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ELECTROPHYSIOLOGICAL RESPONSES TO SOMATOSTATIN OF RAT HYPOPHYSIAL CELLS IN SOMATOTROPH-ENRICHED PRIMARY CULTURES

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SUMMARY

1. Somatotroph cells were obtained from pituitaries of adult male rats by dissociation, separation and enrichment on a continuous gradient of bovine serum albumin at unit gravity. They were kept in culture for 7–15 days before electrophysiological experiments.

2. Immunofluorescent staining of the resulting gradient fractions (numbered F_2 to F_9) indicated that the majority of somatotrophs (75–85%) were located in the heavy fractions (F_8 and F_9). However, a small percentage (15–20%) of cells in these fractions were identified as lactotrophs.

3. Perifusion experiments indicated that on the one hand release of growth hormone from somatotroph-enriched fractions was stable at the level of 6 ng $(2\ min)^{-1}$ $(10^6\ cells)^{-1}$ and was markedly inhibited by somatostatin (1·9 ng $(2\ min)^{-1}$ $(10^6\ cells)^{-1}$) but not by dopamine. On the other hand, in the same cell preparations, basal prolactin release (1·6 ng $(2\ min)^{-1}$ $(10^6\ cells)^{-1}$) was significantly reduced by dopamine (0·08 ng $(2\ min)^{-1}$ $(10^6\ cells)^{-1}$) but remained unchanged by somatostatin treatment.

4. The inhibitory effect of somatostatin on growth hormone release was dose dependent. This effect was not abolished by tetraethylammonium (40 mM) or 4-aminopyridine (5 mM), but somatostatin decreased high-potassium-induced release.

5. In all the cells recorded ($n = 187$), 14% ($n = 26$) displayed a low resting potential (less than −30 mV) and poor membrane resistance (less than 50 MΩ). The recording was unstable and resting potentials decreased regularly to 0 mV in less than 5 min. The other 86% of the cells displayed resting potentials varying from −45 to −65 mV and had a membrane resistance of more than 150 MΩ. Only cells which displayed these membrane characteristics showed clear responses to somatostatin or dopamine, and were therefore chosen for experiments.

6. In all the cells selected for the experiments ($n = 161$), 78% ($n = 126$) showed either triggered or spontaneous action potentials. The action potentials remained insensitive to sodium-free bath solution, but were reversibly blocked by the calcium channel blockers cobalt (5 mM) or nickel (5 mM).

7. When the cells were at resting potential, somatostatin induced a hyperpolarizing response associated with a decrease of membrane resistance. During this

response, spontaneous or triggered action potentials were inhibited. The hyperpolarizing response induced by somatostatin was dose-dependent.

8. The reversal potential of the somatostatin-induced response was found to be near −100 mV, and was shifted to −70 or −50 mV respectively when 30 or 55 mM-extracellular potassium medium was used. Tetraethylammonium (40 mM) and 4-aminopyridine (5 mM) had no effect on the somatostatin-induced response.

9. The effects of somatostatin were also investigated on lactotrophs. In lactotroph-enriched fractions (fractions 3 and 4), cells respond to dopamine (10^{-7} M) but not to somatostatin. Conversely, in somatotroph-enriched fractions, most of the cells (73%, $n = 124$) responded to somatostatin but did not respond to dopamine. Nevertheless, a few cells (13%) responded to dopamine but not to somatostatin. They were presumed to be lactotrophs.

10. The inhibitory effect of somatostatin on growth hormone release and the hyperpolarizing response to somatostatin by somatotrophs could be integrated into a hyperpolarization–inhibition coupling hypothesis.

INTRODUCTION

Pituitary growth hormone (GH) secretion is mainly regulated by two hypothalamic hormones, somatostatin (somatotrophin-release inhibitory factor, SRIF) and GH-releasing factor (GRF) (for review, see Arimura & Culler, 1985).

Different mechanisms have been proposed for the inhibitory effect of SRIF on basal GH release, i.e. suppression of intracellular accumulation of cyclic AMP (Bilezikjian & Vale, 1983) and/or increase of potassium permeability (Pace & Tarvin, 1981). In previous experiments, calcium-dependent action potentials (Israel, Denef & Vincent, 1983) and voltage-dependent calcium channels (DeRiemer & Sakmann, 1986; Yamashita, Shibuya & Obata, 1986) have been reported on rat somatotroph (GH-secreting cell) membranes. We have previously reported that SRIF modified electrical properties of somatotrophs, i.e. SRIF hyperpolarized the cell membrane by decreasing undefined conductances in primary culture rat somatotrophs (Israel *et al.* 1983). Recently, Yamashita *et al.* (1986) have shown a hyperpolarizing effect of SRIF on human adenoma cells which has been identified as being due to an increase in a potassium conductance. Since, on the one hand, the origin of cultured cells (i.e. immature *versus* adult, male *versus* female, normal *versus* tumoral), and on the other hand, the age of the culture, may affect the response of pituitary cells to regulating agents (Israel *et al.* 1983), we decided to reinvestigate the effects of SRIF on somatotroph membrane conductance under stricter conditions.

In the present work, we describe the electrical properties of adult male rat pituitary cells in somatotroph-enriched (75–85%) primary cultures. We show that (i) a majority of cells responded to SRIF by a hyperpolarization concomitant with an increase in potassium conductance; (ii) the potassium-induced conductance was not inhibited by classical inhibitors (tetraethylammonium, TEA; 4-aminopyridine, 4-AP); (iii) the cells which responded to SRIF did not respond to dopamine and conversely, the minority of cells which did respond to dopamine were unresponsive to SRIF; (iv) this SRIF-induced electrical response has been interpreted in terms of hyperpolarization–inhibition coupling.

METHODS

Somatotroph-enriched cells

Pituitary glands were dissected from male adult Wistar rats (2–3 months old, 250–320 g). Cell dissociation and separation methods have been described in a previous paper (Israel *et al.* 1983) as the original technique from Hopkins & Farquhar (1973) adapted by Denef, Hautekeete & Rubin (1976) and Denef, Hautekeete, De Wolf & Vanderschueren (1978). Briefly, the dissociation consisted of tissue mincing and trypsinization (0·5%, for 15 min), then separation for 3 h in 1·1 l of a continuous density gradient of 0·3–2·4% bovine serum albumin (BSA) and collection in fractions of 100 ml numbered 2–9 after rejection of the first 300 ml. After centrifugation, resuspended cells from the different fractions were plated onto 35 mm Petri dishes (NUNC, Denmark) at 10^5 to $1{\cdot}5 \times 10^5$ cells per dish for electrophysiological and 4×10^5 cells per dish for perifusion experiments, with 2 ml of Dulbecco's Modified Eagle's Medium (DMEM) containing 10% newborn calf serum and 2·6 g l^{-1} $NaHCO_3$ at pH 7·45. Antibiotics (35 mg l^{-1} penicillin and 50 mg l^{-1} streptomycin) were added to the culture medium during the first 2 days of culture and the medium was renewed every 2–3 days without antibiotics. All the cells were cultured in a humidified incubator (37 °C; air, 94%; CO_2, 6%).

Identification of cells

Dubois' technique was used in immunofluorescent staining of cultures (Dubois, 1972). This technique has been described in detail in our previous work (Israel, Kirk & Vincent, 1987). Briefly, it consists of cell fixation with 4% paraformaldehyde in phosphate-buffered saline (PBS), incubation in PBS containing 1/20 normal sheep serum and exposure to specific rabbit antisera (anti-rat GH (growth hormone), anti-rat ACTH (adrenocorticotrophic hormone), anti-rat LH (luteinizing hormone), anti-rat PRL (prolactin)). Then cells are left for 1 h at 4 °C with fluorescein-isothiocyanate-labelled anti-rabbit immunoglobulin G. The immunostained cells are observed and photographed under a fluorescent light microscope (Leitz Metallux, MPS 55, Switzerland). The percentage of each cell type is estimated from the photographs. We found that fractions 8 and 9 consisted of up to 80% somatotrophs; this coincided with a previous report (Israel *et al.* 1983). Lactotrophs constituted the other major cell type in these fractions (15–20%). The sizes of the GH-positive and PRL-positive cells were also estimated from the photographs.

