G_o-2 protein mediates the reduction in Ca^{2+} currents by somatostatin in cultured ovine somatotrophs

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1. Somatotroph-enriched cells (up to 85%) were obtained from ovine pituitary glands by means of collagenase dissociation and Percoll-gradient centrifugation. Further identification was based on the reduction in Ca^{2+} currents by 10 nM somatostatin (SRIF).

2. The whole-cell configuration of the patch-clamp technique was employed to study the membrane Ca^{2+} currents with K^+ ions replaced by Cs^+ and the addition of K^+ and Na^+ channel blockers in bath and pipette solutions.

3. A significant reduction in Ca^{2+} currents was obtained in response to local application of SRIF (10 nM) but vehicle application had no effect.

4. Intracellular dialysis of antibodies to α_o, α_i-1–2, or α_i-3 subunits of G proteins into the cells via patch-clamp pipettes was confirmed by immunofluorescent staining of the antibodies. Antibody dialysis did not modify resting voltage-gated Ca^{2+} currents across the cell membrane.

5. Dialysis of anti-α_o antibodies significantly attenuated the reduction in Ca^{2+} currents that was obtained upon application of 10 or 100 nM SRIF. Dialysis of neither anti-α_i-1–2 nor anti-α_i-3 antibodies diminished the effect of SRIF on Ca^{2+} currents.

6. Intracellular dialysis of antisense oligonucleotides directed against the α_o subunit mRNA (α_o ASm, for α_o common) or against the α_i-3 subunit mRNA (α_i-3 AS) blocked expression of α_o or α_i-3 subunits in the cells, respectively, as assessed by fluorescent staining with anti-α_o or anti-α_i-3 antibodies 48 h after dialysis.

7. Dialysis of α_o ASm, but not α_i-3 AS, significantly diminished the inhibitory effect of SRIF on Ca^{2+} currents. This effect of α_o ASm dialysis occurred within 12 h after dialysis and reached a maximum at 48 h; partial recovery was seen at 72 h.

8. Antisense oligonucleotides specific for α_o-1 (α_o-1 AS) or α_o-2 (α_o-2 AS) were dialysed into somatotrophs and only α_o-2 AS significantly attenuated the inhibition of Ca^{2+} currents by SRIF.

9. We conclude that the G_o-2 protein mediates the effect of SRIF on Ca^{2+} currents in ovine somatotrophs in primary culture.

A number of different ion channels in the somatotroph cell membrane are modified by somatostatin (SRIF) leading to a reduction in growth hormone (GH) secretion (Chen, Vincent & Clarke, 1994*b*). One important action of SRIF is to decrease transmembrane Ca^{2+} currents, which has been observed in rat somatotrophs (Nussinovitch, 1989; Chen, Zhang, Vincent & Israel, 1990*a*) and neurones (Ikeda & Schofield, 1989; Surprenant, Shen, North & Tatsumi, 1990). It has been suggested that G proteins mediate this response on the basis of blockade of the response by pertussis toxin treatment (Ikeda & Schofield, 1989; Surprenant *et al.* 1990; Chen *et al.* 1990*a*) and because the SRIF receptor has a structure that typically couples to G

proteins (Bell & Reisine, 1993). Subtypes of the receptor are thought to be coupled to different types of G protein (Bell & Reisine, 1993) and the G_i protein is thought to mediate the effect of SRIF on K^+ channels (Yatani, Codina, Sekura, Birnbaumer & Brown, 1987). Although it has been suggested that G_o protein plays a role in the effect of SRIF to reduce Ca^{2+} currents in GH_3 tumour cells (Kleuss, Hescheler, Ewel, Rosenthal, Schultz & Wittig, 1991), it is not clear which G protein subtype mediates the effect of SRIF on membrane Ca^{2+} currents in 'normal' somatotrophs in primary culture. The present study aimed to resolve this issue by dialysis of antibodies against the α-subunit of G protein and antisense oligonucleotides for the α-subunit of

G protein mRNA sequence into cells to block the function of certain subtypes of G proteins. In cultured ovine somatotrophs, we found that the α_0 subunit of G_0 protein (G_0-2) mediates the reduction in the Ca^{2+} current by SRIF.

METHODS

Cell preparation

Sheep pituitaries were obtained from a local abattoir and then subjected to collagenase–pancreatin treatment to liberate cells as described previously (Chen, Hayward, Zhang, Wu & Clarke, 1994a). The yield was usually more than 10^7 cells per pituitary gland, with greater than 90% viability (Trypan Blue exclusion test). The cell suspension (3–5 ml) was placed, under sterile conditions, above a layered column of Percoll solutions of increasing density and centrifuged as described previously (Chen et al. 1994a). Fractions which contained up to 85% of somatotrophs (1 and 2) were used in these experiments (Chen et al. 1994a). Electrophysiological recordings were made after 4–14 days in culture in a humidified incubator (37 °C, 95% air–5% CO_2). In each case penicillin and streptomycin together were used in the culture for the first 24 h in vitro. The culture medium was then changed every 48 h using Dulbecco's modified Eagle's medium plus 10% sheep serum and 2% fetal calf serum.

