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TWO TYPES OF VOLTAGE-DEPENDENT CALCIUM CURRENT IN RAT SOMATOTROPHS ARE REDUCED BY SOMATOSTATIN

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SUMMARY

1. Somatotrophs were obtained from rat pituitary glands after dissociation, separation and enrichment on a continuous gradient of bovine serum albumin at unit gravity. Somatotrophs were enriched up to 85% in the heavy fractions (F8 and F9).

2. After identification by reverse hemolytic plaque assay, patch-clamp recording in the whole-cell mode was performed on somatotrophs.

3. Under voltage-clamp conditions, two types of Ca^{2+} currents were recorded. From a holding potential of −70 mV, depolarizing voltage steps to potentials more positive than −50 mV activated a current which rapidly inactivated and which was very sensitive to Ni^{2+} but not to Cd^{2+}. This current corresponds to T-type current. Depolarizing steps to potentials more positive than −30 mV from a holding potential of −40 mV triggered a current which slowly inactivated and which was very sensitive to Cd^{2+} but not to Ni^{2+}. This current corresponds to L-type current.

4. Application of somatostatin to the bath solution (10 nM) markedly reduced the amplitudes of both T- and L-type currents. Somatostatin decreased the conductance of L-type current without modifying its time- and voltage-dependent inactivation but its activation was not affected. However, somatostatin decreased the conductance of T-type currents, and also accelerated its time-dependent inactivation. Half-inactivation voltage of T-type current was shifted from −52 to −63 mV by somatostatin but no change was obtained in the current activation curve.

5. All these modifications in Ca^{2+} currents were abolished by a pre-treatment of the cultures with pertussis toxin (100 ng/ml, for 10 h). This pre-treatment also blocked the inhibitory effect of somatostatin on high-K^+-stimulated growth hormone release.

6. Our results show that somatostatin acts on somatotrophs by attenuating the voltage-dependent Ca^{2+} currents. These effects may contribute to a somatostatin-induced reduction in $[Ca^{2+}]_i$ and the subsequent decline in growth hormone release.

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INTRODUCTION

Cytosolic calcium plays a crucial role in the control of neurotransmitter and hormone secretion. In certain cases, such as growth hormone (GH) release from somatotrophs, both spontaneous and stimulated hormone release have been found to be Ca^{2+} dependent (Holl, Thorner & Leong, 1988; Chen, Zhang, Vincent & Israel, 1990). We have also shown that Ca^{2+} influx is absolutely necessary for both human growth hormone-releasing factor (hGRF)-induced GH release and the hGRF-induced electrical response (Chen, Israel & Vincent, 1989*b*). Local application of somatostatin (SRIF) induced a hyperpolarization concomitant with an increase in potassium conductance, which diminished the frequency of spontaneous Ca^{2+}-dependent action potentials and subsequently decreased the opening probability of voltage-dependent Ca^{2+} channels (Yamashita, Shibuya & Ogata, 1986; Chen, Israel & Vincent, 1989*a*). Somatostatin has also been reported to block Ca^{2+} action potentials in GH_3 cells through co-ordinate actions on K^+ and Ca^{2+} conductances (Mollard, Vacher, Dufy & Barker, 1988). Ikeda & Schofield (1989) recently reported that somatostatin reduced a voltage-dependent Ca^{2+} current in sympathetic ganglion neurones. There is no doubt that voltage-dependent Ca^{2+} currents play an important role in the control of hormone release. Bearing in mind the differences which may occur between tumour and normal cells and between neuronal and pituitary cells, it was necessary to reinvestigate the Ca^{2+} currents and the effect of somatostatin in rat somatotrophs. In this study, we employed rat somatotrophs in enriched primary culture identified by reverse hemolytic plaque assay (Neil & Frawley, 1983) to investigate the action of somatostatin on calcium currents using the whole-cell mode of the patch-clamp technique (Hamill, Marty, Neher, Sakmann & Sigworth, 1981).

METHODS

Somatotroph-enriched cultures

Pituitary glands were dissected from adult male Wistar rats killed by decapitation. Cell dissociation, separation and primary culture methods have been described in a previous report (Chen *et al.* 1989*a*). Briefly, dissociation consisted of tissue mincing and trypsinization (0·5% for 15 min) followed by mechanical dispersion with a Pasteur pipette in Ca^{2+}-free medium. The separation was performed in a continuous density gradient of bovine serum albumin (BSA). Somatotrophs were enriched up to 85% of the cells in the heavy fractions (fractions 8 and 9). Cells were plated onto 35 mm Petri dishes (NUNC, Denmark) at a density of $1–1·5 \times 10^5$ or 4×10^5 cells. Electrophysiological or perifusion experiments were performed on 7- to 15-day *in vitro* cultures.

Identification of somatotrophs

Single somatotrophs were identified using a reverse hemolytic plaque assay (RHPA) (Neil & Frawley, 1983; Chen, Zhang, Dayanithi, Vincent & Israel, 1989*c*). Ovine erythrocytes (Biomerieux) were covalently coupled with staphylococcal protein A (Sigma) in the presence of chromium chloride hexahydrate (Sigma) as conjugating agent, incubated at 30 °C for 1 h, washed with saline and with Dulbecco's modified Eagle's medium (DMEM)–0·1% BSA, resuspended and stored at 4 °C for less than 1 week. In a second phase, the cells were suspended in Eagle's BSS (balanced salt solution) (GIBCO) before washing three times with DMEM–0·1% BSA. The final step is the plaque induction which reveals the somatotrophs. Equal volumes of monodispersed pituitary cells and

protein A-coated ovine erythrocytes in suspension were gently mixed and put into a poly-L-lysine-coated dish. They were left for 1 h at 37 °C and then washed in DMEM–0·1 % BSA to remove unattached cells. Rat GH antiserum diluted 1/100 and guinea-pig serum (3 %) were added, and within 1–2 h plaques formed around somatotrophs.