Hormone release studies

Growth hormone release experiments were performed in the origin Petri dishes (4×10^5 cells per dish). A continuous-flow perifusion system which accommodated four dishes at a time (Israel *et al.* 1987) was devised using a four-channel peristaltic pump (Minipuls, Gilson) and maintained at $37 \pm 0{\cdot}5$ °C by a water-heated platform. The perifusion medium was DMEM + 15 mM-HEPES + 12 mM-$NaHCO_3$ with pH adjusted at 7·35–7·45. The substances to be tested (SRIF, TEA, 4-AP and high K^+) were added to the medium as indicated in the Results section. Medium volumes remained at about 1 ml $dish^{-1}$; flow rate was 0·5 ml min^{-1}. Eluate for the first one and a half hour stabilization period was discarded, then fractions were collected every 2 min in tubes containing 100 μl of PBS + 1% BSA, and stored at −20 °C until radioimmunoassay (RIA).

Radioimmunoassay

GH and PRL levels in the effluent medium were determined by a radioimmunoassay using the kits provided by the National Hormone and Pituitary Program and University of Maryland School of Medicine (Baltimore, MD, USA; NIDDK; anti-rat GH serum (NIDDK, monkey, anti-rGH-S-4), rat GH reference (NIDDK, rGH-RP2) and rat GH for RIA (NIDDK, rGH-1-4), anti-rat PRL serum (NIDDK, anti-rPRL-S-9), rat PRL reference (NIDDK, rPRL-RP3) and rat PRL for RIA (NIDDK, rPRL-I-4)). All perifusion samples from one experiment were measured in the same assay and all the measurements were performed in duplicate. Growth hormone and prolactin values were expressed as ng equivalents of rat standard GH-RP-1 and PRL-RP-1.

Hormone release was calculated by substracting the mean basal release (before treatment) from each sample collected during treatment (Nordmann & Zyzek, 1982).

Recordings

Since electrophysiological properties have been shown to evolve with time in culture up to the 7th day (Israel *et al.* 1983), we carried out all experiments on 7- to 15-day-old cells, whose electrical properties are stable. Culture medium was replaced by recording medium (DMEM + 12 mM-$NaHCO_3$ and 15 mM-HEPES) about 15 min before the start of electrophysiological experiments. Microelectrodes were pulled from capillary tubes (Phymep F10, Clark Electromedical Instruments, England) with a horizontal puller (BB-CH, Mecanex, Geneva, Switzerland). Their resistances varied from 80 to 120 MΩ when filled with a 4 M-potassium acetate solution. A single-microelectrode bridge amplifier (Dagan cell explorer 8700; DAGAN Corp., Minneapolis, MN, USA) was used for the recording of potential and injection of transmembrane current. The drugs used were dissolved in the recording medium and were locally applied close to the cell by ejection from a micropipette (≈ 2 μm in diameter) connected to an air-pressure system. This technique of drug application has been discussed in detail by Palmer, Wuerthele & Hoffer (1980), particularly the concentration of the drug which reaches the cell membrane. The pressure was adjusted to 0·1 bar, which was sufficient for drug ejection with the least pressure artifact. Controls were performed with normal medium, confirming that pressure ejection did not trigger an artificial response.

In the experiments shown below, the channel blockers tested were applied directly to the cell via a glass delivery pipette with a tip diameter of 20–30 μm, so that substances reached the cell membrane by unit gravity, when the pipette was pushed near the cell.

Chemical materials used

Culture medium and sera were from GIBCO (95051 Cergy-Pontoise, France). Cobalt ($CoCl_2$), HEPES, nickel ($NiCl_2$), tetraethylammonium chloride (TEA), 4-aminopyridine (4-AP), dopamine (DA) and somatostatin (SRIF) were purchased from Sigma (Chemical Company, St Louis, MO, USA).

RESULTS

Distribution of cells

The enrichment of the pituitary cells was confirmed with immunofluorescent staining of cultures from any given fraction. The percentage of somatotrophs was higher in fractions 8 and 9, constituting 75–85% ($n = 6$) of the total cell count, lactotrophs represented 15–20% and others (gonadotrophs, corticotrophs, thyrotrophs) less than 5% of the total cells. After immunofluorescent staining, the average cell size for each cell type was estimated and found to be 11–13 μm ($n = 25$) for lactotrophs and 11–14 μm ($n = 85$) for somatotrophs.

Hormone release

Perifusion experiments indicated that detectable amounts of GH and PRL were continuously secreted for periods of up to 4 h in all dishes tested. Basal GH release was stable with mean values of 6 ng $(2\ min)^{-1}$ $(10^6\ cells)^{-1}$ ($5{\cdot}97 \pm 3{\cdot}32$, mean ± S.E.M.; $n = 140$) and basal PRL release from the same preparations was 1·6 ng $(2\ min)^{-1}$ $(10^6\ cells)^{-1}$ ($1{\cdot}62 \pm 0{\cdot}71$, mean ± S.E.M.; $n = 80$). In the same cell culture, SRIF (2×10^{-7} M) and DA (10^{-7} M) were tested on PRL and GH release. Figure 1 showed that SRIF reduced GH release to very low levels (10% of the basal release) but did not modify PRL release, whereas DA significantly inhibited PRL release to less than 10% of the basal value without any effect on GH release. Note that both SRIF-induced inhibition of GH release and DA-induced inhibition of PRL release had slight rebounds before returning to basal level.

Perifusion of SRIF-containing (10^{-7} M) medium markedly decreased GH release to

