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Hyperpolarization of the membrane potential caused by somatostatin in dissociated human pituitary adenoma cells that secrete growth hormone

(somatotropin release-inhibiting factor/potassium conductance/calcium channel/cobalt)

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ABSTRACT **Membrane electrical properties and the response to somatostatin were examined in dissociated human pituitary adenoma cells that secrete growth hormone (GH). Under current clamp condition with a patch electrode, the resting potential was −52.4 ± 8.0 mV, and spontaneous action potentials were observed in 58% of the cells. Under voltage clamp condition an outward K^+ current, a tetrodotoxin-sensitive Na^+ current, and a Ca^{2+} current were observed. Cobalt ions suppressed the Ca^{2+} current. The threshold of Ca^{2+} current activation was about −60 mV. Somatostatin elicited a membrane hyperpolarization associated with increased membrane permeability in these cells. The reversal potential of somatostatin-induced hyperpolarization was −78.4 ± 4.3 mV in 6 mM K^+ medium and −97.2 ± 6.4 mV in 3 mM K^+ medium. These reversal potential values and a shift with the external K^+ concentration indicated that membrane hyperpolarization was caused by increased permeability to K^+. The hyperpolarized membrane potential induced by somatostatin was −63.6 ± 5.9 mV in the standard medium. This level was subthreshold for Ca^{2+} and Na^+ currents and was sufficient to inhibit spontaneous action potentials. Hormone secretion was significantly suppressed by somatostatin and cobalt ions. Therefore, we suggest that Ca^{2+} entering the cell through voltage-dependent channels are playing an important role for GH secretion and that somatostatin suppresses GH secretion by blocking Ca^{2+} currents. Finally, we discuss other possibilities for the inhibitory effect of somatostatin on GH secretion.**

Somatostatin (somatotropin release-inhibiting factor, SRIF) is a potent inhibitor of growth hormone (GH) secretion. It also inhibits the secretion of other pituitary hormones (1, 2) and extrapituitary hormones (3). GH secretion is known to be regulated by the level of intracellular cyclic AMP (cAMP), which is raised by a stimulatory hormone, such as GH-releasing factor (4). It has been claimed that somatostatin exerts its inhibitory action through the reduction of cAMP production (5–7).

Ca^{2+} is another important factor that controls GH secretion from the anterior pituitary. It has been shown that Ca^{2+} is required for GH release evoked by high concentrations of extracellular K^+ (8) and GH-releasing factor (4, 9). Various anterior pituitary cell lines have been shown to have action potentials that are dependent on Ca^{2+} or Na^+ (10). The influx of Ca^{2+} is postulated to be controlled at least partly by the action potential. The membrane-signal transduction mechanism for somatostatin action has not yet been fully clarified in the pituitary. In the rat pancreas islet it has been reported that somatostatin activates a K^+ conductance and inhibits glucose-induced insulin release (11). In analogy, a similar mechanism of somatostatin may exist in the pituitary. For the investigation of the mechanism of somatostatin action in the pituitary, human GH-producing pituitary tumor cells are useful because the presence of somatostatin receptors in the cell membrane has been demonstrated (12–14) and the inhibition of GH secretion by somatostatin has been reported (15–19). In the present study we investigated the response of the membrane potential to somatostatin in dissociated human pituitary adenomas that secreted GH, and we found that somatostatin increased the K^+ conductance.

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MATERIALS AND METHODS

Cell Culture. Three GH-producing human pituitary adenomas were obtained at transsphenoidal surgery. The methods for preparing monolayer cells were essentially the same as described by Ishibashi and Yamaji (9, 17). The tissue was minced into small pieces (less than 1 mm in diameter) and was treated with collagenase (0.2 mg/ml) and hyaluronidase (1 mg/ml). These dissociated cells (about 2×10^5 cells per ml) were then seeded in multiwell culture dishes (24 holes) for hormone assay, and the remaining cells were seeded in 35-mm culture dishes for electrophysiological studies. The monolayer cells were cultured at 37°C under 5% CO_2 in humidified air in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated fetal calf serum. The culture medium was changed every other day. Hormone release assays were carried out within 10 days after cell preparation, and electrophysiological studies were done within 4 weeks.

