GHRH evoked a significant reduction in the amplitude of the K^+ current. The effect of GHRH occurred immediately, reaching the maximal reduction within 5 min, and the amplitude of the peak current recovered completely 10 min after the removal of GHRH. The total voltage-gated K^+ current and I_K current were evoked by depolarising pulses from a holding potential of − 80 mV and − 40 mV, respectively. These two holding potentials were then used to differentiate between the two components of the K^+ current, I_A and I_K, respectively. Typical current traces of a somatotrope are shown in Fig. 3(a). Fig. 3(b) shows the current-voltage relationships for this cell first held at − 80 mV (Fig. 3(b-i)) and then at − 40 mV (Fig. 3(b-ii)). Means ± s.e.m. of current recorded with a test pulse to + 60 mV from a group of 13 cells are shown in Fig. 3(c). GHRH treatment reduced I_A by 37.6% ± 6.3% (hp = − 80 mV, peak amplitude of the K^+ current) and I_K by 32.8% ± 8.98% (hp = − 40 mV, amplitude of steady-state K^+ current). Ten minutes after GHRH was removed and the cells perfused with bath solution, the peak amplitude of the I_A had fully recovered, whereas I_K was only partially recovered (88% of control). Several pharmacological reagents, such as 8-bromo-AMP (a membrane permeable cAMP analogue, 100 μmol/L), Rp-cAMP (a membrane permeable cAMP antagonist, 100 μmol/L), H_{89} (a selective PKA inhibitor, 1 μmol/L) or internally dialysis of PKI (a selective PKA inhibitory peptide, 10 μmol/L) did not change either the recorded K^+ current or the K^+ current response to GHRH[12, 13].

Although the treatment of the somatotrope cultures with calphostin C for 10 min did not significantly change the mean amplitude of the peak K^+ current, it abolished the GHRH-induced decrease in the K^+ current amplitude (Fig. 4(a), $n = 5$). Similarly, incubation of the somatotropes with chelerythrine for 10 min did not affect the amplitude of total K^+ current but preventing the GHRH-induced K^+ current response (Fig. 4(b), $n = 4$). Pre-treatment of cells with phorbol 12, 13-dibutyrate (PDBu) for 16 h was used to down-regulate PKC systems in the cultured cells as we have used previously[24]. Pre-treatment of cells with 4α-phorbol 12, 13-didecanoate (4αPDD) for 16 h was used as a control treatment as this phorbol has no biological activity[25]. This down-regulation of PKC did not change the voltage-gated K^+ current amplitude recorded with a test pulse to + 60 mV from a hp of − 80 mV ($n = 6$ for each group, Fig. 4(c)). The K^+ current response to GHRH was, however, totally abolished in cells pre-treated with PDBu. Treatment with 4αPDD had no effect on either the peak K^+ current amplitude or the response to GHRH (Fig. 4(c)). In contrast, a short term stimulation of PKC by 0.5 μmol/L PDBu for 10 min significantly reduced total K^+ current similar to that induced by

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GHRH but without clear recovery even 20 min after removal of PDBu (Fig. 4(d)). Again, the control 4αPDD was without effect. $PKC_{19\text{-}36}$, a selective PKC inhibitory peptide, was dialyzed into the recorded cell at a concentration of 10 μmol/L via classic WCR patch-clamp pipette. No change was found in the recorded K^+ current amplitude following this dialysis but the K^+ current response to GHRH was blocked (Fig. 4(e)). Under the same recording conditions but using a vehicle to replace PKC_{19-36}, GHRH induced a significant reduction in the total K^+ current (Fig. 4(e)).