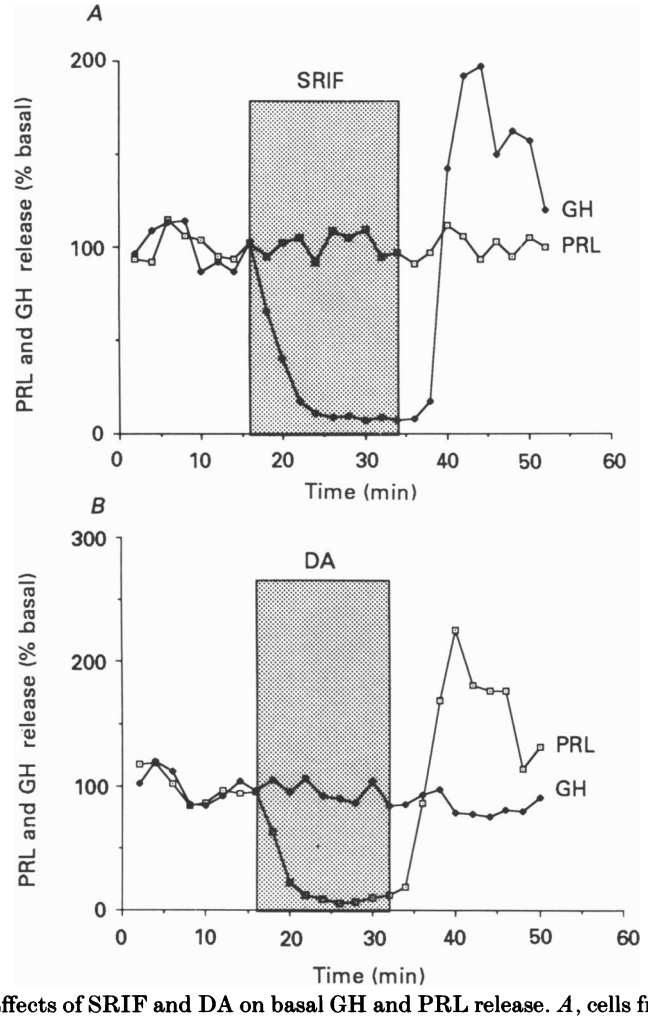

Fig. 1. Effects of SRIF and DA on basal GH and PRL release. *A*, cells from somatotroph-enriched cultures (F_8 and F_9) were continuously perifused and medium was collected every 2 min (0·5 ml min^{-1}). During 18 min of SRIF (2×10^{-7} M) treatment (shaded area), GH release was markedly reduced to less than 15% of the basal release. After cessation of treatment, GH release showed a slight rebound before returning to basal level. However, prolactin release was not modified by this treatment. This curve is representative of six experiments. *B*, perifusions were performed on the same cell preparation. Dopamine-containing medium (10^{-7} M) was used in perifusion for 16 min (shaded area) during which time prolactin release was reduced to less than 10% of the control level. At the same time, GH release was not affected. Note the significant rebound of prolactin release following dopamine treatment. This curve is representative of five experiments.

30% (from $6·2 \pm 2·8$ to $1·9 \pm 0·43$ ng $(2\ min)^{-1}$; mean ± S.E.M.; $n = 10$) of basal level (Fig. 2). A similar inhibition was observed when the potassium channel blocker tetraethylammonium (TEA; 40 mM) was added in the SRIF-containing medium ($2·8 \pm 0·6$ ng $(2\ min)^{-1}$) or when 4-aminopyridine (4-AP; 5 mM) was used ($2·0 \pm 0·5$ ng $(2\ min)^{-1}$), although TEA or 4-AP alone could slightly increase GH release respectively

to 6·6 ng (2 min)$^{-1}$ and to 6·5 ng (2 min)$^{-1}$. In normal perifusion conditions, medium containing SRIF reduced basal GH release to 52% at 10^{-10} M and 22% at 10^{-7} M (Fig. 3*A* and *C*). A high external potassium concentration (30 mM) induced a marked increase in GH release which was nevertheless diminished by an increase in SRIF concentration (Fig. 3*B* and *C*).

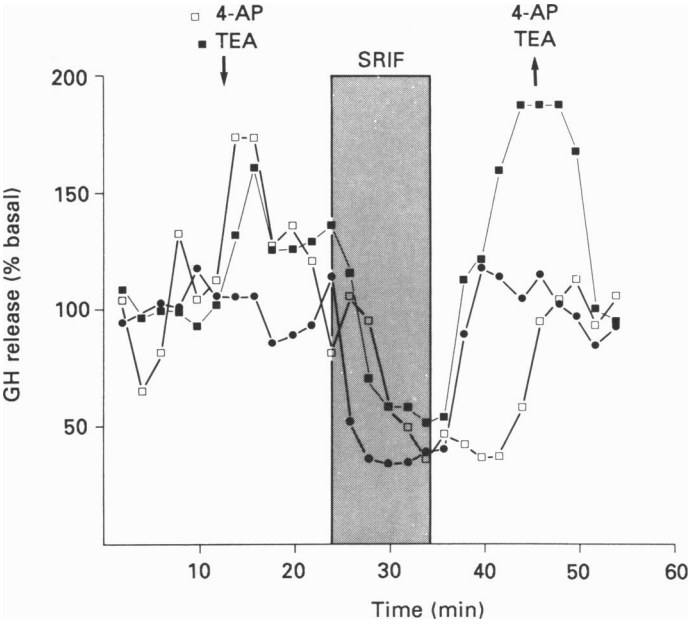

Fig. 2. Effect of SRIF on spontaneous growth hormone release with or without TEA (40 mM) and 4-AP (5 mM) from somatotroph-enriched cells in primary cultures. SRIF (10^{-7} M) was added for a 10 min period (shaded area) to perifusion medium (●), perifusion medium with TEA (■) or 4-AP (□). TEA or 4-AP slightly increased the GH release. However, during SRIF treatment, growth hormone release is decreased to less than 50% in all conditions. This curve was representative of five similar experiments.

Membrane electrical properties

After 7 days in culture, 187 cells were recorded. The electrical properties of the cells did not vary significantly between the 7th and the 15th day in culture, nor from one Petri dish to another. Membrane resting potential varied from −45 to −65 mV except in twenty-six injured cells which displayed a low membrane potential (less than −30 mV) and which progressively decreased to 0 mV within 5 min. These twenty-six cells were excluded. From the remaining 161 cells, membrane input resistances were measured and found to be between 150 and 500 MΩ (214 ± 34 MΩ, $n = 161$). One hundred and twenty-six cells (78%) showed either triggered (77%) or spontaneous (23%) action potentials and were thus classified as excitable cells. Membrane resting potential (−54 ± 8·3 mV) and resistance (227 ± 37 MΩ) of the excitable cells were not significantly different from those of non-excitable cells. Slight depolarizing waves were often observed in spontaneous cells (Fig. 4*A*, left), which might trigger all-or-none action potentials at depolarization of the membrane

potential to threshold, which varied from −45 mV to −50 mV according to the cell. The mean amplitude of action potentials measured from 126 cells was 32·7 ± 4·2 mV and their mean duration was 11·9 ± 2·0 ms ($n = 44$). No differences in these parameters were detected between spontaneous and non-spontaneous cells. Frequencies of spontaneous action potentials could reach more than 10 Hz and varied from one cell to another.