Electrophysiological experiments

Transmembrane currents and voltage were recorded using the 'gigaseal' patch-clamp technique (Hamill *et al.* 1981). Electrodes were pulled by a two-stage process from borosilicate micropipettes and had a tip diameter after fire-polishing of 1–1·5 μm corresponding to an initial input resistance of 2–5 MΩ. The recording solution for filling the electrode contained 120 mM-CsCl, 20 mM-TEA-Cl, 10 mM-EGTA, 1 mM-$MgCl_2$, 10 mM-glucose, 10 mM-HEPES, 5 mM-ATP, 0·2 mM-cyclic AMP, 0·4 mM-GTP (pH 7·35, 300 mosM), and that for the bath solution contained 130 mM-NaCl, 5 mM-$CaCl_2$, 5 mM-KCl, 0·5 mM-$MgCl_2$, 10 mM-TEA-Cl, 15 mM-glucose, 15 mM-HEPES, 1 μM-TTX (pH 7·35, 315 mosM). All recordings were made at room temperature (20–25 °C) using a high-gain voltage-clamp amplifier (RK 300, Biologic). The junction potential between the electrode solution and the bath was adjusted to zero and series resistances were compensated using the procedure described in the RK 300 manual. Cell membrane resistance was estimated from the steady-state responses to hyperpolarizing voltage commands at resting potentials. Data were stored in digital form on a video cassette recorder using a DC converter (Sony) after being filtered by an 8-pole Bessel filter (Frequency Devices). A PDP 11/73 computer and a Cambridge Electronic Design 502 analogue–digital interface with direct memory access to a 160 MB Winchester hard disc were used for data analysis. Acquisition of data, current averaging, and subtraction of capacitative and ohmic leak were performed using suitable programs: the ANADISK and DA23 Library written by Dr T. D. Lamb (Physiological Laboratory, University of Cambridge, UK).

Hormone release

The release experiments were performed as previously described (Chen *et al.* 1989*a*, *b*). Somatostatin (10 nM) was added to the medium 2 min prior to the high-K^+ medium (containing 10 nM-somatostatin) and removed from the medium 4 min after cessation of the high-K^+ treatment. In the group treated by pertussis toxin, 10 h before the experiment the toxin was added to the culture medium at a final concentration of 100 ng/ml. Growth hormone levels in the effluent medium were determined by radioimmunoassay using kits provided by the National Hormone and Pituitary Program and University of Maryland School of Medicine (Baltimore, MD, USA).

Chemical materials

Culture medium and sera were from Gibco (95051 Cergy-Pontoise, France). Nickel ($NiCl_2$), cadmium ($CdCl_2$), HEPES, tetraethylammonium chloride (TEA-Cl), somatostatin, pertussis toxin and all salts for patch solutions were purchased from the Sigma Chemical Company (St Louis, USA).

RESULTS

Wash-out effect on L-type currents

It is difficult to study Ca^{2+} currents using the whole-cell-mode patch-clamp technique, because Ca^{2+} currents, especially L-type Ca^{2+} currents, fade as a function of the recording time. This 'wash-out' effect is induced by the loss of some intracellular components. To overcome this inconvenience, cyclic AMP, ATP and GTP were added to the electrode solution (see Methods section). It was observed that the absence of cyclic AMP, ATP and GTP in the electrode solution led to a significant loss of long-lasting current starting about 10 min after setting up the whole-cell recording condition; however, addition of these agents in the pipette solution markedly prolonged the stability of the long-lasting current which started to decline

only about 45 min after the beginning of whole-cell recording (results not shown), which is, therefore, long enough to test the effect of somatostatin (see below).

Activation of voltage-dependent Ca^{2+} currents

After identification of somatotrophs by RHPA, 'giga-seal' patch-clamp recording was performed using the whole-cell configuration. The protocol used for differ-