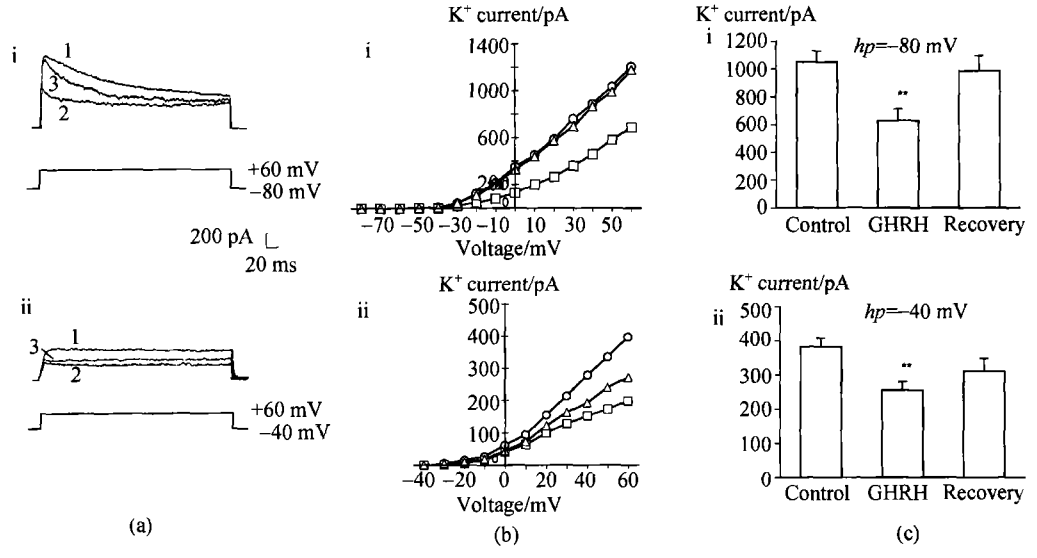

Fig. 3 Effect of GHRH on voltage-gated K^+ currents. (a) The voltage-gated K^+ currents of human somatotropes recorded using different hp of −80 mV or −40 mV. Data are shown for a representative cell. (i) The peak K^+ current traces evoked by a depolarising pulse to +60 mV from a hp of −80 mV under control (curve 1), during application of 10 nmol/L GHRH (curve 2), and 10 min after the removal of GHRH (curve 3). (ii) The steady state K^+ current traces evoked by a depolarising pulse to +60 mV from a hp of −40 mV under control (curve 1), during application of 10 nmol/L GHRH (curve 2), and 10 min after the removal of GHRH (curve 3). (b) The current-voltage relationships of the peak or steady state K^+ current recorded with a hp of −80 mV or −40 mV. Data were obtained from the same cell in panel (a). (i) Comparison of peak K+ currents at a hp of −80 mV during a depolarising pulse up to +60 mV with a 10 mV interval measured under control (○), during application of GHRH (□), and 10 min after the removal of GHRH (△). (ii) Comparison of steady state K^+ currents at a hp of −40 mV during a depolarising pulse up to +60 mV with a 10 mV interval measured under control (○), during application of GHRH (□), and 10 min after the removal of GHRH (△). Note that only a partial recovery after the removal of GHRH was observed when K^+ current was recorded at a hp of −40 mV. (c) (i) Statistical data (mean ± s. e. m., $n = 13$) for the peak K^+ current measured following depolarising pulse to +60 mV from a hp of −80 mV. GHRH (10 nmol/L) significantly (**, $P < 0.01$) reduced the amplitude of the K^+ current with a full recovery 10 min after removal of GHRH. (ii) Statistical data (mean ± s. e. m., $n = 5$) for the steady state K^+ current measured following depolarising pulse to +60 mV from a hp of −40 mV. GHRH (10 nmol/L) significantly (**, $P < 0.01$) reduced the amplitude of K^+ current with a partial recovery 10 min after removal of GHRH. Adapted from Ref. [12] with permission.

In summary, GHRH is shown to increase voltage-gated Ca^{2+} currents whereas to reduce voltage-gated K^+ currents in somatotropes. The increase in Ca^{2+} currents by GHRH is mediated by cAMP/PKA system and the decrease in K^+ currents by GHRH requires activation of PKC system . Those