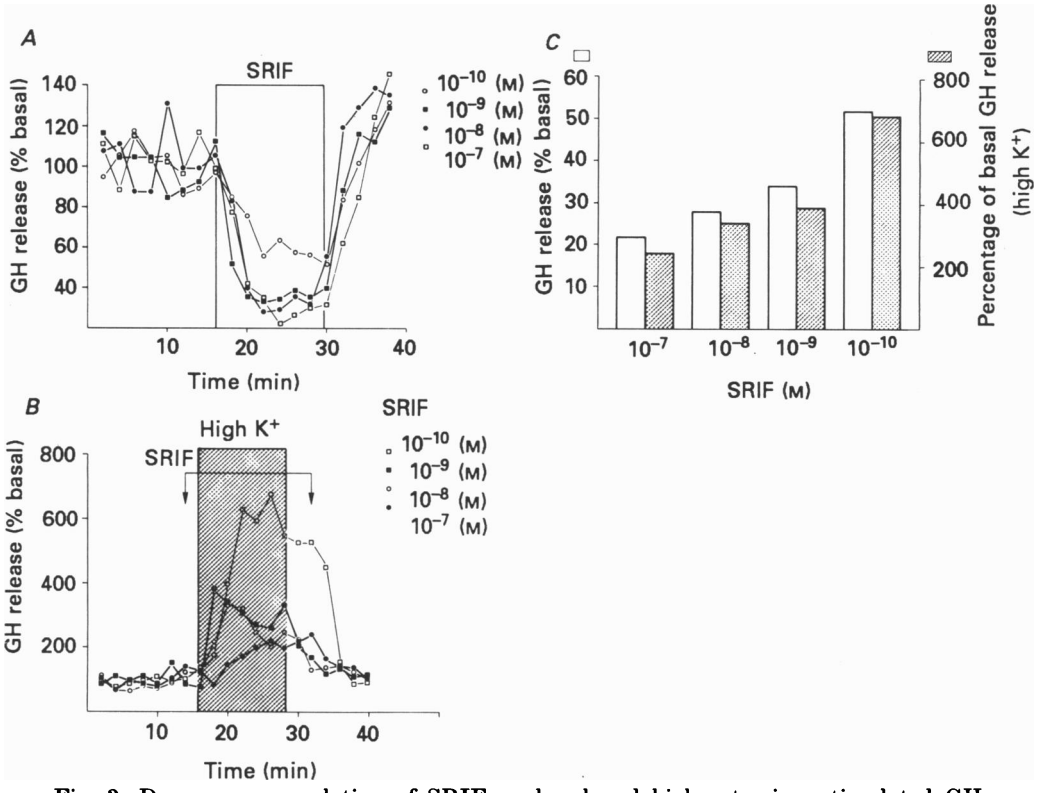

Fig. 3. Dose–response relation of SRIF on basal and high-potassium-stimulated GH release. All the curves represented at least five similar experiments. *A*, inhibitory effects induced by different concentration of SRIF (enclosed area). Even with 10^{-10} M-SRIF, GH release is reduced to approximately 50% of basal release and with 10^{-7} M it is reduced to almost 20%. *B*, inhibitory effects on high-potassium-stimulated (shaded area) GH release by different concentrations of SRIF (arrow). As SRIF was increased from 10^{-10} to 10^{-7} M, high-potassium-stimulated GH release is also decreased from 670 to 240%. *C*, dose–response relation of SRIF on GH basal release (□, left ordinate) and high-potassium-stimulated GH release (▨, right ordinate).

Some non-spontaneous cells showed action potentials at cessation of applied hyperpolarizing currents and were thus called 'off spikes' (Fig. 4*A*, right).

To characterize the ionic properties of inward currents implicated in the depolarizing phase of the action potential, specific current blockers were used. The calcium current blockers, cobalt or nickel at 5 mM, completely and reversibly inhibited the action potentials (Fig. 4*B*, left and right).

Sodium-free extracellular medium (sodium was replaced by choline) was used to

identify sodium current involvement in action potentials. This modified medium did not abolish action potentials which were still inhibited by nickel (Fig. 4*B*, right). Tetrodotoxin, known to inhibit the sodium current, was also used and did not block the action potential (result not shown), confirming observations from a previous report (Israel *et al.* 1983).

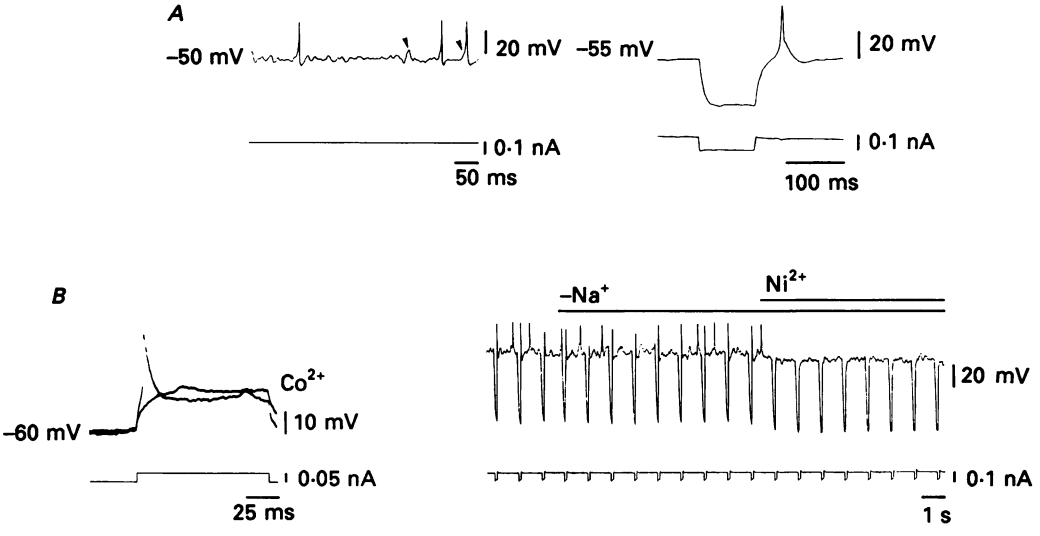

Fig. 4. Electrical characteristics recorded from presumptive somatotrophs. *A*, left, shows an example of spontaneous action potentials. A slow depolarizing wave can be seen (arrow-head) and if it takes the membrane potential above threshold, an all-or-none action potential will take place. *A*, right, is a typical recording from an excitable cell with an action potential (upper trace) following a hyperpolarizing pulse (lower trace), which was called an 'off-spike' in the text. *B*, left, effect of cobalt on the action potential. When the cell was at resting potential (−60 mV), an action potential was evoked by a depolarizing pulse (lower trace) and was completely blocked by cobalt (5 mM in the delivery pipette). *B*, right, spontaneous and off-spikes (left) were not affected with extracellular sodium-free medium but were blocked by nickel (right).

Effects of SRIF on the electrical properties of the cells

In all the experiments reported here, SRIF (10^{-7} M) was applied directly to the cell through a glass delivery pipette with low pressure.

Among 124 cells tested, 90 cells (73%) responded to SRIF. This response consisted of a rapid hyperpolarization accompanied by a significant decrease of membrane resistance. Thus from the resting potential, the cell membrane could be hyperpolarized by about −15 mV, which led to the cessation of action potentials (Fig. 5, upper and middle panels). This response was concomitant with a membrane resistance decreasing which could reach about 50% of the control value (Fig. 5, lower panel).

The amplitude of the SRIF-induced response was dose-dependent (Fig. 6). The dose–response curve was obtained by increasing ejection time with constant pressure. The doses of SRIF were therefore expressed in units of kilopascals (kPa)

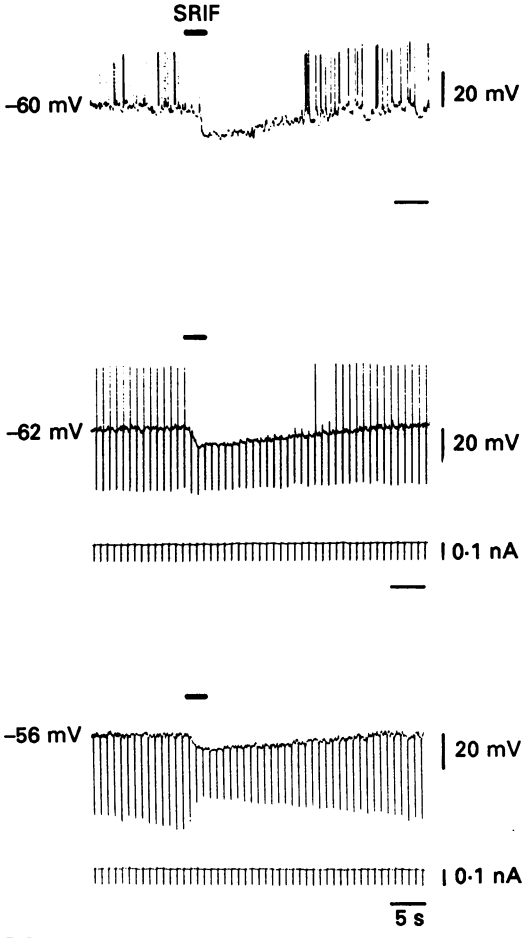

Fig. 5. Effect of SRIF on the electrical properties of excitable and unexcitable cells. Upper trace, a spontaneously firing cell at resting potential. SRIF (10^{-7} M in the delivery micropipette) induced a hyperpolarizing response, during which action potentials were totally abolished. Middle trace, action potentials were triggered by hyperpolarizing pulses (off-spikes) at resting potential and were suppressed during the SRIF response. In this cell, SRIF evoked a hyperpolarizing response characterized by a decrease of the membrane resistance as indicated by the attenuation of the deflections induced by hyperpolarizing currents (0·1 nA, 100 ms, 1 Hz). Lower trace, the cell studied was unexcitable at resting potential: application of SRIF produced a hyperpolarization with a decrease of membrane resistance.

multiplied by the duration of the ejection in seconds. In agreement with experiments from McCaman, MacKenna & Ono (1977) and Legendre, Dupouy & Vincent (1988), we have also assumed that a direct relationship exists between the number of molecules delivered and the pulse duration. The response first appeared at the point of 0·4 kPa s and rapidly increased with ejection duration, to reach a plateau at ejection times of more than 200 ms(= 2 kPa s).