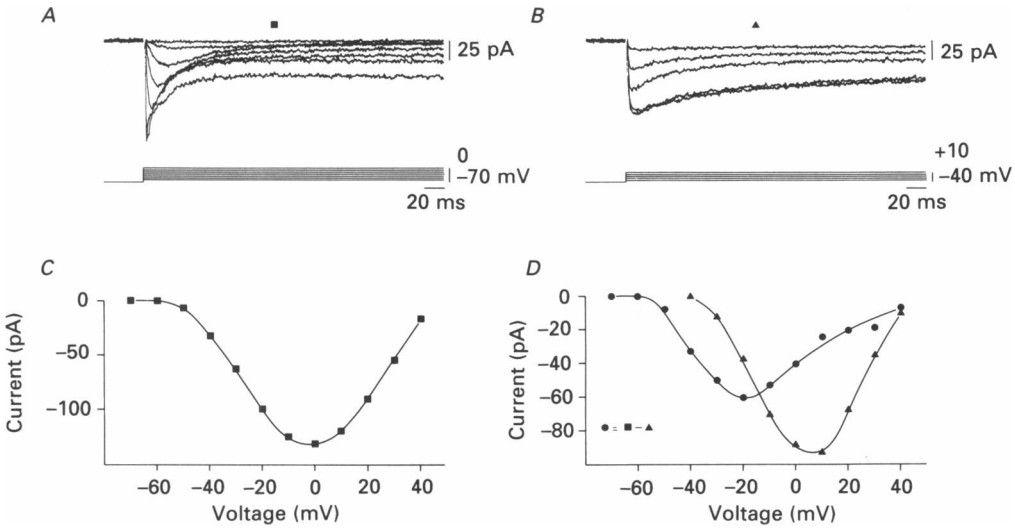

Fig. 1. Two components of Ca^{2+} current were observed under whole-cell recording conditions. *A*, currents (upper traces) evoked with pulses from a holding potential (HP) of −70 mV (lower traces) to test potentials between −60 and 0 mV. Current traces have been corrected for linear leak and capacitance current, and are blanked for the first 800 μs following the depolarizing step. *B*, currents (upper traces) evoked with pulses from HP = −40 mV to test potentials between −30 and +10 mV as indicated (lower traces). Current traces have been corrected as described in *A*. *C*, peak current (■) plotted against test potential for the records shown in *A*. The currents here present two components and thus are called total currents. *D*, separation of the two types of Ca^{2+} current. Triangles indicate the long-lasting current (L-type) recorded in *B*. Subtraction of L-type current from total current shows the transient component (T-type) of the Ca^{2+} current (●).

entiating the components of the calcium currents was first described by Nowycky, Fox & Tsien (1985). Step pulses were successively applied at holding potentials of −70 and −40 mV. The data illustrated in Fig. 1 indicate that two types of voltage-dependent calcium channels exist in somatotroph cells. When the membrane potential was set at −40 mV, depolarizing steps up to −30 mV elicited a long-lasting calcium current. In contrast, from a −70 mV holding potential, depolarizing steps up to −50 mV evoked a calcium current with a rapidly decaying transient component. Subtraction of the long-lasting current form the total current recorded at a holding potential of −70 mV gave the transient current.

Pharmacological study of the currents

As indicated by Fox, Nowycky & Tsien (1987*a*, *b*), differential sensitivities to nickel (Ni^{2+}) and cadmium (Cd^{2+}) exist between T- and L-type currents and this pharmacological character can be used to identify these subtypes of current. Figure 2

shows that addition of Ni^{2+} (20 μM) to the bath solution markedly inhibited the transient Ca^{2+} current but not the long-lasting components in accordance with the supposition that the transient current brought up from a holding potential of −70 mV is type T. On the other hand, bath solution containing Cd^{2+} (40 μM) significantly reduced the long-lasting component appearing with depolarizing steps from holding potentials of either −70 mV (Fig. 2*B*) or −40 mV (Fig. 2*C*), but did not affect the transient one. Therefore, the long-lasting component is similar to the L-type current in neurones.

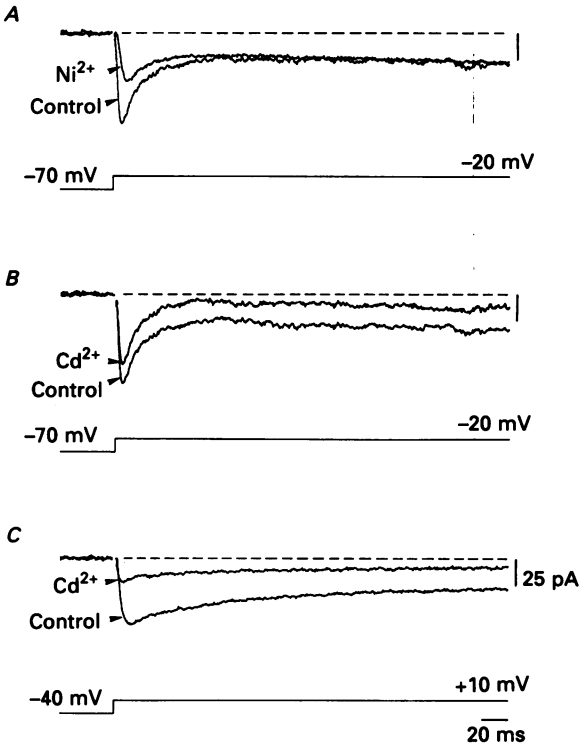

Fig. 2. Differential blocking of T- and L-type currents by Ni^{2+} (20 μM) and Cd^{2+} (40 μM). Records were obtained in a control run and after addition of Ni^{2+} or Cd^{2+} to the bath as indicated. *A*, the voltage protocol used evoked two currents but only T-type current was markedly inhibited by Ni^{2+}. *B*, two currents were recorded as shown in *A*, and Cd^{2+} addition reduced only the long-lasting component. *C*, the voltage protocol evoked only L-type current, which was significantly diminished by Cd^{2+}.

Somatostatin decreased both T- and L-type Ca^{2+} currents

Addition of somatostatin to the bath solution (10 nM) significantly reduced both L- and T-type Ca^{2+} currents by up to 50%. Figure 3*A* and *B* respectively illustrate the effects of the application and removal of somatostatin on L-type and T-type current. Note that the T-type current was obtained by the subtraction of the L-type current from the total Ca^{2+} current and that a small proportion of the L-type component remained at positive test pulses, due to the voltage-dependent inactivation of L-type current.