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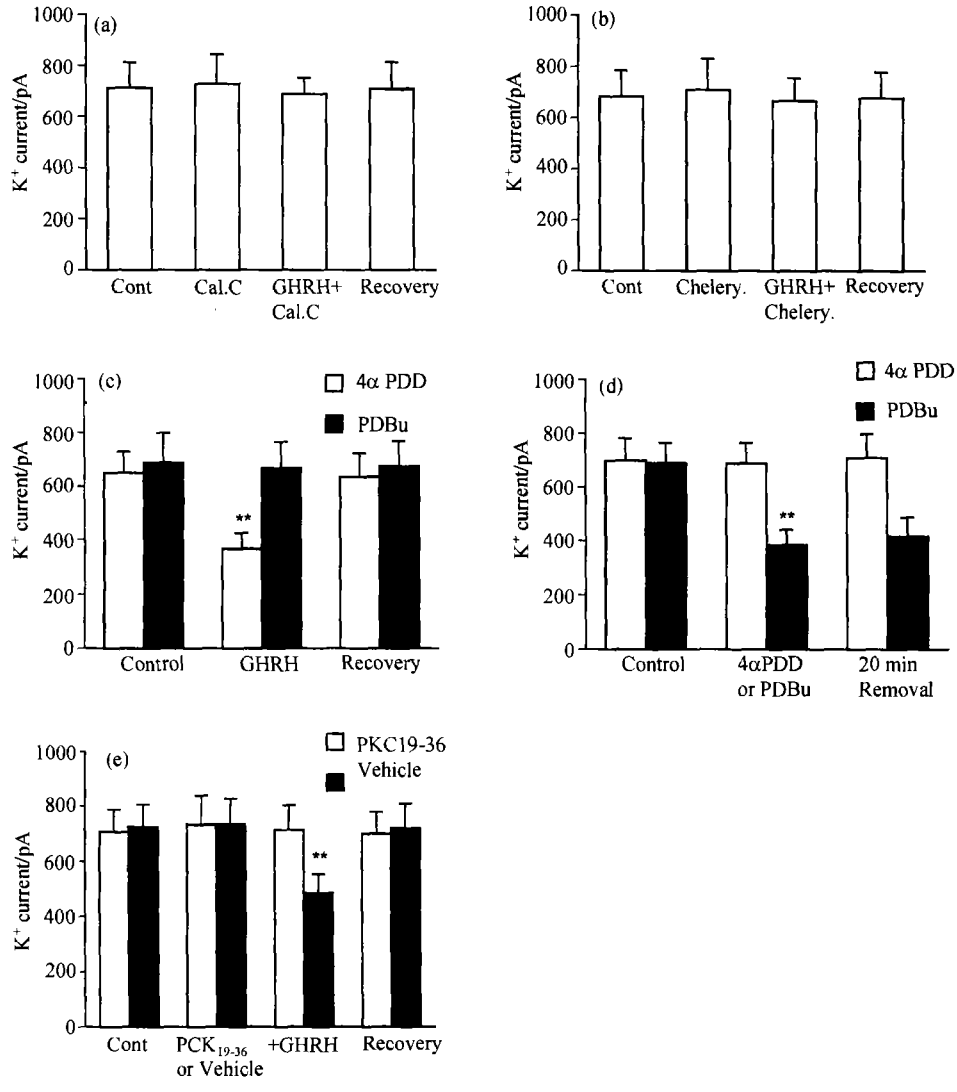

Fig. 4 Involvement of PKC system in the K^+ current response to GHRH. Statistical data (mean ± s.e.m., $n = 5$) for the K^+ current evoked by depolarizing pulse from a hp of − 80 mV to + 60 mV. The applications of 100 nmol/L calphostin C (a) or 1 μmol/L chelerythrine (b) did not significantly modify the recorded K^+ current. Both calphostin C and chelerythrine prevented GHRH from reducing the amplitude of the K^+ current. In the continued presence of calphostin C or chelerythrine, the amplitude of the K^+ current was unaffected by removal of GHRH. (c) After pretreatment of cells with 4αPDD (0.5 μmol/L, open columns) or PDBu (0.5 μmol/L, filled columns) for 16 h, voltage-gated K^+ currents were recorded from two treatment groups ($n = 6$ for each group). Statistical data (mean ± s.e.m., $n = 6$) for the K^+ current evoked by depolarizing pulse from a hp of − 80 mV to + 60 mV. No difference in voltage-gated K^+ currents was observed between two treatments under control condition. The application of GHRH (10 nmol/L) significantly and reversibly reduced the recorded K^+ current in 4αPDD-treated cells but did not modify the K^+ current in PDBu-treated cells. (d) Local application of PDBu (0.5 μmol/L, filled columns) but not 4αPDD (0.5 μmol/L, open columns) for 10 min from application pipette decreased the voltage-gated K^+ current. Statistical data (mean ± s.e.m., $n = 6$) for the K^+ current evoked by depolarizing pulse from a hp of − 80 mV to + 60 mV are given in this panel. Note that the recovery of K^+ current was not observed at least 20 min after removal of PDBu. (e) PKC inhibitory peptide, $PKC_{19\text{-}36}$, is not membrane permeable. An internal dialysis was therefore used to introduce it to the recorded cell. Conventional WCR configuration was employed in this study as detailed in the Materials and Methods. Columns in this panel show statistical data (mean ± s.e.m., $n = 4$) for the K^+ current evoked by depolarizing pulse from a hp of − 80 mV to + 60 mV. Inclusion of $PKC_{19\text{-}36}$ (10 μmol/L) in internal solution (filled columns) for 15 min did not significantly modify the recorded K^+ current and GHRH did not modify the amplitude of the K^+ current in presence of $PKC_{19\text{-}36}$ in the recorded cells. In a group of cells recorded without $PKC_{19\text{-}36}$ in electrode (open columns), GHRH reversibly reduced the K^+ current. Adapted from Ref. [12] with permission.