The amplitude of the SRIF-induced response varied with cell potential, i.e. it was

increased when the cell was depolarized and was decreased when the cell was hyperpolarized. The reversal potential of the SRIF-induced response was found to be near −100 mV (Fig. 7*A* and *B*). High concentrations of potassium, 30 or 55 mM (in this case SRIF was dissolved in the same high-potassium-containing medium), induced a shift of the reversal potential to more positive levels: nearly −70 mV for 30 mM and −50 mV for 55 mM (Fig. 7*B* and *C*).

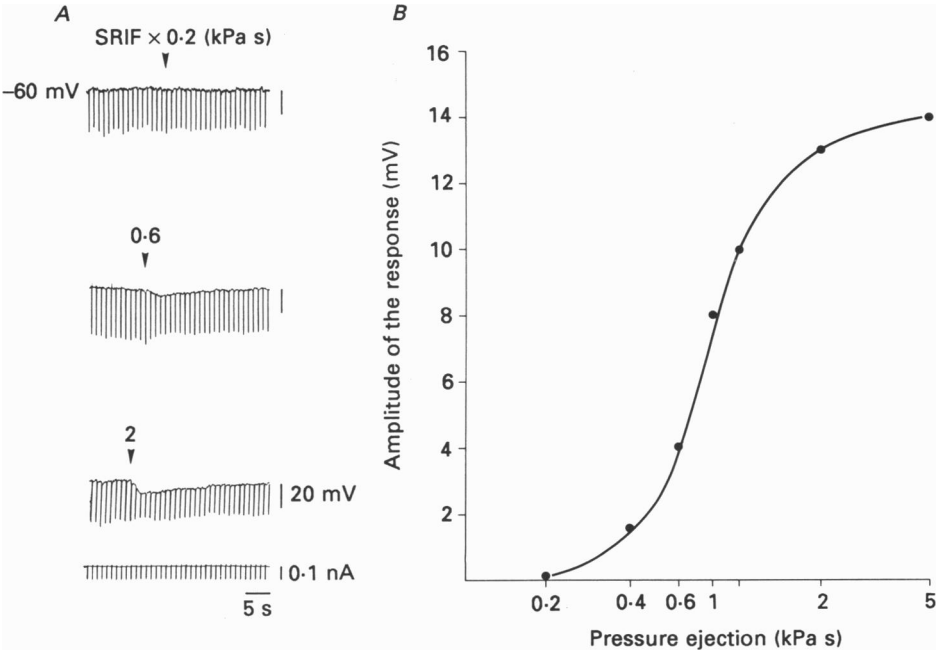

Fig. 6. Dose–response relation for SRIF. *A*, SRIF was delivered from a micropipette (2 μm in diameter) close to a single cell. As shown (injection: 0·2, 0·6 and 2 kPa s), the amplitude of hyperpolarization was increased as a function of ejection time. *B*, the absolute amplitudes of the induced responses were plotted *versus* the pressure (kPa) multiplied by ejection time (s) (resting potential of the cell: −60 mV). The plateau was reached at 2 kPa s.

Pharmacological studies of SRIF-induced responses

The above results indicate that SRIF may induce a hyperpolarizing response through an increase of potassium conductance. In order to identify the ionic currents implicated in this response, we used specific pharmacological agents of the different types of potassium channels.

TEA was administered at a concentration of 40 mM, which is believed to completely block the TEA-sensitive potassium channels. This induced no significant effect on the hyperpolarizing response induced by SRIF (Fig. 8*A*) on the four cells tested.

A high concentration of 4-AP (5 mM) in the delivery pipette was found to slightly increase the membrane resistance but had only a weak effect on the SRIF-induced response (Fig. 8*B*). This was shown on three cells.

Comparative study of effects induced by dopamine and SRIF

There exists evidence for control of prolactin release by SRIF (Vale, Rivier, Brazeau & Guillemin, 1974) and modification of growth hormone release by dopamine (Serri, Deslauriers & Brazeau, 1987); consequently a comparative study was performed with SRIF and DA on the presumed two cell types.

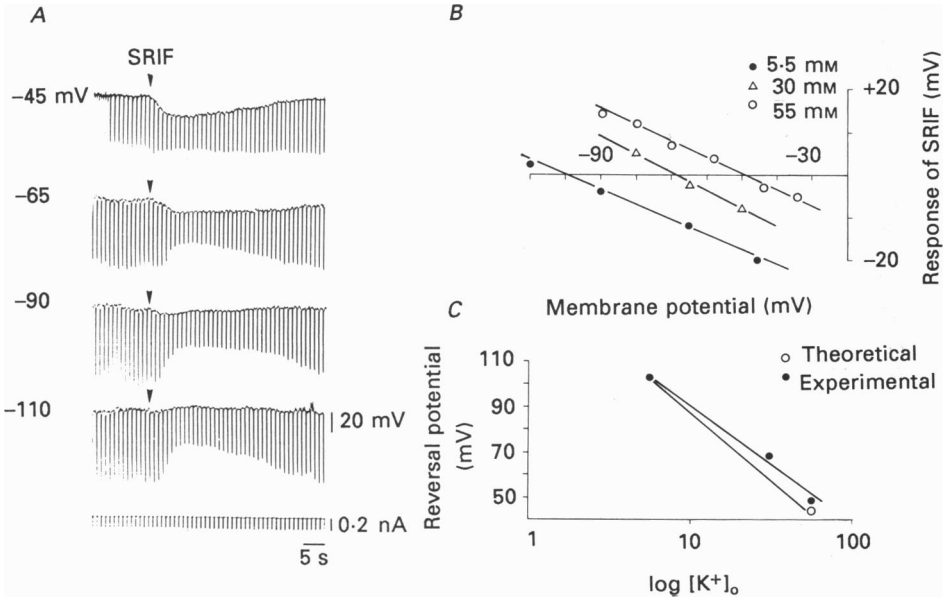

Fig. 7. Ionic properties of SRIF-induced response. *A*, SRIF was tested at different membrane potentials (−45, −65, −90, −110 mV) on the same cell. The amplitude of the responses decreased as the membrane potential was held at more negative levels and was reversed at high membrane potentials. *B*, the amplitude of the SRIF response was plotted *versus* the membrane potential. The experiment was performed in normal medium with 5·5 mM-potassium (●) as shown in *A*, and in high-potassium medium (△, 30 mM; and ○, 55 mM). The reversal potentials were found to be near −100 mV in normal medium, while in 30 and 55 mM-potassium medium, the reversal potentials were found to be near −70 and −50 mV. *C*, the reversal potential was plotted as a function of the log $[K^+]_o$ (●) and the theoretical relation from the Nernst equation was drawn (○). This figure shows the good correlation between the experimental and theoretical relations.

SRIF-induced hyperpolarizations were shown on cells from the somatotroph-enriched fractions, whereas at the same time DA had no effect on these cells (Fig. 9*A*). On the other hand, in lactotroph-enriched fractions (F_3 and F_4), and in very few cells from somatotroph-enriched (F_8 and F_9) fractions (two of twelve cells tested), dopamine (10^{-7} M) induced typical responses as previously described by Israel *et al.* (1987), thus further identifying the target cells as prolactin cells. Ejection of SRIF on the same cell had no influence on membrane potential or conductance (Fig. 9*B*).