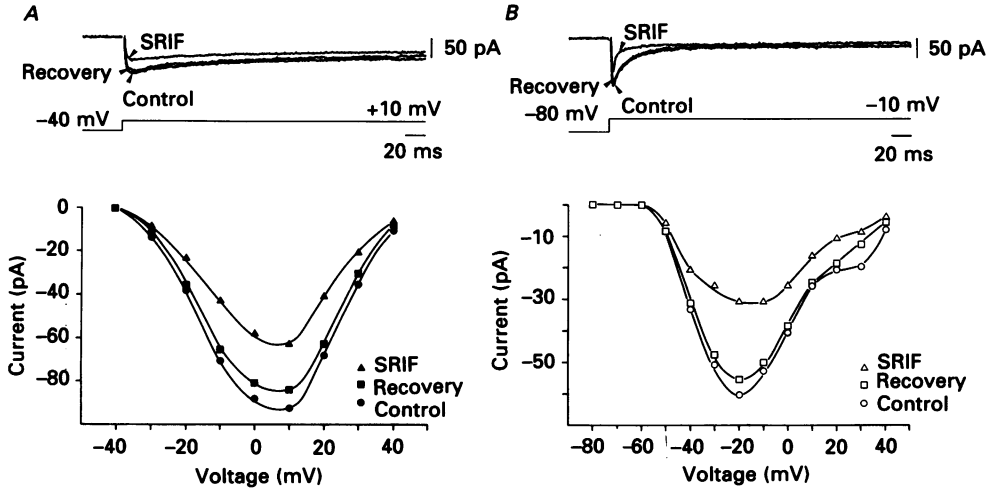

Fig. 3. Effects of somatostatin (SRIF) on Ca^{2+} currents. *A*, from HP = −40 mV, a depolarizing pulse to +10 mV induced L-type current. Upper traces show an example of L-type current prior, in the presence of and after removal of somatostatin. Bath solution containing somatostatin (10 nM) markedly reduced the amplitude of L-type current, and the *I–V* curve shown below was calculated with data obtained from the same cell. *B*, from HP = −80 mV in the same cell as in *A*; T-type current was also present. Upper traces illustrate the current elicited by a pulses from −80 to −10 mV and the decrease in transient T-type current caused by addition of SRIF. The *I–V* curve shown below was obtained after subtraction of L-type current, leaving the T-type current.

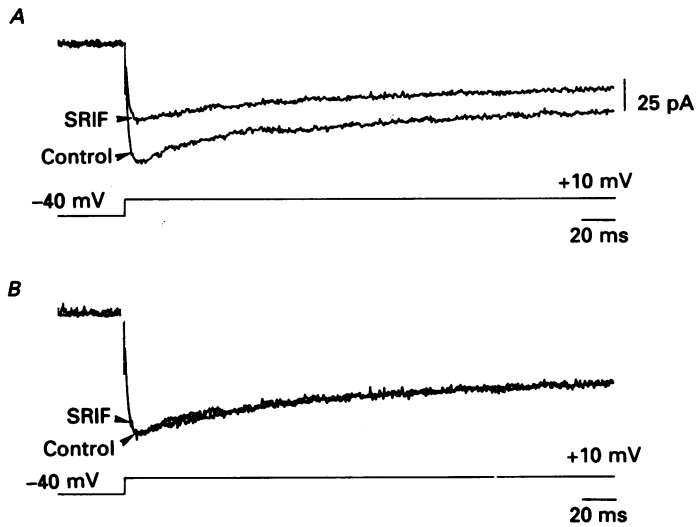

Fig. 4. Time-dependent inactivation of L-type current. *A*, from HP = −40 mV, a test potential to +10 mV evoked L-type current. This current was markedly decreased by somatostatin (10 nM). *B*, after augmentation by computer of inhibited current shown in *A* to control level, the two currents displayed the same inactivation time course.