It has been reported that GH-secreting human adenomas, which secrete GH and a small amount of prolactin *in vitro*, are almost exclusively constituted by cells immunoreactive to anti-GH antibody. Other pituitary hormones either are not detectable or are detectable in extremely low concentrations in comparison to GH (20). The fact that the cells are immunoreactive to anti-GH antibody implies that these cells produce GH. The adenomas used in the present study released GH *in vitro* (the mean GH secretion was 496.5 ng/ml per 2 hr in no. 1, 545.5 ng/ml per 2 hr in no. 2, and 539 ng/ml per 2 hr in no. 3). Prolactin was undetectable in two adenomas (nos. 1 and 3). In one adenoma (no. 2) released prolactin was less than 10% of GH (47.5 ng/ml per 2 hr). Therefore it was considered that most cells examined in the present study were GH-producing cells. Fibroblasts that contaminated our cultures were easily discriminated under a microscope as described by Adams *et al.* (15).

Electrophysiological Analysis. For electrophysiological study, whole cell variations of the patch electrode voltage clamp technique (21) were employed. The L/M-EPC 5 amplifier (List Electronics, Federal Republic of Germany)

Abbreviation: GH, growth hormone.

was used. The standard solution in the patch electrode contained 141.3 mM KCl, 1 mM $MgCl_2$, 0.5 mM EGTA (K salt), 20 mM Hepes (K salt, pH 7.4). The composition of the patch electrode solution for blocking the outward current was 120 mM CsCl, 1 mM $MgCl_2$, 20 mM EGTA, 10 mM Hepes (Na salt, pH 7.4). The resistance of patch electrode ranged from 5 to 10 MΩ and the seal resistance was usually over several tens of GΩ. The composition of the standard extracellular medium was 128.8 mM NaCl, 6 mM KCl, 1 mM $MgCl_2$, 2.5 mM $CaCl_2$, 5.6 mM glucose, and 20 mM Hepes (Na salt, pH 7.4). Other media were made by replacing NaCl or $CaCl_2$ (or both) of the standard medium isoosmotically. To block Ca^{2+} channels, 5 mM $CoCl_2$ or 0.5 mM $LaCl_3$ was added to the medium. Somatostatin was dissolved in the extracellular medium (at 1 μM), and a glass capillary with a tip diameter of a few micrometers was filled with this solution. The glass capillary was placed 30–40 μm away from the recording cell and somatostatin was administered by applying positive pressure to the inside of the capillary for 500 msec. The liquid junction potential was corrected as described by Hagiwara and Ohmori (22). The detail of the above methods has been described elsewhere (23). Unless otherwise noted, experiments were carried out at room temperature (23–25°C).

Hormone Assay. The cells grown in multiwell culture dishes were washed two times with serum-free DMEM containing 0.5% human serum albumin. The cells were incubated with 1 ml of the same medium containing somatostatin (0.1 μM) or $CoCl_2$ (5 mM). After incubation for 2 hr, the medium was aspirated and centrifuged at 180 × *g* for 5 min. GH in the supernatant was assayed by double-antibody RIA (a kit supplied by S. Raiti of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases). Data were expressed as mean ± SEM, and statistical differences among data were tested by using Student's *t* test.

RESULTS

Action Potentials in Human GH-Producing Cells. The resting potential of human GH-producing pituitary cells was −52.4 ± 8.0 mV (mean ± SD) (n = 66) in the standard medium when recorded under current clamp with a patch electrode containing the standard internal solution (see *Materials and Methods*). This value was more negative than the values reported by others (24, 25) employing the glass microelectrode technique, probably because the leakage current was smaller in this case with the current clamp recording with a patch electrode. Spontaneous action potentials were observed in 58% of the cells (Fig. 1*A*). In the remaining cells spontaneous action potentials were not observed, but regenerative changes were evoked either by depolarizing current pulses (Fig. 1*B*) or after cessation of hyperpolarizing current pulses (Fig. 1*C*).