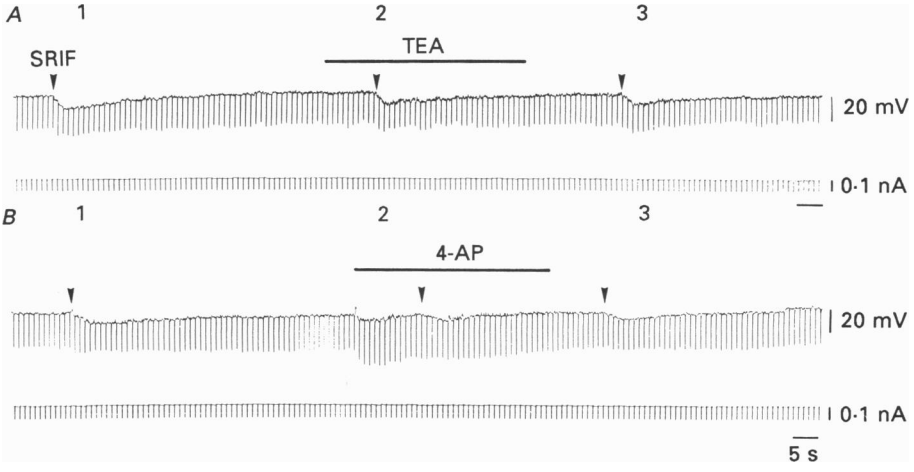

Fig. 8. Pharmacological study of the SRIF response induced on somatotrophs. This experiment consisted in three successive applications of SRIF on a same cell and was performed in control condition (1), with ion current blockers (2) and after their removal (3). *A*, effect of tetraethylammonium (TEA). Under constant application of TEA (2, continuous bar, 40 mM), ejection of SRIF induced a response identical to control before (1) and after TEA (3). *B*, effect of 4-aminopyridine (4-AP). 4-AP treatment (continuous bar, 5 mM) affected slightly the response to SRIF (2). Note the increase of membrane resistance due to the 4-AP application.

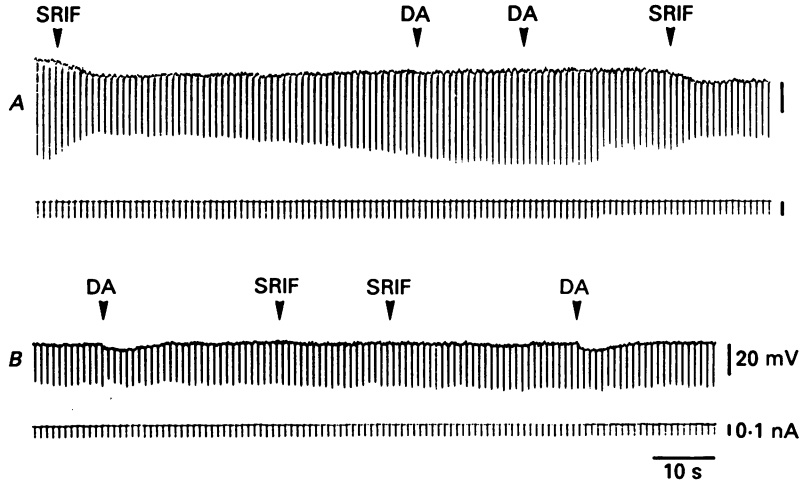

Fig. 9. Different responsiveness to SRIF and DA between presumed somatotroph and lactotroph. *A*, in somatotroph-enriched fraction, SRIF (10^{-7} M) induced a hyperpolarizing response, whereas DA (10^{-7} M) had no effect on the same cell. *B*, in lactotroph-enriched fractions or on very few cells from somatotroph-enriched fractions (here is shown a result from somatotroph-enriched fractions), DA induced a hyperpolarizing response, whereas SRIF (10^{-7} M) had no effect on the same cell.

DISCUSSION

The present study was performed on the somatotroph-enriched primary cultures from male adult rats. It is well known that secretory granules packaged in the Golgi region are stored intracellularly until release (Palade, 1975), which requires an increase of intracellular free calcium (Douglas, 1981). It has been suggested that calcium may enter the secretory cells via membrane calcium channels with detectable electrical activity (such as action potential, membrane noise etc.). In view of this stimulus–secretion hypothesis, the electrophysiological properties of several secretory cell types, including pituitary cells, have been studied (for review, see Vincent & Dufy, 1982) and is the reason behind this present experiment.

Identification of somatotrophs

Although the experiments were carried out on the somatotroph-enriched fraction (75–85%), there are other pituitary endocrine cells present, mainly lactotrophs (15–20%). Somatostatin (SRIF) may affect hormone release other than growth hormone (Vale *et al.* 1974; Richardson & Schonbrunn, 1981), and SRIF receptors have been identified on lactotroph membranes (Epelbaum, Enjalbert, Krantic, Musset, Bertrand, Rasolonjanahary, Shu & Kordon, 1987). Dual actions of dopamine on GH release *in vitro* have been demonstrated recently (Serri *et al.* 1987). In addition, there is also evidence which shows the co-localization of prolactin and growth hormone within specific adenohypophyseal cells (mammo-somatotrophs) in the rat (Nikitovitch-Winer, Atkin & Maley, 1987) and a possible transformation of somatotrophs into mammotrophs in male rats (Stratman, Ezrin & Seller, 1974). Thus a SRIF effect on lactotrophs, and conversely a DA effect on somatotrophs or some co-effects on mammo-somatotrophs, was researched in this experiment. Results from our experiments support a lack of SRIF-induced effect on lactotrophs: DA could markedly inhibit prolactin but not GH release; conversely, SRIF could inhibit GH release with no effect on prolactin release. Because of this, we believe that the effect of SRIF shown in our experiments is not obtained from lactotrophs. Also, immunofluorescent staining showed the population to consist of 80% somatotrophs; thus the probability of impaling a somatotroph was far greater than that of impaling a lactotroph.

Passive membrane electrical properties

In the present study, the majority of the cells were excitable showing stimulated or spontaneous action potentials. This is in agreement with the results from GH-secreting (Yamashita *et al.* 1986) and prolactin-secreting human tumoral cells (Israel, Jaquet & Vincent, 1985), GH_3B_6 cells (Dufy, Vincent, Fleury, Du Pasquier, Gourdji & Tixier-Vidal, 1979), normal rat lactotrophs (Israel *et al.* 1987) and bovine lactotrophs (Ingram, Bicknell & Mason, 1986). These action potentials were resistant to TTX and persisted in Na^+-free bath solution. It could thus be proposed that the sodium ion is not involved in the inward currents of spikes. Large sodium currents have, however, been described in GH-secreting tumoral cells (Yamashita *et al.* 1986), in bovine lactotrophs (Cobbett, Ingram & Mason, 1987) and rat (DeRiemer & Sakmann, 1986) somatotrophs. It was shown that sodium plays no major role in

prolactin release and its role remains undefined (Cobbett *et al.* 1987). As shown in the present report, action potentials in somatotroph-enriched cells were completely and reversibly blocked by the calcium current blockers cobalt and nickel. Therefore, calcium current is the main factor implicated in the depolarizing phase of the action potential. Holding the potential at −40 mV markedly reduced the amplitude of the action potential, which implicates the low-voltage-activated calcium channel in generation of calcium-dependent action potentials as suggested by DeRiemer & Sakmann (1986). It has been proposed that the calcium which enters the cell during the calcium-dependent action potential could increase the amount of cytosolic calcium and in this way contribute to the hormonal release process.