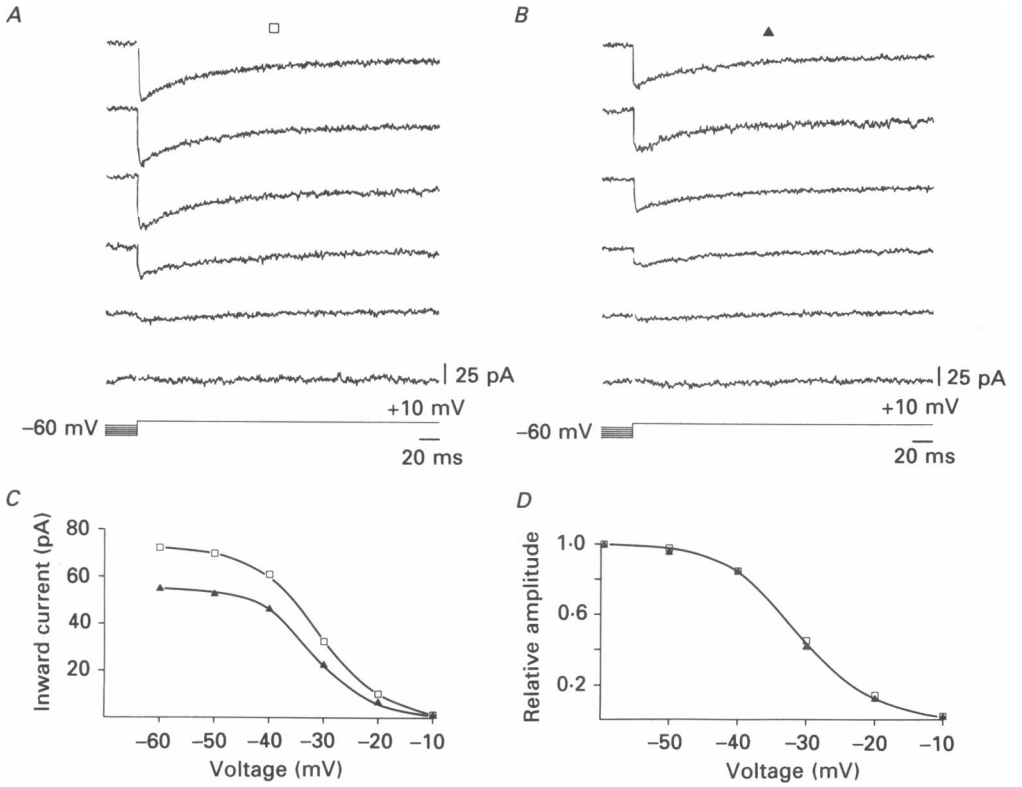

Fig. 5. Voltage-dependent inactivation of L-type current. *A* and *B*, L-types currents evoked by depolarizations to +10 mV from different holding potentials separated by 10 mV for at least 20 s in the absence (*A*) or presence (*B*) of somatostatin in the bath solution (10 nM). *C*, peak-current amplitudes plotted against the holding potential from data illustrated in *A* (□) and in *B* (▲). *D*, peak-current-to-maximal-peak-current ratios plotted against the holding potentials in *A* (□) and in *B* (▲).

Modification of activation and inactivation parameters of L-type current by somatostatin

Bath solution containing somatostatin reduced the amplitude of L-type current; however, its time-dependent inactivation was not modified. Figure 4 shows that after scaling the inhibited L-type current to the level of the control, the two recordings were superimposed for 300 ms (Fig. 4*B*). Therefore, the time-dependent inactivation was the same during control and somatostatin treatment. Somatostatin did not modify the voltage-dependent inactivation either (Fig. 5).

In Figure 3, the threshold and peak position of L-type current were shown to be unchanged by somatostatin. The relative amplitudes of L-type current with or without somatostatin showed that the activation of L-type current was not markedly changed by somatostatin, even at a concentration of 100 nM (Fig. 6).

Modification of activation and inactivation parameters of T-type current by somatostatin

For T-type Ca^{2+} current, somatostatin accelerated the time-dependent inactivation. Figure 7*A* illustrates that the inactivation of current took place more quickly in the presence of somatostatin. Figure 7*B* represents the inactivation time constant obtained at −30, −20 and −10 mV, showing that a marked shift was produced by somatostatin.

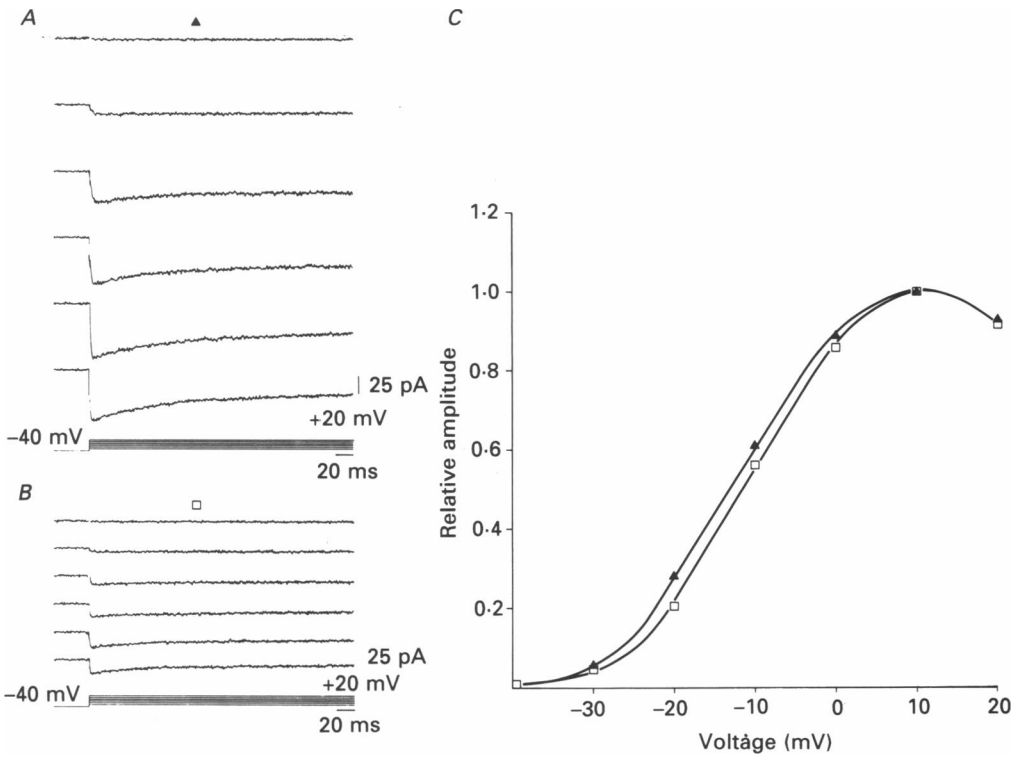

Fig. 6. Voltage-dependent activation of L-type current. *A*, L-type currents evoked by depolarizing pulses from a HP = −40 mV to test potential spaced by 10 mV. *B*, under application of somatostatin (100 nM), L-type currents were greatly reduced. However, the threshold was not changed. *C*, peak-current-to-maximal-peak-current, plotted against the test potential (▲, from data in *A*; □, from data in *B*).