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signalling systems are illustrated in Fig. 5, which demonstrates a diversity of intracellular signalling systems employed by GHRH to regulate voltage-gated Ca^{2+} or K^+ channels. Such an increase in Ca^{2+} current and decrease in K^+ current cause an elevation of Ca^{2+} influx and thereby the GH secretion.

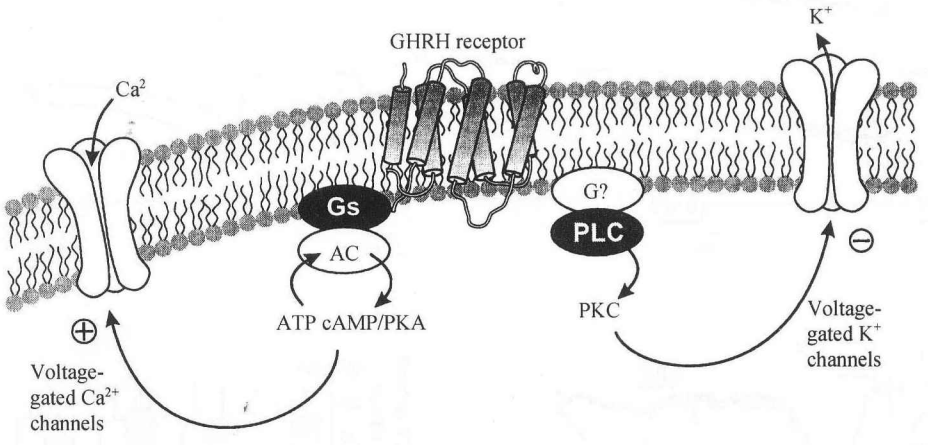

Fig. 5 Mechanism of the action of GHRH on Ca^{2+} and K^+ channels-coupling with PKA and PKC systems. This diagram illustrates the coupling of the Ca^{2+} and K^+ channels with GHRH receptors. Using the extracellular and intracellular applied pharmacological reagents against different steps in PKA or PKC signalling systems, we have demonstrated that cAMP-PKA system mediates the action of GHRH on voltage-gated Ca^{2+} currents and PKC system is essential for the action of GHRH on voltage-gated K^+ currents in somatotropes.

2 Effect of SRIF on voltage-gated Ca^{2+} and K^+ currents

2.1 Ca^{2+} currents

As discussed above, two types of Ca^{2+} currents were recorded as T and L types with L current as the majority[7]. Local administration of SRIF reduced both T and L currents without preference. We have shown that the reduction in Ca^{2+} currents by SRIF was completely abolished by the treatment of the culture with pertussis toxin (100 ng/mL) for 10 h[8]. This result indicates that the functional expression of SRIF on Ca^{2+} currents is mediated through activation of pertussis toxin sensitive G proteins. We have also shown that[26] with WCR using anti-αo antibodies in the pipette, a reduction in Ca^{2+} currents by SRIF was obtained within 5 min after establishing WCR (Fig. 6). This response diminished after 20 min of dialysis of anti-αo antibodies, when SRIF was given a second time. With anti-αi_{1-2}, anti-αi_3 or heat-inactivated anti-αo antibodies in the electrode solution there was no effect on the reduction in Ca^{2+} currents by SRIF either just after WCR formation or after 20 min of dialysis (Fig. 6). In order to identify the subtype of Go proteins involved in the Ca^{2+} current response to GHRH, we developed antisense strategies to specifically target on αo1 or αo2 sub-units whereas the specific antibodies were not available[26]. At the time of dialysis with antisense oligonucleotides (1 μmol/L), recordings were made of voltage-gated Ca^{2+} currents and the response to SRIF. In order to study the time course of the effect of antisense dialysis, αo antisense (ASm) was dialysed into the cell when initial response to SRIF was recorded[26]. Cell dialysis lasted for 5 min, which was sufficient for the dialysis of the antisense oligonucleotides. At the end of the dialysis period, the patch