Antisense oligonucleotides

Oligodeoxyribonucleotides were synthesized on a PCR-MATE Synthesizer system (Applied Biosystems, CA, USA) and purified using an oligonucleotide purification cartridge (OPC; Applied Biosystems). Purified oligonucleotides were reconstituted in distilled water at a concentration of 100 μM, as verified by spectrophotometry. Four antisense oligonucleotides were used as anti-α_0 (α_0 ASm; 3'-CCC CCC TCC TAC CCT ACA-5'), anti-α_1-3 (α_1-3 AS; 3'-CAG TAC CCG ACG TGC AAC-5'), anti-α_0-1 (α_0-1 AS; 3'-TTG TGG ATA CTT CTA CGT CGA CGG A-5') and anti-α_0-2 (α_0-2 AS; 3'-TCA CGG AAG TGT CTT CGA CAC CGA G-5') which have been reported previously (Hsu et al. 1990; Kleuss et al. 1991; Baertschi, Audigier, Lledo, Israel, Bockaert & Vincent, 1992). A concentration of 1 μM was used in the electrode solution for dialysis.

Cell dialysis and experimental design

Cell dialysis via patch pipettes of molecules of various molecular weight was carefully studied by Pusch & Neher (1988). From their equations, 72% of the antisense concentration in the patch pipette was dialysed into the cell within 5 min using an electrode of resistance of 5 MΩ. We therefore used a dialysis time of 5–8 min to allow adequate (about 0·8 μM) transfer of the antisense oligonucleotides into the cell. During this dialysis time, the first series of whole-cell recordings (WCRs) were made to determine the Ca^{2+} current responses to the local application of 10 nM SRIF. The medium was then changed and cells were incubated for 12–72 h before a second series of WCRs were performed on the same cells and the Ca^{2+} current responses to SRIF were reassessed.

Specific antibodies to α_0, α_1-1–2 or α_1-3 subunits were used in the electrode solution at a concentration of 1:100 in the presence of bovine serum albumin (0·5%). Anti-α_0 antibodies (GC/2; supplied by DuPont), were specific for the α_0 subunit without cross-reactivity to α_1-1, α_1-2, α_1-3 or α_s subunits. Anti-α_1-1–2 antibodies (AS/7; DuPont) had no cross-reactivity to α_0, α_s or α_1-3 subunits.

Anti-α_1-3 antibodies (to C-terminal 345–354; CalBiochem) had no cross-reactivity to α_1-1, α_1-2, α_0 or α_s subunits. Because of large molecular weights of these antibodies, 20 min of dialysis time was required. Transmembrane Ca^{2+} currents were recorded by depolarizing the membrane potential to 0 or +20 mV for 200 ms from a holding potential of −80 mV. Depolarizing pulses were applied at 1 min intervals. The first application of SRIF was made within 5 min of establishing a WCR and a second application was made after 20 min (i.e. 20 min after antibody dialysis).

Immunocytochemistry

An immunofluorescent staining technique was used to verify the dialysis of antibodies into the cells. After electrophysiological recordings with electrode solution containing antibodies (1:100), recorded cells were washed in phosphate-buffered saline (PBS) and fixed in 4% paraformaldehyde for 15–30 min. The cells were then incubated in 50:50 acetone–methanol for 3 min, washed with PBS, and then incubated for 30 min in PBS-containing 5% horse serum to eliminate non-specific binding. After further washing with PBS, the cells were exposed to a 1:30 (as suggested by supplier) dilution of fluorescein isothiocyanate (FITC)-conjugated anti-rabbit IgG antibodies for 1 h at room temperature, then mounted under coverslips in 70% glycerine for storage at 4 °C prior to viewing. Negative control was performed on the dishes without antibody dialysis and no cells stained positive. Only cells dialysed with rabbit antibodies stained positive.

A similar technique was used to verify the intracellular dialysis of antisense oligonucleotides. After the second series of recordings, the cells were washed in PBS, fixed in 4% paraformaldehyde for 15–30 min and then incubated in 50:50 acetone–methanol for 3 min. The cells were washed in PBS, incubated for 30 min in PBS containing 5% horse serum to eliminate non-specific binding and washed again in PBS. The cells were then exposed for 12 h at 4 °C to the relevant primary antibody (against the α_0 or α_1-3 subunits of G proteins, as mentioned above), diluted to 1:100 in PBS containing 0·5% BSA (several dilutions such as 1:200, 1:500 and 1:1000 were also tested with less satisfaction). FITC-conjugated anti-rabbit IgG antibodies were applied for 1 h at room temperature, and the cells were mounted under coverslips in 70% glycerine for storage at 4 °C prior to viewing. Antibodies to α_0 or α_1-3 recognized all the endocrine cells in culture with a very weak staining of flat cells (which might be fibroblasts and which served as a negative control). Cells dialysed with antisense oligonucleotides to α_0 or α_1-3 mRNA for 48 h also showed a weak staining with corresponding primary antibodies (see Results for details).