In addition to the modification of time-dependent inactivation, somatostatin also changed the voltage-dependent inactivation of T-type current. The half-maximal steady-state inactivation potential was found to be −52 mV under control conditions and −63 mV in the presence of somatostatin (Fig. 8).

Activation of T-type current, in contrast, was not modified by somatostatin (results not shown).

Effects of pertussis toxin pre-treatment on somatostatin-induced inhibition

Pre-treatment of the culture with pertussis toxin (100 ng/ml) for 10 h completely abolished the reduction of Ca^{2+} current by somatostatin (Fig. 9*A*). In order to

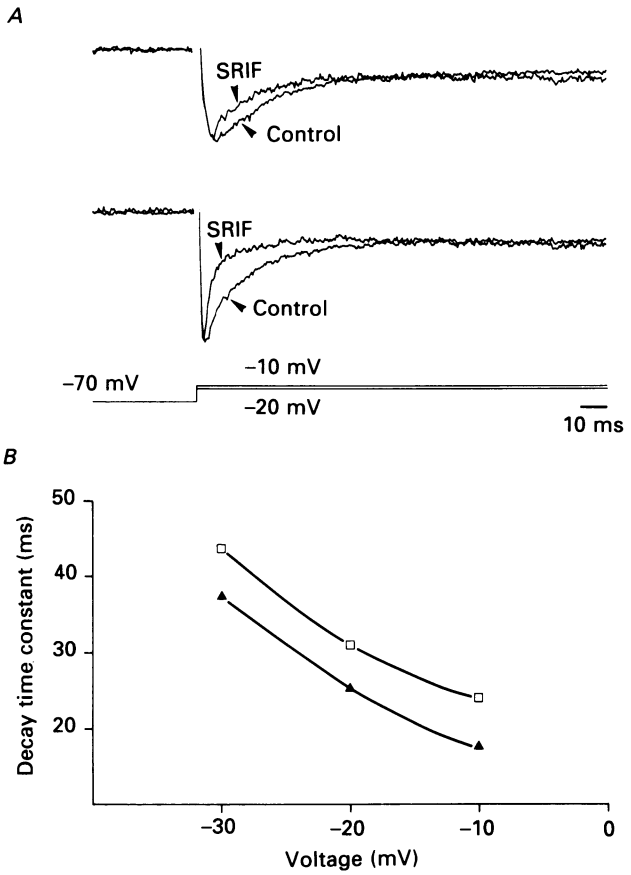

Fig. 7. Time-dependent inactivation of T-type current. *A*, from a HP = −70 mV, test potentials to −20 mV (upper) or −10 mV (lower) evoked mainly T-type current. It has been shown above that somatostatin reduced this T-type current. After augmentation by computer, somatostatin-reduced current (marked with SRIF) decays more quickly than control current. *B*, voltage dependence of time constants of T-type currents from test potentials of −30, −20 and −10 mV. □, control; ▲, somatostatin. Data from the same cell were illustrated in *A*.

examine the relationship between the reduced effect induced by somatostatin on Ca^{2+} currents and GH release, a high-K^+ medium was used to stimulate GH release, since high-K^+ is thought to open voltage-dependent Ca^{2+} channels. Figure 9*B* shows that a high-K^+ (30 mM) medium induced a marked increase in GH release which was nevertheless significantly diminished by somatostatin. Pre-treatment with pertussis toxin completely blocked this effect.

DISCUSSION

Ca^{2+} currents on normal rat somatotrophs

We have described two types of Ca^{2+} currents in normal rat somatotrophs. Previous reports on the properties of Ca^{2+} channels in chick sensory neurones

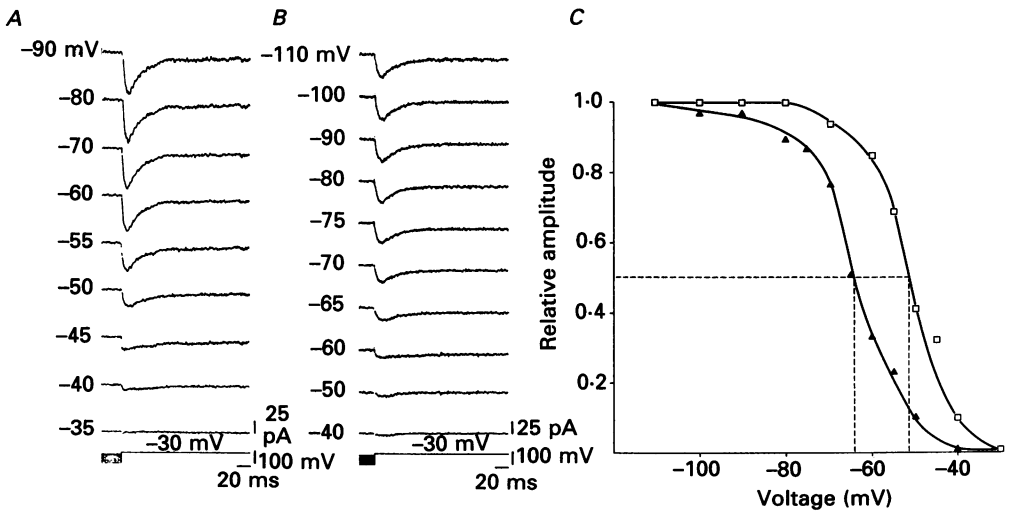

Fig. 8. Voltage-dependent inactivation of T-type current. *A*, isolated T-type currents evoked by depolarizations to a test potential of −30 mV from various holding potentials as indicated. *B*, same experimental protocol on same cell as in *A*, but with somatostatin (10 nM) in bath solution. *C*, peak-current-to-maximal-peak-current ratios plotted against the holding potential. Data were obtained from *A* (□) and *B* (▲) and the half-maximal inactivation holding potential is −52 mV for control (□, *A*) and −63 mV for somatostatin-containing medium (▲, *B*).