Membrane Currents in Human GH-Producing Cells. Fig. 2 shows records of voltage-gated channel currents in the standard medium obtained under voltage clamp conditions. With a patch electrode containing the standard internal solution, a major membrane current was the outward current (Fig. 2*A*). The threshold for the activation of the outward current was about −55 mV. The amplitude of the outward current increased and the kinetics became faster as the amplitude of depolarizing potential steps was increased. When the patch electrode contained 20 mM EGTA and Cs^+, the outward current was almost completely suppressed. Therefore it was concluded that the outward current was carried through K^+ channels, although the K^+ channels were not characterized further. After suppression of the outward current, the inward current with fast kinetics became prominent (Fig. 2*B*).‡ The amplitude and the kinetics of this inward current were voltage dependent. This inward current was totally eliminated, either when tetrodotoxin was added to the standard medium at 1 μM or when external Na^+ was isoosmotically replaced with choline. Therefore it was concluded that this fast inward current was carried through Na^+ channels. In the medium containing tetrodotoxin or in Na^+-free medium, another inward current with different kinetics was observed (Fig. 2*C*). Compared to the Na^+ channel current, the amplitude was smaller and the kinetics were slower. Similar inward currents with slow kinetics were observed in solution containing Ba^{2+} in place of Ca^{2+} (data not shown). This current was not observed in the medium containing 5 mM Co^{2+} or 0.5 mM La^{3+} (Fig. 2*D*). Therefore it was concluded that this slow inward current was carried through Ca^{2+} channels. The existence of Na^+ and Ca^{2+} channels in human GH-producing cells was also reported by Ozawa and Saito (24).

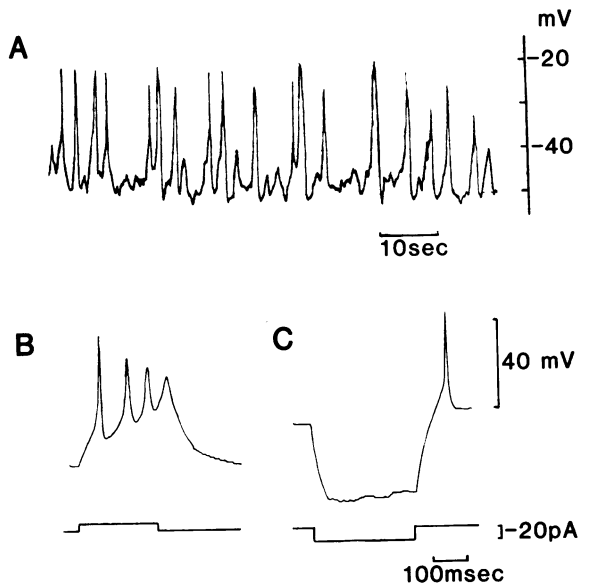

FIG. 1. (*A*) Spontaneous action potentials in GH-secreting adenoma cell. (*B*) Evoked action potentials in a cell that did not show spontaneous action potential. The depolarizing current pulse of 10 pA was applied. The resting potential was −53 mV. (*C*) An off-response evoked at the cessation of a hyperpolarizing current pulse of 20 pA. Spontaneous action potentials were not observed in this cell. The resting potential was −56 mV. Temperature, 25°C; current clamp with a patch electrode containing the standard internal solution.

Fig. 3 shows the current–voltage (*I–V*) relationship of membrane currents in Fig. 2. The threshold for activation of the Ca^{2+} and K^+ currents ranged between −55 and −60 mV, while that of the Na^+ current was shifted to the positive direction by about 10 mV.

Somatostatin Increases Permeability to K^+. Fig. 4*A* depicts the membrane potential changes in human GH-producing pituitary cells caused by somatostatin. The membrane resistance was monitored by applying hyperpolarizing current pulses. Before application of somatostatin, this cell showed a regenerative potential change after cessation of applied hyperpolarizing currents (Fig. 1*C*). Brief application of somatostatin hyperpolarized the membrane potential from −52

‡Recording of the Na^+ channel current was carried out at lower temperature (about 13°C), because the maximum peak amplitude of Na^+ channel current was usually greater than 500 pA at 23–25°C, which caused an error in the clamped potential level. At lower temperature, the amplitude of Ca^{2+} channel current was small, as seen in Fig. 2*B*.