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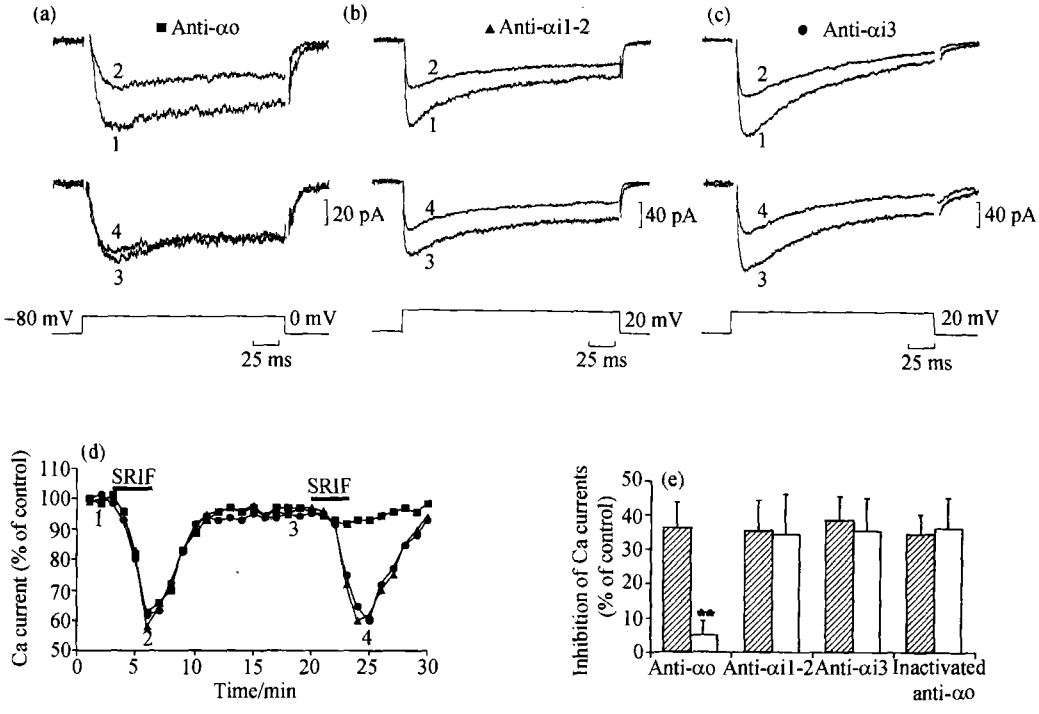

Fig. 6 Effect of intracellular dialysis of anti-α sub-unit of G - protein antibodies on SRIF-induced reduction in Ca^{2+} current. Ca^{2+} current was evoked by depolarising pulses from a holding potential of − 80 mV to 0 mV, with an electrode solution containing anti-αo sub-unit antibodies for intracellular dialysis (a). Ca^{2+} current was evoked by depolarising pulses from a holding potential of − 80 mV to 20 mV with electrode solution containing anti-αi1-2 sub-unit antibody (b) or anti-αi3 sub-unit antibody (c) for intracellular dialysis. In (a) ~ (c), trace 1 represents the control current; trace 2 is the current after the first application of 10 nM SRIF; trace 3 is the current recorded about 20 min after establishing WCR; and trace 4 is the current after the second application of 10 nmol/L SRIF (following trace 3). (d) Ca^{2+} current-time relationships during intracellular dialysis of anti-αi3 sub-unit (●), anti-αo sub-unit (■) or anti-αi1-2 sub-units (▲) antibodies. Ca^{2+} current was recorded every min and shown as a percentage of control current (peak value, 100%). Numbers on the curve represent the times at which inward currents were passed to derive the data shown in panels (a) ~ (c). (e) (Mean ± s.e.m.) percentage inhibition of peak Ca^{2+} current by 10 nmol/L SRIF during the first application of SRIF (filled columns) or 20 min after establishing WCR (open columns) during the intracellular dialysis with anti-αo ($n = 8$), anti-αi1-2 ($n = 5$), anti-αi3 ($n = 8$) or heat-inactivated anti-αo ($n = 8$) antibodies. ** : $P < 0.01$. Adapted from Ref. [26] with permission.