Electrophysiological recording

Transmembrane currents were recorded using the 'gigaseal' patch-clamp technique in the whole-cell recording (WCR) configuration (Hamill, Marty, Neher, Sakmann & Sigworth, 1981) and the peak value of each current trace is reported in this paper. All recordings were made using the Axopatch-1C amplifier and electrodes were pulled by a Sutter P-87 microelectrode puller from borosilicate micropipettes coated with wax and fire-polished. The tip resistance of the electrode filled with internal solution ranged from 2 to 3 MΩ. Recordings were made on the stage of an Olympus inverted microscope. The bath solution was composed of the following (mM): NaCl, 120; tetraethylammonium chloride (TEACl), 20; $CaCl_2$, 2·5; KCl, 5; $MgCl_2$, 0·5; Hepes, 10; glucose, 10; and 1 μM TTX; pH 7·4 and 310 mosmol l^{-1}. The electrode solution
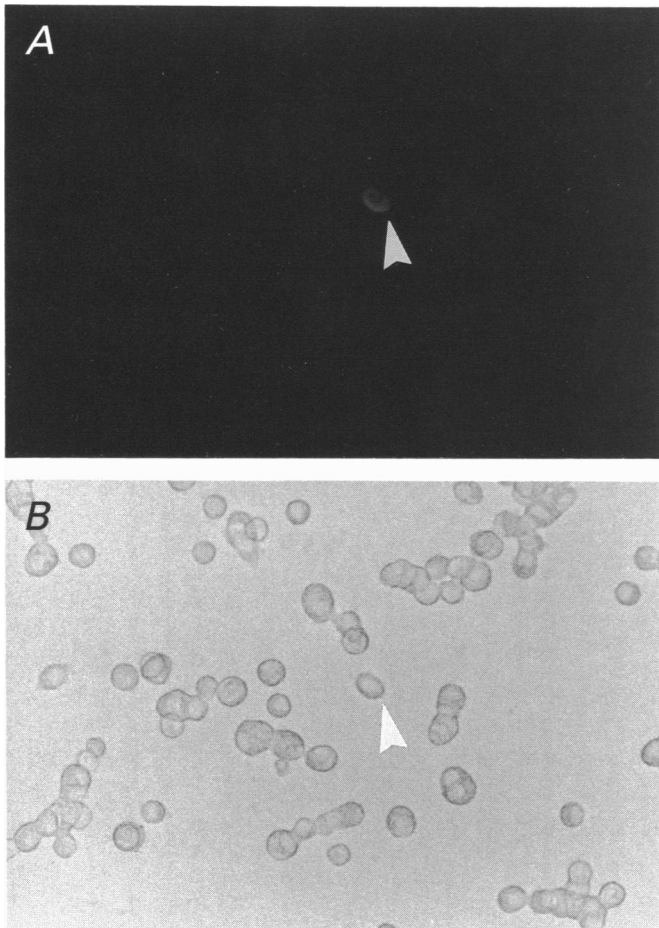
was composed of the following (mM): CsCl, 120; TEACl, 15; EGTA, 10; MgCl$_2$, 1; Hepes, 10; and glucose, 10. An ATP regenerative system (2 mM ATP, 5 mM sodium phosphocreatine and 20 U ml^{-1} creatine phosphokinase) and antibodies or antisense oligonucleotides were added to the electrode solution just before recording and the electrode solution was then adjusted to pH 7·4 and 300 mosmol l^{-1}.

Original cell culture dishes were fixed on the stage of the microscope and a peristaltic pump was used to perifuse the cells at a rate of 1 ml min^{-1}. Short-term SRIF application was performed using pressure injection through a large-bore (approximately 10 μm) pipette located about 0·5 cm from the recorded cell. Injection with vehicle did not cause any change in Ca^{2+} currents. Long-term SRIF treatment was achieved by changing the perifusion medium.
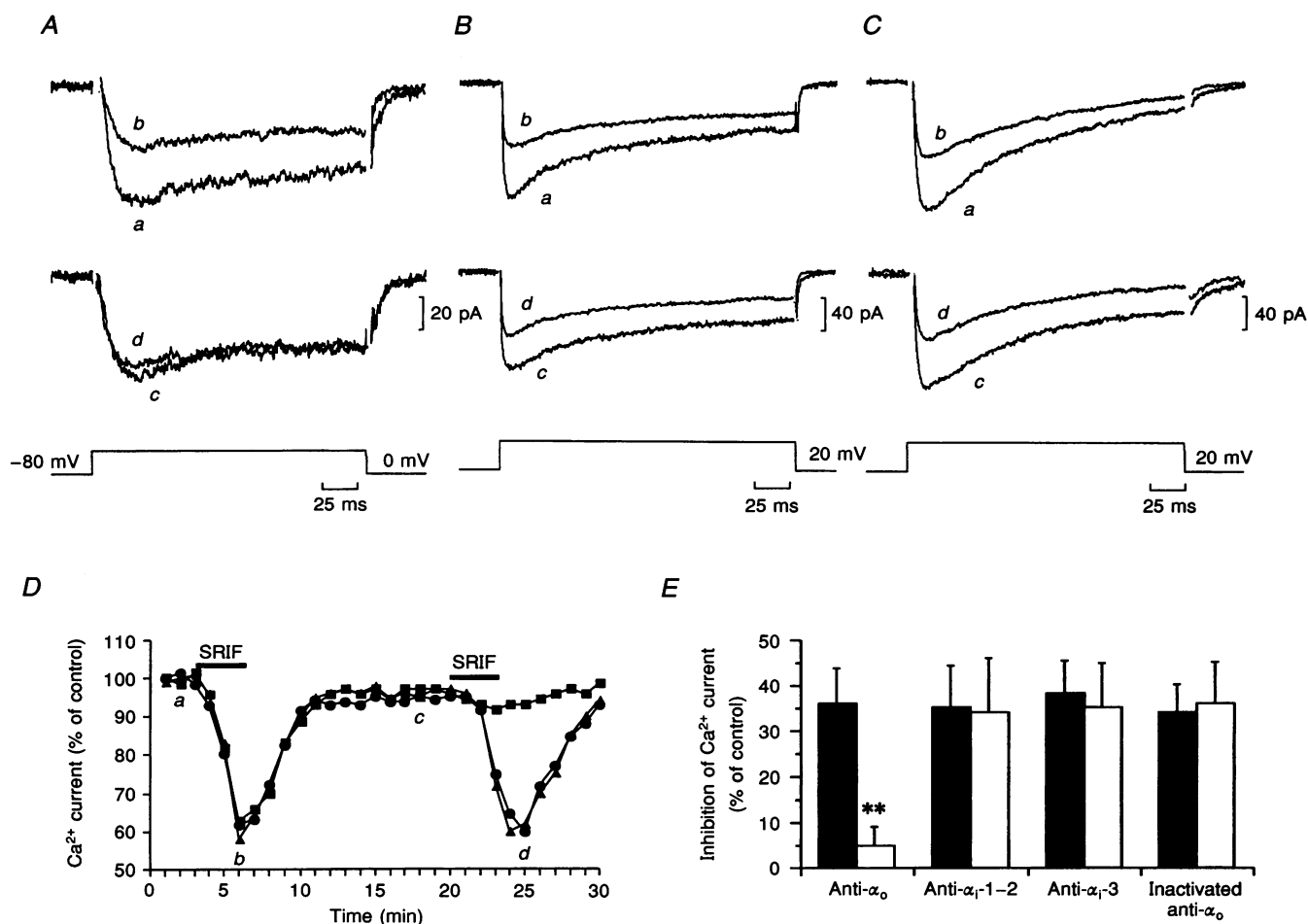
Figure 1. Effect of intracellular dialysis of the anti-α-subunit of G-protein antibodies on SRIF-induced reduction in Ca^{2+} current