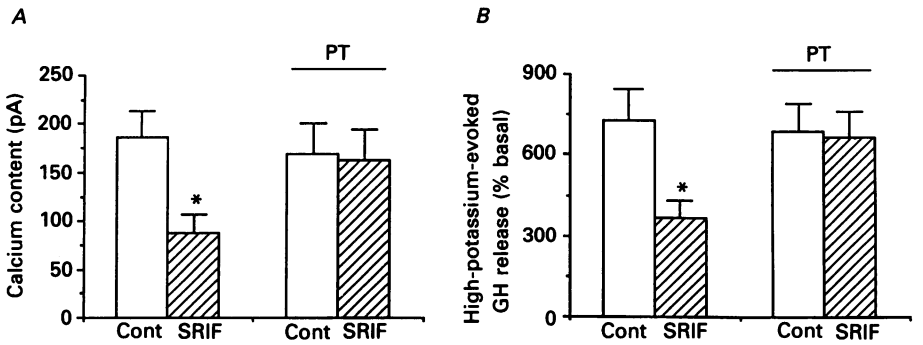

Fig. 9. Pertussis toxin (PT) treatment (100 ng/ml, 10 h) abolished the Ca^{2+} current-blocking and high-K^+-stimulated GH release reducing effects of somatostatin. *A*, comparison of the mean ± S.E.M. ($n = 10$) between Ca^{2+} current amplitude elicited by steps from HP = −70 mV to 0 mV in controls (Cont) and after somatostatin (SRIF). Somatostatin (10 nM) decreases Ca^{2+} current by about 50% (left) and this blocking effect was completely abolished by pre-treatment with PT (right). *B*, comparison of reducing effect of somatostatin (10 nM) on high-K^+ (30 mM)-stimulated GH release (mean ± S.E.M., $n = 4$). Under control conditions, somatostatin reduced high-K^+-stimulated GH release from 730 to 320% (left) of the basal release. However, in PT-treated cultures, this reducing effect was completely abolished (right). * $P < 0{\cdot}01$.

(Nowycky *et al.* 1985; Fox *et al.* 1987*a*, *b*) described the existence of three types of Ca^{2+} currents named L, T and N currents; each of them displays a unique set of kinetic, ionic and pharmacological properties. However, some investigators have questioned the distinction between L- and N-type channels (Tsien, Lipscombe, Madison, Bley & Fox, 1988; Swandulla & Armstrong, 1989). So far, the N-type Ca^{2+} current has only been recorded in neurones, and in the present work only two types of Ca^{2+} currents corresponding to T- and L-type currents were present. This is in agreement with the previous reports which described voltage-dependent Ca^{2+} currents in GH_3B_6 cells (Matteson & Armstrong, 1986), pars intermedia cells of the rat pituitary gland (Cota, 1986), rat anterior pituitary cells (Deriemer & Sakmann, 1986) and ovine anterior pituitary cells (Sikdar, Waring & Mason, 1986; Mason & Rawlings, 1988). Distinction between T- and L-type currents has been based on several criteria, including activation kinetics, gating and pharmacology. We therefore studied these three parameters and confirmed the co-existence of T- and L-type currents in normal somatotroph membranes. Firstly, T-type Ca^{2+} current was only available from relatively negative holding potentials (−70 mV) and decayed rapidly upon activation, whereas only a slowly decaying L-type Ca^{2+} current could be recorded at a holding potential of −40 mV. Secondly, activation threshold (loosely defined as the point where current through the channels becomes evident) for T-type current is about −50 mV, whereas it is −30 mV for L-type current. Thirdly, addition of Ni^{2+} into the bath solution at a concentration which blocked 70% of T-type current did not markedly modify L-type current, whereas Cd^{2+} at a concentration which blocked 75% of L-type current did not inhibit T-type current. These data confirmed the existence of two types of Ca^{2+} currents in normal rat somatotrophs. Unlike T- and L-type currents, N-type current cannot be isolated by working over a narrow voltage range since it requires negative holding potentials and relatively positive test potentials. N-type current is therefore the hardest to study with whole-cell recordings. However, in the present experiments, N-type current must be very slight if it even exists. This is based on the following evidence: (i) subtraction of the L-type *I–V* curve from the total *I–V* curve gave only one peak at −20 mV, which is far from the peak position of N-type current (+10 mV); (ii) the peak position at +10 mV was obtained at a holding potential of −40 mV, at which only L-type current could be recorded; (iii) Cd^2 blocked L-type current but did not affect the transient component recorded at a holding potential of −70 mV. However, T- and L-type currents identified in rat somatotrophs were quite different to Ca^{2+} currents recorded in neurones (Fox *et al.* 1987*a*, *b*). For T-type current, thresholds were −50 mV in somatotrophs and −70 mV in chick sensory neurones and the inactivation ranges were −80 to −30 mV in somatotrophs and −100 to −60 mV in chick sensory neurones. For L-type current, the threshold was −30 mV in somatotrophs and −10 mV in sensory neurones.