pipette was carefully withdrawn, and usually the cell resealed itself. Distinctive marks were made on recorded dishes to locate the dialysed cells for subsequent recording. The cells were then incubated in the presence of serum for 12, 24, 48, and 72 h, the cells that survived dialysis were re-patched for a second time for the response to SRIF. A sharp reduction in the effect of SRIF on the Ca^{2+} current was observed after 24 h of incubation, reached a peak after 48 h incubation, and partially recovered after 72 h of incubation[26]. The incubation time of 48 h was then selected to study the effect of different antisense oligonucleotides dialysis on the response to SRIF. After dialysis of αo ASm or αo_2 AS, there was a reduced effect of SRIF on Ca^{2+} currents compared with the initial response to SRIF (Fig. 7). In contrast, after dialysis of αi_3 AS or αo_1 AS, the SRIF response was not altered (Fig. 7). No

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kinetic changes were obtained after dialysis of antisense oligonucleotides.

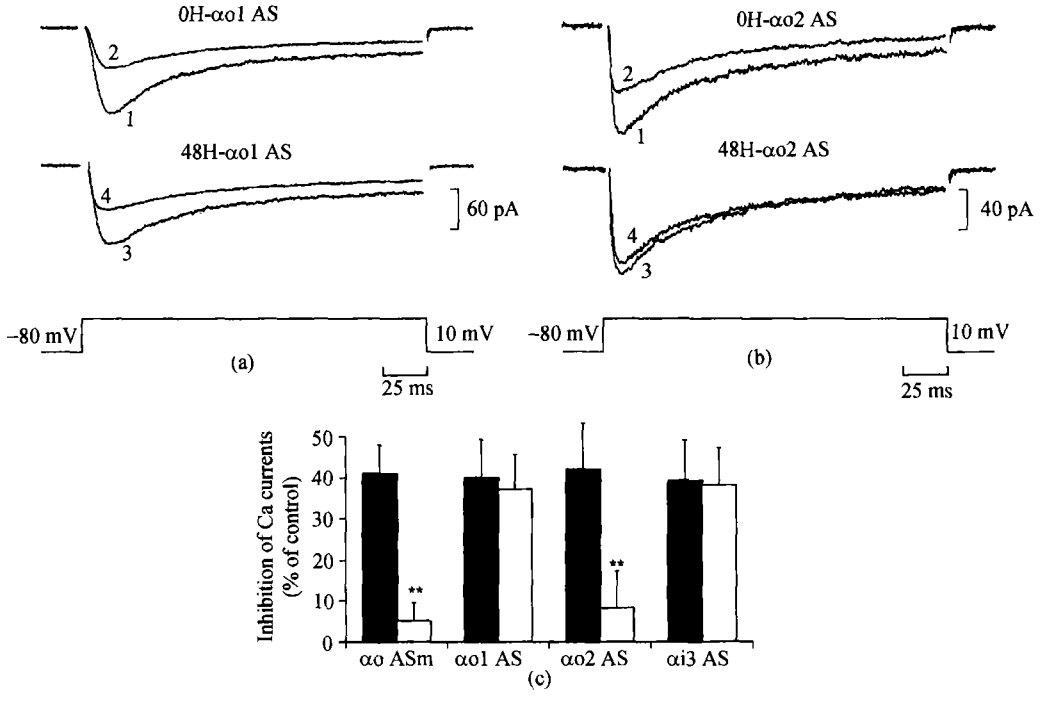

Fig. 7 Ca^{2+}-current inhibition by SRIF in ovine somatotrophs 48 h after dialyses of antisense oligonucleotides directed against mRNAs encoding αo, αo1, αo2, and αi3 sub-units. Ca^{2+} current was evoked by depolarising pulses from a holding potential of -80 mV to 10 mV with an electrode solution containing αo1 AS (a) or αo2 AS (b) for intracellular dialysis. Trace 1 represents the control current; trace 2 is the current after the application of 10 nmol/L SRIF during initial recording; trace 3 is the current recorded 48 h after dialysis; and trace 4 is the current after the second application of 10 nmol/L SRIF. (c) (Mean ± s.e.m.) percentage inhibition of peak Ca^{2+} current by SRIF during the dialysis of antisense oligonucleotides (filled columns) or 48 h after the dialysis (open columns) with αo ASm ($n = 7$), αo1 AS ($n = 4$), αo2 AS ($n = 4$), or αi3 AS ($n = 5$). **: $P < 0.01$. Adapted from Ref. [26] with permission.