A, Ca^{2+} current was evoked by depolarizing pulses from a holding potential of −80 mV to 0 mV as indicated at the bottom of the panel, with an electrode solution containing anti-α$_o$ subunit antibodies for intracellular dialysis. *B*, Ca^{2+} current was evoked by depolarizing pulses from a holding potential of −80 mV to 20 mV with electrode solution containing anti-α$_1$-1-2 subunit antibodies for intracellular dialysis. *C*, Ca^{2+} current was evoked by depolarizing pulses from a holding potential of −80 mV to +20 mV with electrode solution containing anti-α$_1$-3 subunit antibodies for intracellular dialysis. In *A–C*, trace *a* represents the control current; trace *b* is the current after the first application of 10 nM SRIF; trace *c* is the current recorded about 20 min after establishing WCR; and trace *d* is the current after the second application of 10 nM SRIF (following trace *c*). *D*, Ca^{2+} current–time relationships during intracellular dialysis of antibodies to the α$_1$-3 subunit (●), the α$_o$ subunit (■) or the α$_1$-1-2 subunit (▲). Ca^{2+} current was recorded every minute and shown as a percentage of control current (peak value, 100%). Letters on the curve represent the times at which inward currents were passed to derive the data shown in panels *A*, *B* and *C*. *E*, means ± S.E.M. percentage inhibition of peak Ca^{2+} current by 10 nM SRIF during the first application of SRIF (■) or 20 min after establishing WCR (□) during the intracellular dialysis with anti-α$_o$ (anti-α$_o$, $n = 8$), anti-α$_1$-1-2 (anti-α$_1$-1-2, $n = 5$), anti-α$_1$-3 (Anti-α$_1$-3, $n = 8$) or heat-inactivated anti-α$_o$ (inactivated anti-α$_o$, $n = 8$) antibodies. ** $P < 0·01$.

**Figure 2. Immunofluorescent staining of anti-α subunit antibodies**

Identification of antibodies in a cell dialysed by anti-α_o subunit antibodies raised from rabbit. *A* shows fluorescent staining of a cell with FITC-conjugated anti-rabbit IgG antibodies. *B* shows the same field under light microscopy. Note that other cells in the field were not stained by anti-rabbit IgG antibodies, indicating the specificity of the staining for dialysed antibody.

Data analysis and chemicals

Figures showing Ca^{2+} current traces represent one example from a group of experiments. The group data are presented as means ± s.e.m. calculated from at least three experiments. The effects of treatments (percentage change) were considered significant at the $P < 0.05$ level using the Kruskal–Wallis H test. Microphotographs are examples representing a group of experiments.