Effects of SRIF

SRIF, like other regulatory peptides, is supposed to bind first to a specific membrane-bound receptor located on the surface of the target cell. Since its discovery in 1973 (Brazeau, Rivier, Vale & Guillemin, 1974; Vale, Brazeau, Rivier, Brown, Boss, Rivier, Burgus, Ling & Guillemin, 1975), much evidence has accumulated showing that the effect of SRIF is a rapid, dose-dependent decrease in the intracellular concentration of cyclic AMP, which accompanies a reduction in basal hormone secretion (Bilezikjian & Vale, 1983; Cronin, Rogol, Myers & Hewlett, 1983). SRIF also inhibits the GH release induced by prostaglandins and phosphodiesterase inhibitors (Vale *et al.* 1975; Szabo & Frohman, 1975). However, SRIF can suppress GH release in response to exogenous cyclic AMP derivates (Hayasaki-Kimura & Takahashi, 1979; Sheppard, Spence & Kraicer, 1979), which suggests that reduction of cyclic AMP synthesis is not the sole mechanism by which SRIF inhibits GH release. In addition to this, Epelbaum *et al.* (1987) found that the SRIF binding sites were negatively but loosely coupled to adenylate cyclase.

SRIF may also inhibit calcium influx into the cell and thereby prevent stimulated hormone release. It has been shown that somatostatin reduces the GH release induced by the calcium ionophore A23187 (Hayasaki-Kimura & Takahashi, 1979; Kraicer & Spence, 1981) and by high potassium, which is believed to act directly on the cellular membrane causing a transient increase in calcium uptake (Schofield & Bicknell, 1978). There are two possibilities: SRIF acts directly at the membrane calcium channel to reduce the amount of calcium entering the cell or acts on the cell membrane potential thus indirectly influencing the voltage-dependent calcium currents. The present experiments clearly show a hyperpolarization due to an increase of membrane conductance in response to SRIF, which parallels experiments on human GH adenoma cells (Yamashita *et al.* 1986). The same kind of effect was found with the DA-induced response from human tumoral (Israel *et al.* 1985) and rat (Israel *et al.* 1987) and bovine (Ingram *et al.* 1986) normal prolactin cells.

The reversal potential of the SRIF response (−100 mV) and its shift to more positive levels in high external potassium strongly indicate the participation of potassium ions. It has also been suggested that SRIF increases a potassium conductance in pancreatic β-cells (Pace & Tarvin, 1981) and increases the non-inactivating, voltage-dependent outward potassium current in hippocampal neurones (Moore, Madamba, Joëls & Siggins, 1987) and in guinea-pig submucous plexus neurones (Mihara, North & Surprenant, 1987). Therefore, this electrical response to SRIF might be the general consequence of binding of SRIF with its receptors.

Because of the SRIF-induced hyperpolarization, calcium-dependent action potentials were inhibited as voltage-dependent calcium channels, open at resting potential, were believed to be closed. This leads to a decrease of calcium influx, which may explain in part the inhibitory effect of SRIF. This is corroborated by the inhibitory effect of SRIF on potassium-stimulated release (see Fig. 3*B* and *C*); a similar explanation was proposed from perifusion experiments of other groups (Kraicer & Chow, 1982).

The differences between this experiment and previous reports, which showed a SRIF-induced hyperpolarization concomitant with a decrease of membrane conductance by Israel *et al.* (1983), are probably due to differences in culture age. In the present experiment, all the electrical recordings were performed after at least 7 days of culture and in fact most were in culture for more than 10 days. In the previous experiment, SRIF was used at a high concentration (10^{-5} M) and perhaps triggered a non-specific response. However, a similar observation has been reported for lactotroph cells obtained from juvenile animals (14-day-old females), i.e. dopamine had a hyperpolarizing effect on certain lactotrophs via a decrease of membrane conductance (Israel *et al.* 1987). This response was not affected by sodium channel blockers but was reversed by cobalt or cadmium, suggesting the participation of calcium ions (unpublished results). Thus a direct inhibition of calcium currents by SRIF, although not found in the present experimental conditions, cannot be excluded, but may be particular to very young cells.

Pharmacological study of potassium channels implicated in the SRIF response

To study the characteristics of potassium channels involved in the SRIF-induced response, two relatively specific blockers were used to inhibit two classical types of potassium currents (see review by Adams, Smith & Thompson, 1980).

TEA is known to block the delayed voltage-dependent potassium current; TEA has no effect on the response to SRIF even at 40 mM. Similar observations were reported for the characterization of DA-induced responses in normal rat (Israel *et al.* 1987) and bovine (Ingram *et al.* 1986) prolactin cells.

4-AP is known to block the voltage-dependent transient potassium current; this blocker, even at high concentration (5 mM), has only a slight inhibitory effect on the SRIF-induced response. However, this concentration is relatively high and its partial inhibition of the SRIF-induced hyperpolarization may be unspecific. A similar finding has already been reported for the DA-induced response (Israel *et al.* 1987).

This, in conjunction with the ionic characterization of the response (see Fig. 7), implicates the possible involvement of a non-voltage-dependent potassium current, insensitive to TEA and 4-AP.

A third potassium current is known to be activated by intracellular calcium ions (calcium-dependent potassium current); the implication of such a current in this response seems unlikely, because an increase in intracellular calcium (released from intracellular storage sites) would logically lead to a simultaneous increase of hormone release and an activation of calcium-dependent potassium channels. A similar mechanism has been proposed for TRH-induced PRL release in GH_3 cells (Dubinsky & Oxford, 1985).

It thus seems that SRIF binding with its receptor could open a potassium channel

which does not belong to any of the 'classical' types identified by patch-clamp experiments. For further comprehension of the ionic mechanisms involved in this control, the additional information provided by voltage-clamp and patch-clamp techniques must be sought.

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REFERENCE

ADAMS, D. A., SMITH, S. J. & THOMPSON, S. H. (1980). Ionic current in molluscan soma. *Annual Review of Neuroscience* **3**, 141–167.

ARIMURA, A. & CULLER, M. D. (1985). Regulation of growth hormone secretion. In *The Pituitary Gland*, ed. IMURA, H., pp. 221–259. New York: Raven Press.

BILEZIKJIAN, L. M. & VALE, W. (1983). Stimulation of adenosine 3′,5′-monophosphate production by growth hormone-releasing factor and its inhibition by somatostatin in anterior pituitary cells in vitro. *Endocrinology* **113**, 1726–1731.

BRAZEAU, P., RIVIER, J., VALE, W. & GUILLEMIN, R. (1974). Inhibition of growth hormone secretion in the rat by synthetic somatostatin. *Endocrinology* **94**, 184–187.

COBBETT, P., INGRAM, C. D. & MASON, W. T. (1987). Sodium and potassium currents involved in action potential propagation in normal bovine lactotrophs. *Journal of Physiology* **392**, 273–299.

CRONIN, M. J., ROGOL, A. D., MYERS, G. A. & HEWLETT, E. L. (1983). Pertussis toxin blocks the somatostatin-induced inhibition of growth hormone release and adenosine 3′,5′-monophosphate accumulation. *Endocrinology* **113**, 209–215.

DENEF, C., HAUTEKEETE, E., DE WOLF, A. & VANDERSCHUEREN, B. (1978). Pituitary basophils from immature male and female rats: distribution of gonadotrophs and thyrotrophs as studied by unit gravity sedimentation. *Endocrinology* **103**, 724–735.

DENEF, C., HAUTEKEETE, E. & RUBIN, L. (1976). A specific population of gonadotrophs purified from immature female rat pituitary. *Science* **194**, 848–851.