2.2 K^+ currents

SRIF causes a reversible increase in voltage-gated K^+ currents including both I_A and I_K currents[17, 27]. In ovine somatotropes, those two types of K^+ currents were recorded with I_K as the majority[27, 28]. Local administration of SRIF (10 nmol/L) increased both I_A and I_K without preference and this increase was reversible after removal of the SRIF[27]. When the GTP-γ-s was included in the electrode solution (200 μmol/L), application of SRIF increased the K^+ currents but this increase was not reversible after removal of the SRIF. When the same concentration of GDP-β-s was included in the electrode solution, the increase in the K^+ current by SRIF was totally abolished. These effects of GTP-γ-s and GDP-β-s dialyses on the K^+ current response to SRIF strongly indicate an involvement of G-proteins.

With WCR using anti-αi_{1-2} or anti-αi_3 antibodies in the pipette, an increase in K^+ currents by SRIF was obtained within 5 min after establishing WCR (Fig. 8). This response diminished after

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25 min of dialysis of anti-αi_3 antibodies, when SRIF was given a second time (Fig. 8). With anti-αi_{1-2} antibodies in the electrode solution there was no modification of the increase in K^+ currents by SRIF either just after WCR formation or after 25 min of dialysis (Fig. 8). Dialysis of these antibodies did not change the basal K^+ current recorded by depolarising membrane potentials from a holding potential of -80 mV. These data therefore indicate that Gi_3 protein mediates the action of SRIF on the voltage-gated K^+ current[27].

We conclude that the effect of SRIF on voltage-gated K^+ currents in ovine somatotropes is mediated by αi_3 sub-unit of Gi_3 proteins and on voltage-gated Ca^{2+} currents is by αo_2 sub-unit of Go_2 proteins. The mechanism of action of SRIF on Ca^{2+} and K^+ currents is summarised and illustrated in Figure 9.