Culture media were obtained from Cytosystems (Castle Hill, Australia), sera and pancreatin were from Gibco (Gaithersburg, MD, USA), collagenase was from Worthington Biochemical Corporation (Freehold, NJ, USA) and SRIF was from Auspep (Parkville, Australia). Antibodies to α_o (GC/2) and α_1-1–2 (AS/7) subunits of G proteins were purchased from Dupont (Boston, MA, USA) and antibodies to the α_1-3 subunit of G protein were from Calbiochem (San Diego, CA, USA; cat. no. 371729). Antisense oligonucleotides synthesis and purification materials were obtained from Applied Biosystems. TEA, DNase, and all salts for experimental solutions were purchased from Sigma.
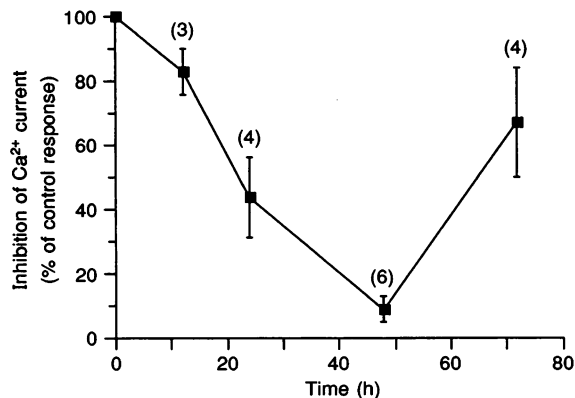
Figure 3. Time course of peak Ca^{2+} current inhibition by SRIF (10 nM) in ovine somatotrophs dialysed with α_o ASm

The α_o ASm was dialysed at time zero. Means ± s.e.m. are shown with the number of cells in parentheses.

RESULTS

Effect of antibodies dialysis on the Ca^{2+} current response to SRIF

SRIF normally causes a reduction in Ca^{2+} influx into somatotrophs (Chen *et al.* 1990*a*). In ovine somatotrophs, two types of Ca^{2+} current were recorded as T- and L-types with the L-type being larger (Chen & Clarke, 1995). Local administration of SRIF reduced both T- and L-currents without preference. Dialysis of antibodies or antisense oligonucleotides did not affect the kinetics of the Ca^{2+} current (see below). The peak Ca^{2+} current (T-type and L-type) was then investigated in this experiment to simplify the data. With WCR using anti-α_o antibodies in the pipette, a reduction in Ca^{2+} current by SRIF (10 nM) was obtained within 5 min of establishing WCR (Fig. 1*A*, upper traces). This response diminished when SRIF was given a second time after 20 min of dialysis with anti-α_o
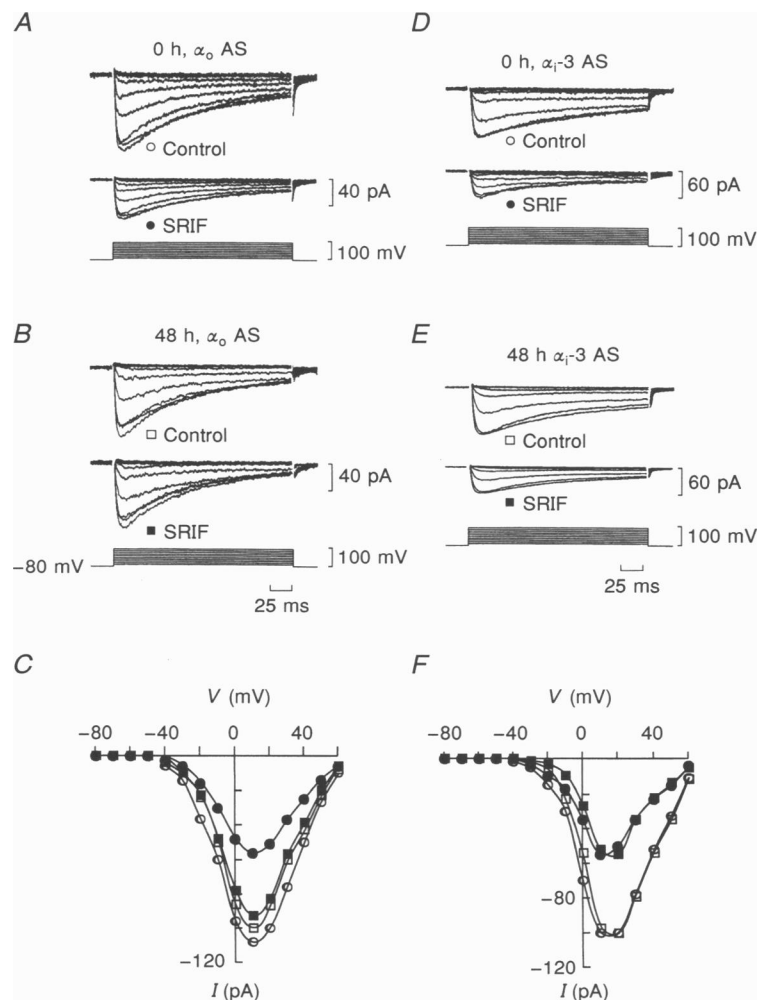
Figure 4. Effect of intracellular dialysis of antisense oligonucleotides to the α-subunit of G proteins on SRIF-induced reduction in Ca^{2+} current