Effects of somatostatin

Application of somatostatin into the bath solution markedly reduced both T-type and L-type currents in GH_3 cells (Mollard *et al.* 1988). However, this inhibitory effect on Ca^{2+} currents in normal somatotrophs had not yet been identified. Somatostatin

decreased the T-type current through different mechanisms: (i) somatostatin reversibly decreased the amplitude of the current; (ii) somatostatin accelerated the time-dependent inactivation and reduced the inactivation time constant; (iii) somatostatin shifted the voltage-dependent steady-state inactivation curve to a more negative half-maximal inactivation potential. For L-type current, somatostatin induced only the reduction of current amplitude but did not affect the voltage- or time-dependent inactivations. Ikeda & Schofield (1988) reported that somatostatin specifically induced a rapid, reversible and concentration-dependent reduction of the Ca^{2+} current, i.e. that the rising phase of the Ca^{2+} current was slower. However, Ikeda and Schofield did not separate the sub-types of Ca^{2+} current and therefore no conclusion could be drawn. Mollard *et al.* (1988) also described the reduction of Ca^{2+} currents (T- and L-types) by somatostatin in GH_3 cells, but these authors did not study the kinetic parameters of these currents either. Our report supports these pervious findings and extends this mechanism to normal rat somatotrophs *in vitro*.

Somatostatin has also been demonstrated to increase potassium currents, including voltage-independent (Chen *et al.* 1989*a*), inwardly rectifying (Mihara, North & Suprenant, 1987; Inoue, Nakajima & Nakajima, 1988) and voltage-dependent (Mollard *et al.* 1988) potassium conductance. Therefore, the binding of somatostatin with its receptor may have multiple effects on membrane ionic channels, leading to a reduction of the intracellular Ca^{2+} concentration followed by a decrease in hormone or neurotransmitter secretion.

Coupling role of G-protein

Recent reports concerning guinea-pig submucous plexus neurones (Mihara *et al.* 1987), rat locus coeruleus neurones (Inoue *et al.* 1988), rat sympathetic ganglion neurones (Ikeda & Schofield, 1989) and GH_3 tumoural cells (Lewis, Weight & Luini, 1986; Mollard *et al.* 1988) indicate that the functional expression of somatostatin is mediated through activation of GTP-binding proteins (G-protein).

Certain types of G-proteins (G_i, G_o, etc.) are known to be ADP-ribosylated by pertussis toxin rendering the G-proteins incapable of reacting with GTP (Bokoch, Katada, Northup, Ui & Gilman, 1984). It was previously shown that the inhibition of cellular excitability by somatostatin in GH_3 cells can be blocked by pre-treatment with pertussis toxin (Mollard *et al.* 1988). In the present experiments, pertussis toxin treatment abolished the inhibitory effect of somatostatin on voltage-dependent Ca^{2+} currents and on high-K^+-stimulated GH release. These results strongly suggest that similar transduction mechanisms may underlie the functional expression of somatostatin in normal somatotrophs, i.e. the binding of somatostatin to its receptor led to the reduction of voltage-dependent Ca^{2+} currents through a pertussis-toxin-sensitive G-protein.

It is an open question whether the same G-protein mediated these two effects (decrease in Ca^{2+} conductance and increase in K^+ conductance) or whether these expressions in normal somatotrophs are related to two different G-proteins.

Physiological considerations

Somatostatin is a potent inhibitor of GH secretion in pituitary somatotrophs (Chen *et al.* 1989*a*). However, the post-receptor events in this cell type are only partially understood so far. Somatostatin has, to a certain extent, an inhibitory

action on cyclic AMP production (Sheppard, Spence & Kraicer, 1979), but additional mechanisms, such as Ca^{2+} mobilization, have also been reported (Dorflinger & Schonbrunn, 1983; Sheppard, Moore & Kraicer, 1985; Epelbaum, Enjalbert, Krantic, Musset, Bertrand, Rasolonjanahary, Shu & Kordon, 1987). We have previously shown that somatostatin increased membrane potassium conductance, leading to the hyperpolarization of the membrane potential. As a result of this response, Ca^{2+} influx through voltage-dependent Ca^{2+} channels (via action potentials or slow voltage fluctuations) was reduced. We have also demonstrated that a Ca^{2+} influx was involved in both basal and GRF-stimulated GH release (Chen, Zhang, Vincent & Israel, 1990). Here, we reported a direct effect of somatostatin on voltage-dependent Ca^{2+} currents, which resulted in the reduction of Ca^{2+} influx through these channels. The role of G-protein in the augmentation of K^+ conductance has been demonstrated (Mihara *et al.*, 1987; Inoue *et al.* 1988). Since pertussis toxin treatment of somatotrophs blocked the inhibitory effects of somatostatin on GH release and Ca^{2+} currents, it is suggested that the binding of somatostatin with its receptor, through a G-protein-mediated mechanism, decreases the Ca^{2+} influx through membrane Ca^{2+} channels followed by decrease in intracellular Ca^{2+} and a decrease in GH secretion.

In summary, we have shown that somatostatin reduced the amplitudes of both T- and L-type Ca^{2+} currents through different mechanisms. These effects and the reduction in GH release are blocked by pertussis toxin treatment, suggesting that the coupling between the somatostatin receptor and functional expression appears to involve a GTP-binding protein.

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