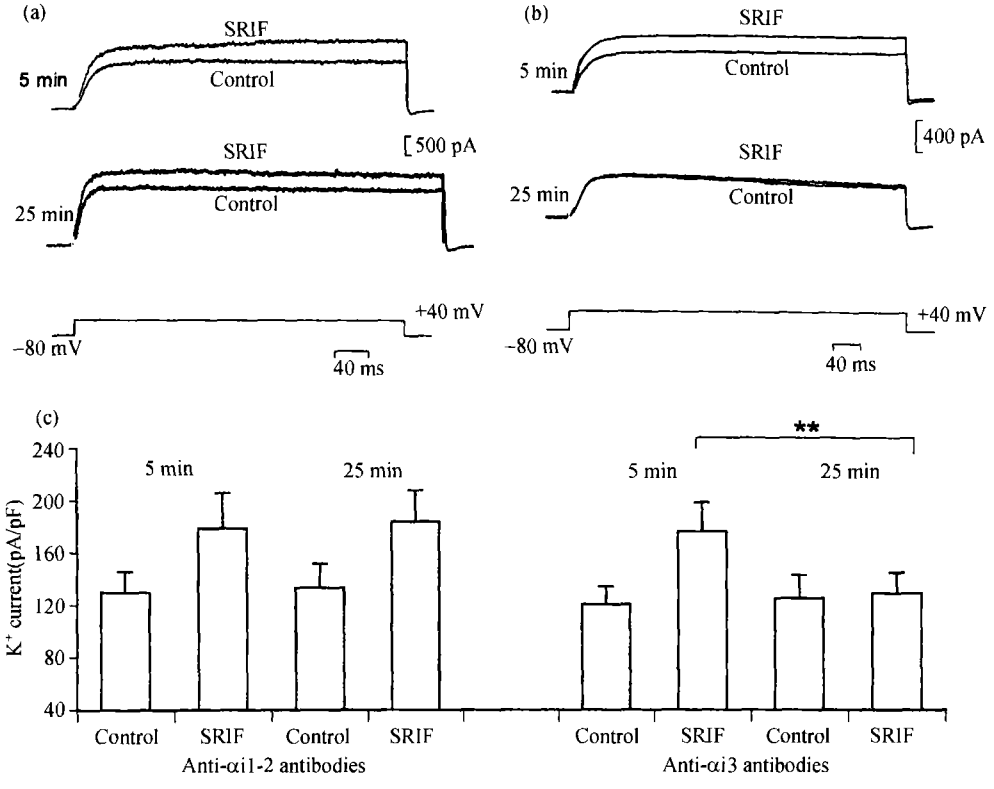

Fig. 8 Effect of intracellular dialysis of anti-αi1-2 or anti-αi3 sub-units of G-protein antibodies on SRIF-induced increase in the K^+ current. The K^+ current was evoked by depolarizing pulses from a holding potential of -80 mV to $+40$ mV with an electrode solution containing anti-αi1-2 antibody (a) or anti-αi3 antibody (b). SRIF was applied twice within 5 min after establishing WCR and at 25 min after establishing WCR. Trace 'SRIF' is the current after the application of 10 nmol/L SRIF. (c) is the K^+ current (calculated for unit membrane capacitance, pF) evoked by the same depolarising pulse with an electrode solution containing antibodies against αi1-2 or αi3 sub-unit of G-protein for intracellular dialysis. Data shown are mean($\pm$ s.e.m.) peak K^+ current (marked by control) and the K^+ current in presence of 10 nmol/L SRIF (marked by SRIF) during the first application of SRIF (5 min) or 25 min after establishing WCR (25 min) during the intracellular dialysis with anti-αi1-2 ($n = 5$), anti-αi3 ($n = 8$) antibodies. **: $P < 0.01$. Adapted from Ref. [27] with permission.

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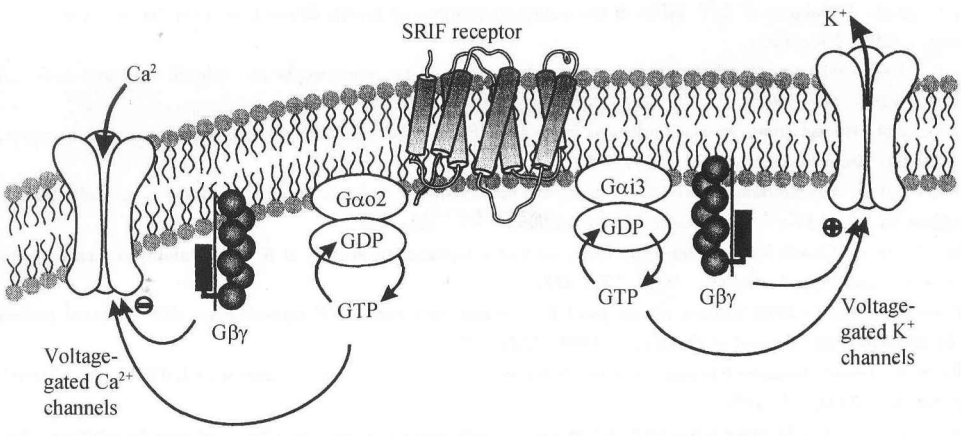

Fig. 9 Mechanism of the action of SRIF on Ca^{2+} and K^+ channels-coupling with Go_2 and Gi_3 proteins. This diagram illustrates the coupling of the Ca^{2+} and K^+ channels with somatostatin receptors. Using the intracellular dialysis of antibodies and antisense oligonucleotides against different α sub-units of G proteins, we have demonstrated that αo_2 sub-unit of Go_2 protein mediates the action of SRIF on voltage-gated Ca^{2+} currents and αi_3 sub-unit of Gi_3 protein mediates the action of SRIF on voltage-gated K^+ currents in ovine somatotrophs.

3 Final conclusion

Our study clearly demonstrated the modification of voltage-gated Ca^{2+} and K^+ channels by GHRH and SRIF in opposite directions. The detailed signalling systems also have been studied using extracellular applied pharmacological reagents, intracellular applied peptidergic protein kinase inhibitors, antibodies or antisense oligonucleotides. Detailed studies of certain signalling molecules at single cell level are clearly able to resolve which signalling systems mediate which responses and the dialyses of specific peptidergic inhibitors, antibodies or/and antisense oligonucleotides via patch-clamp electrode provide a powerful approach in this regard. The technique of intracellular dialysis can also be used in other studies, such as direct dialysis of intracellular signalling molecules or the peptide fragment of receptor responsible for the cascade of stimulation by activation of the receptor.

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