A, during the dialysis of the α_o subunit antisense, Ca^{2+} current was evoked with pulses from a holding potential of −80 mV to test potential between −70 and +20 mV (as indicated at the bottom of the panel) in the control condition (upper traces) and in the presence of 10 nM SRIF (lower traces). *B*, after 48 h of incubation, the same cell as in *A* was repatched and Ca^{2+} current was recorded with pulses from a holding potential of −80 mV to test potential between −70 and +20 mV in the absence (upper traces) or presence (lower traces) of 10 nM SRIF. *C*, current–voltage relationships for peak Ca^{2+} current shown in *A* as control (O) and in the presence of SRIF (●) and in *B* as control (□) and in the presence of SRIF (■). *D*, during the dialysis of antisense oligonucleotides to the α_i-3 subunit, Ca^{2+} current was evoked with pulses from a holding potential of −80 mV to a test potential between −70 and +20 mV in control condition (upper traces) and in the presence of 10 nM SRIF (lower traces). *E*, after 48 h of incubation, the same cell as in *D* was repatched and Ca^{2+} current was recorded with pulses from a holding potential of −80 mV to test potential between −70 and +20 mV in the absence (upper traces) or presence (lower traces) of 10 nM SRIF. *F*, current–voltage relationships for peak Ca^{2+} current shown in *D* as control (O) and in the presence of SRIF (●) and in *E* as control (□) and in the presence of SRIF (■).

antibodies (Fig. 1A, lower traces). With anti-α_1-1–2 or anti-α_1-3 antibodies in the electrode solution, the reduction in Ca^{2+} current by SRIF (10 nM) after 20 min of dialysis was similar to the control responses (Fig. 1B and C). Dialysis of anti-α_0, anti-α_1-1–2 or anti-α_1-3 antibodies did not change the basal Ca^{2+} current recorded by depolarizing the membrane potential from a holding potential of −80 mV to 0 (Fig. 1A and D) or +20 mV (Fig. 1B, C and D). The percentage change in Ca^{2+} current as a function of time is shown in Fig. 1D. Mean data for eight cells (anti-α_0, anti-α_1-3 and inactivated anti-α_0 antibodies) or five cells (anti-α_1-1–2 antibodies) are shown in Fig. 1E indicating that anti-α_0 antibodies significantly ($P < 0.01$) decreased the Ca^{2+} current response to SRIF. The effect of dialysis of anti-α_0 antibodies blocked the response to SRIF even when a very high dose of SRIF (1 μM) was used (9·2 ± 2·4 % of initial response, $n = 3$). When anti-α_1-1–2, anti-α_1-3 or heat (60 °C for 15 min)-inactivated anti-α_0 antibodies were

included in electrode solution, the modification of the SRIF response did not occur (Fig. 1E).

The dialysis of antibodies into cells could be verified by immunofluorescent staining with FITC-conjugated anti-rabbit IgG antibodies. The staining of the recorded cells indicates the antibodies were dialysed into the cell during the WCR (Fig. 2). Similar results were obtained for all cells in this experiment.

Effect of dialysis with antisense oligonucleotides on the Ca^{2+} current response to SRIF

At the time of dialysis with antisense oligonucleotides (α_0 ASm, α_0-1 AS, α_0-2 AS or α_1-3 AS, 1 μM), recordings were made of voltage-gated Ca^{2+} currents and the response to SRIF application. In order to study the time course of the effect of antisense dialysis, α_0 ASm was dialysed into the cell when initial response to SRIF was recorded. At the end of the dialysis period, the patch pipette was carefully
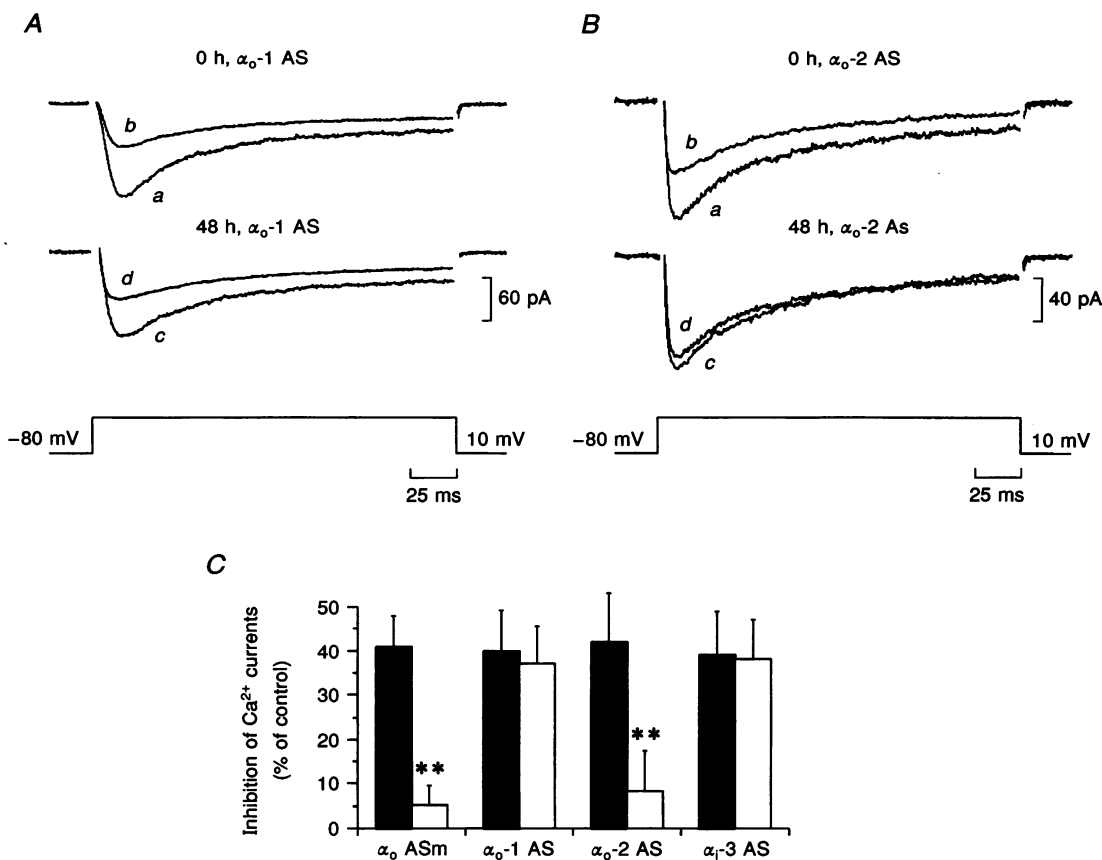