DERIEMER, S. A. & SAKMANN, B. (1986). Two calcium currents in normal rat anterior pituitary cells identified by a plaque assay. *Experimental Brain Research*, series **14**, 139–154.

DOUGLAS, W. W. (1981). Aspects of the calcium hypothesis of stimulus–secretion coupling: electrical activity in adenohypophyseal cells, and membrane retrieval after exocytosis. *Methods in Cell Biology* **23**, 485–501.

DUBINSKY, J. M. & OXFORD, G. S. (1985). Dual modulation of K^+ channels by thyrotropin-releasing hormone in clonal pituitary cells. *Proceedings of the National Academy of Sciences of the USA* **82**, 4282–4286.

DUBOIS, M. P. (1972). Nouvelles données sur la localisation au niveau de l'adénohypophyse des hormones polypeptidiques: ACTH, MSH, LPH. Etude en immunofluorescence de l'hypophyse in situ de bovins, ovins et porcins et d'homogreffes hypophysaires dans l'hypothalamus de rat. *Lille Médical* **17**, 1391.

DUFY, B., VINCENT, J. D., FLEURY, H., DU PASQUIER, P., GOURDJI, D. & TIXIER-VIDAL, A. (1979). Dopamine inhibition of action potentials in a prolactin secreting cell line is modulated by oestrogen. *Nature* **282**, 855–857.

EPELBAUM, J., ENJALBERT, A., KRANTIC, S., MUSSET, F., BERTRAND, P., ROSOLONJANAHARY, R., SHU, C. & KORDON, C. (1987). Somatostatin receptors on pituitary somatotrophs, thyrotrophs and lactotrophs: pharmacological evidence for loose coupling to adenylate cyclase. *Endocrinology* **121**, 2177–2185.

HAYASAKI-KIMURA, N. & TAKAHASHI, K. (1979). Studies on action of somatostatin on growth hormone release in relation to calcium and cAMP. *Proceedings of the Society for Experimental Biology and Medicine* **161**, 312–318.

HOPKINS, C. R. & FARQUHAR, M. G. (1973). Hormone secretion by cells dissociated from rat anterior pituitaries. *Journal of Cell Biology* **59**, 276–303.

INGRAM, C. D., BICKNELL, R. J. & MASON, W. T. (1986). Intracellular recordings from bovine anterior pituitary cells: Modulation of spontaneous activity by regulators of prolactin secretion. *Endocrinology* **119**, 2508–2518.

ISRAEL, J. M., DENEF, C. & VINCENT, J. D. (1983). Electrophysiological properties of normal somatotrophs in culture: an intracellular study. *Neuroendocrinology* **37**, 193–199.

ISRAEL, J. M., JAQUET, J. & VINCENT, J. D. (1985). The electrical properties of isolated human prolactin-secreting adenoma cells and their modification by dopamine. *Endocrinology* **117**, 1448–1455.

ISRAEL, J. M., KIRK, C. & VINCENT, J. D. (1987). Electrophysiological responses to dopamine of rat hypophysial cells in lactotroph-enriched primary cultures. *Journal of Physiology* **390**, 1–22.

KRAICER, J. & CHOW, A. E. H. (1982). Release of growth hormone from purified somatotrophs: Use of perifusion system to elucidate interrelations among Ca^{2+}, adenosine 3′,5′-monophosphate, and somatostatin. *Endocrinology* **111**, 1173–1180.

KRAICER, J. & SPENCE, J. W. (1981). Release of growth hormone from purified somatotrophs: use of high K^+ and the ionophore A23187 to elucidate interrelations among Ca^{2+}, adenosine 3′,5′-monophosphate, and somatostatin. *Endocrinology* **108**, 651–657.

LEGENDRE, P., DUPOUY, B. & VINCENT , J. D. (1988). Excitatory effect of noradrenaline on pacemaker cells in spinal cord primary cultures. *Neuroscience* **24**, 647–658.

MCCAMAN, R. E., MACKENNA, E. F. & ONO, J. K. (1977). A pressure system for intracellular and extracellular ejections of picoliter volumes. *Brain Research* **136**, 141–147.

MIHARA, S., NORTH, R. A. & SURPRENANT, A. (1987). Somatostatin increases an inwardly rectifying potassium conductance in guinea-pig submucous plexus neurones. *Journal of Physiology* **390**, 335–355.

MOORE, C. D., MADAMBA, S. G., JOËLS, M. & SIGGINS, G. R. (1987). Somatostatin augments the M-current in hippocampal neurons. *Science* **239**, 278–280.

NIKITOVITCH-WINER, M. B., ATKIN, J. & MALEY, B. E. (1987). Colocalization of prolactin and growth hormone within specific adenohypophyseal cells in male, female, and lactating female rats. *Endocrinology* **121**, 625–630.

NORDMANN, J. J. & ZYZEK, E. (1982). Calcium efflux from the rat neurohypophysis. *Journal of Physiology* **325**, 281–299.

PACE, C. S. & TARVIN, J. T. (1981). Somatostatin: mechanism of action in pancreatic islet beta-cells. *Diabetes* **30**, 836–842.

PALADE, G. (1975). Intracellular aspects of the process of protein secretion. *Science* **189**, 347–350.

PALMER, M. R., WUERTHELE, S. M. & HOFFER, B. J. (1980). Physical and physiological characteristics of micropressure ejection of drugs from multibarreled pipettes. *Neuropharmacology* **19**, 931–938.

RICHARDSON, U. I. & SCHONBRUNN, A. (1981). Inhibition of adrenocorticotropin secretion by somatostatin in pituitary cells in culture. *Endocrinology* **108**, 281–290.

SCHOFIELD, J. G. & BICKNELL, R. J. (1978). Effect of somatostatin and verapamil on growth hormone release and ^{45}Ca fluxes. *Molecular and Cellular Endocrinology* **9**, 255–268.

SERRI, O., DESLAURIERS, N. & BRAZEAU, B. (1987). Dual action of dopamine on growth hormone release in vitro. *Neuroendocrinology* **45**, 363–367.

SHEPPARD, M. S., SPENCE, J. W. & KRAICER, J. (1979). Release of growth hormone from purified somatotrophs: Role of adenosine 3′,5′-monophosphate and guanosine 3′,5′-monophosphate. *Endocrinology* **105**, 261–268.

STRATMAN, I. E., EZRIN, C. & SELLER, E. A. (1974). Estrogen-induced transformation of somatotrophs into mammotrophs in the rat. *Cell and Tissue Research* **155**, 229–239.

SZABO, M. & FROHMAN, L. A. (1975). Effects of porcine stalk median eminence and prostaglandin E_2 on rat growth hormone secretion in vivo and their inhibition by somatostatin. *Endocrinology* **96**, 955–961.

VALE, W., BRAZEAU, P., RIVIER, C., BROWN, M., BOSS, B., RIVIER, J., BURGUS, R., LING, N. & GUILLEMIN, R. (1975). Somatostatin. *Recent Progress in Hormone Research* **31**, 365–392.

VALE, W., RIVIER, J., BRAZEAU, P. & GUILLEMIN, R. (1974). Effects of somatostatin on the secretion of thyrotropin and prolactin. *Endocrinology* **95**, 968–977.

VINCENT, J. D. & DUFY, B. (1982). Electrophysiological correlates of secretion in endocrine cells. In *Cellular Regulation of Secretion and Release*, ed. Conn, P. M., pp. 107–145. New York: Academic Press.

YAMASHITA, N., SHIBUYA, N. & OBATA, E. (1986). Hyperpolarization of the membrane potential caused by somatostatin in dissociated human pituitary adenoma cells that secrete growth hormone. *Proceeding of the National Academy of Sciences of the USA* **83**, 6198–6202.