Figure 5. Ca^{2+} current inhibition by SRIF in ovine somatotrophs 48 h after dialyses of antisense oligonucleotides directed against mRNAs encoding α_0, α_0-1, α_0-2, and α_1-3 subunits

A, Ca^{2+} current was evoked by depolarizing pulses from a holding potential of −80 mV to 10 mV as indicated at the bottom of the panel with an electrode solution containing α_0-1 AS for intracellular dialysis. B, Ca^{2+} current was evoked by depolarizing pulses from a holding potential of −80 mV to 10 mV with an electrode solution containing α_0-2 AS for intracellular dialysis. For A and B, trace a represents the control current; trace b is the current after the application of 10 nM SRIF during initial recording; trace c is the current recorded 48 h after dialysis; and trace d is the current after the second application of 10 nM SRIF. C, means ± s.e.m. percentage inhibition of peak Ca^{2+} current by 10 nM SRIF during the dialysis of antisense oligonucleotides (■) or 48 h after the dialysis (□) with α_0 ASm ($n = 7$), α_0-1 AS ($n = 4$), α_0-2 AS ($n = 4$), or α_1-3 AS ($n = 5$). ** $P < 0.01$.

Figure 6. Immunofluorescent staining of α_o subunit in a cell dialysed with α_o antisense oligonucleotides

A shows immunofluorescent staining for α_o in a field of cells around one cell (arrowhead) that was dialysed with α_o antisense. The dialysed cell shows weak staining whereas other surrounding cells show strong staining. *B* shows the same field viewed under normal light microscopy; the dialysed cell is indicated by the arrowhead.
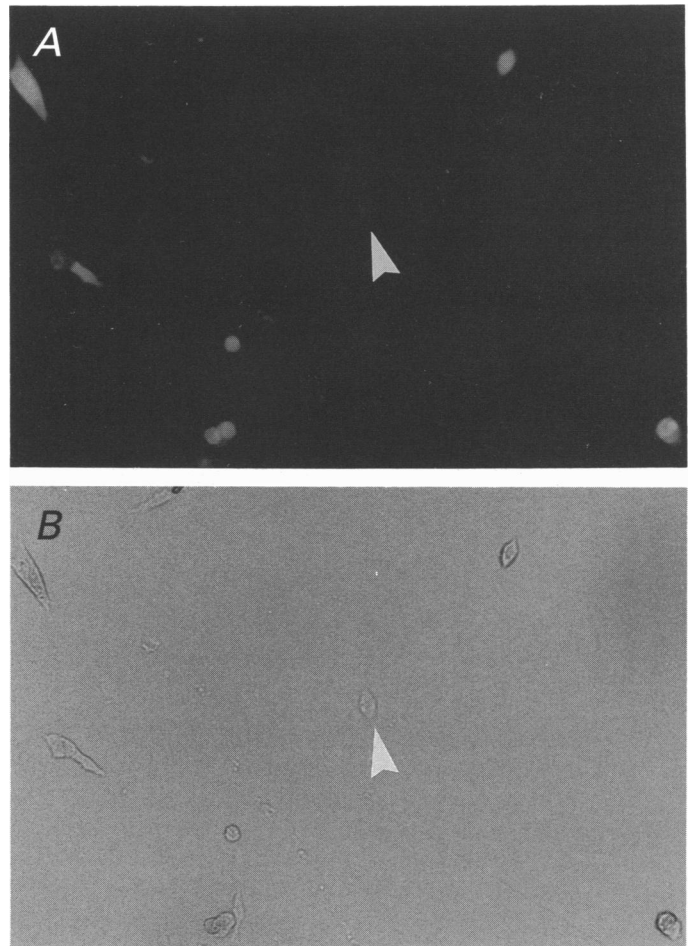
withdrawn and the cell membrane usually resealed. Distinctive marks were made on the outside of the bottom of the culture dishes to locate the dialysed cells for subsequent recording. The cells were then incubated in the presence of serum for 12, 24, 48 or 72 h, and the cells that survived dialysis were re-patched to measure the response to SRIF (100 nM). The effect of dialysis on the response to SRIF was first seen after 24 h of incubation and was maximal after 48 h of incubation; partial recovery was observed by 72 h (Fig. 3). An incubation time of 48 h was used to study the effects of dialysis of different antisense oligonucleotides on the response to SRIF.

After a 48 h incubation, twenty out of thirty-three cells survived dialysis and were recorded a second time. The other thirteen cells disappeared and had either moved or were dead (with a ghost remaining). After dialysis of α_o ASm (Fig. 4*B*), there was a reduced effect of SRIF on Ca^{2+} currents compared with the initial response to SRIF (Fig. 4*A*). Current–voltage relationships for response to SRIF are shown in Fig. 4*C* where the peak current was measured. After dialysis of α_1-3 AS, the SRIF response was not altered (Fig. 4*D* and *E*). An example of current–voltage relationships for these cells during dialysis (control) or 48 h after dialysis of α_1-3 AS is shown in

Fig. 4*F*. No kinetic changes were obtained after dialysis of antisense oligonucleotides.

The function of two subtypes of α_o subunits was studied using α_o-1 AS and α_o-2 AS dialysis (Fig. 5). The response to SRIF was not attenuated 48 h after dialysis of α_o-1 AS (Fig. 5*A*) but was diminished by the dialysis of α_o-2 AS (Fig. 5*B*). Statistical analysis of all of the data from these cells studied is given in Fig. 5*C*. The SRIF response was reduced by 87% ($n = 7$) 48 h after dialysis of α_o ASm and by 82% ($n = 4$) 48 h after dialysis of α_o-2 AS. A similar reduction (89%) in the response to a maximal dose of SRIF (1 μM) was obtained after dialysis of α_o ASm (data not shown). In contrast, the SRIF response in a group of five cells dialysed with α_1-3 AS and in a group of four cells dialysed with α_o-1 AS was not changed. The second response to SRIF was 96% of the initial response with α_1-3 AS dialysis and 91% of the initial response with α_o-1 AS dialysis (Fig. 5*C*).

The cells that were successfully recorded 48 h after α_o ASm dialysis were stained with anti-α_o antibodies and FITC-conjugated anti-rabbit IgG antibodies. An example of the staining obtained is seen in Fig. 6. There was reduced staining of α_o subunits in the single cell dialysed with

α_o ASm whereas other surrounding endocrine cells provided a strong fluorescent signal. Dialysis of cells with α_1-3 AS did not affect the staining of α_o subunits but reduced the staining of α_1-3 subunits (data not shown).

DISCUSSION

The receptors for SRIF on somatotrophs are of the type that contain seven transmembrane domains suggesting that they couple to G proteins (Bell & Reisine, 1993). There is also functional evidence for this from various studies. The effect of SRIF on voltage-gated Ca^{2+} currents in rat somatotrophs is prevented by pertussis toxin pretreatment (Chen *et al.* 1990*a*). G proteins also mediate the effects of SRIF on K^+ channels (Yatani *et al.* 1987) and GH secretion (Chen *et al.* 1990*a*). The α_1 subunit of G_i protein also mediates the effect of SRIF on cAMP production in neurones (Yasuda *et al.* 1992). It has been suggested that the G_o protein mediates the effect of SRIF on Ca^{2+} channels in GH_3 cell lines (Kleuss *et al.* 1991). The present study provides direct evidence that the α_o subunit of G protein mediates the effect of SRIF on Ca^{2+} currents in ovine somatotrophs. On the one hand, dialysis of specific antibodies and antisense oligonucleotides to the α_o subunit prevents the effect of SRIF. On the other hand, heat-inactivated anti-α_o antibodies and specific α_1-1–2 or α_1-3 antibodies or α_1-3 antisense oligonucleotides did not modify the response to SRIF. G_i proteins do not appear to mediate the effect of SRIF on Ca^{2+} currents. Further specific studies were performed by intracellular dialysis of antisense oligonucleotides directed against mRNAs of the α_o-1 or α_o-2 subunits. After dialysis with α_o-2 AS, inhibition of the Ca^{2+} current by SRIF was significantly reduced. Dialysis with α_o-1 AS, however, did not significantly reduce the response to SRIF. All these data suggest that the G_o-2 protein couples the SRIF-induced inhibition of the Ca^{2+} current in ovine somatotrophs.

Several types of SRIF receptor have been cloned and classified as SSTR1–5 with SSTR2 being divided into SSTR2A and SSTR2B (Reisine *et al.* 1993). SSTR2 and SSTR4 have been located in pituitary cells and are coupled to G_i-1, G_i-3 and G_o proteins (Reisine *et al.* 1993). These G proteins are presumably involved in various effects of SRIF, which include a reduction in cAMP levels (Epelbaum *et al.* 1987), an increase in K^+ currents (Chen, Israel & Vincent, 1989; Chen, Zhang, Vincent & Israel, 1990*b*) and a decrease in Ca^{2+} currents (Chen *et al.* 1990*a*). A G protein-dependent and cGMP-dependent protein kinase-mediated decrease in Ca^{2+} currents by SRIF has also been reported in neurones (Meriney, Gray & Pilar, 1994), adding complexity to the mechanism of action of SRIF. Detailed study of the subtypes of G proteins at single cell level is clearly able to resolve which subunits mediate which responses and dialysis of antibodies and/or antisense oligonucleotides via patch-clamp electrode provides a powerful approach in this regard.

We conclude that the effect of SRIF on voltage-gated Ca^{2+} currents in somatotrophs is mediated by the α_o-2 subunit of G_o proteins. G_i-1–3 proteins are not involved in the coupling of the inhibition of Ca^{2+} currents by SRIF.

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