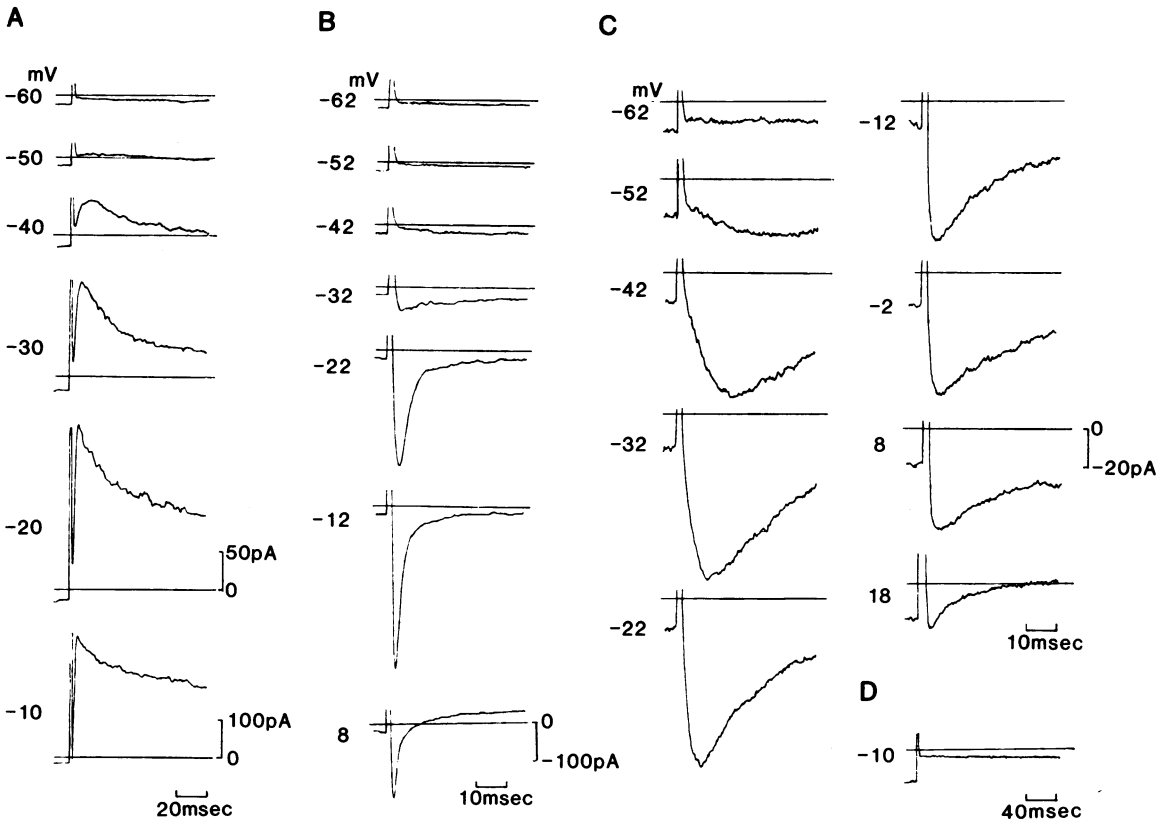

FIG. 2. (*A*) Membrane currents under the voltage clamp with a patch electrode containing the standard internal solution, in the standard medium. Holding potential was −80 mV and the fixed potentials are indicated. Temperature, 24°C. (*B*) Inward currents recorded with the patch electrode containing Cs^+ and 20 mM EGTA, in the standard medium. Holding potential, −82 mV. Temperature, 13°C. (*C*) Ca^{2+} currents. The internal solution of the patch electrode was the same as in *B*, and the cells were in the standard medium with 1 μM tetrodotoxin added. Holding potential, −82 mV. Temperature, 23°C. (*D*) Membrane current in 20 mM Ba^{2+}, Na^+-free medium containing 5 mM Co^{2+}. Holding potential, −80 mV. Temperature, 23°C.

to −69 mV and inhibited action potentials evoked by release of hyperpolarizing currents. The membrane hyperpolarization was accompanied by a decrease in the input resistance. The peak amplitude of somatostatin-induced membrane potential changes was plotted against the membrane potential in Fig. 4*C*. The intersect of the straight line with the abscissa indicates the reversal potential of the somatostatin-induced membrane hyperpolarization, which was −74 mV. Fig. 4*B* shows the somatostatin-induced current obtained under the voltage clamp condition in the same cell. Somatostatin caused an outward current at the holding potential of −41 and −60 mV, while it caused an inward current at −100 mV. The amplitude of somatostatin-induced current was also plotted in Fig. 4*C*. The reversal potential under the voltage clamp was almost identical to that under the current clamp. Similar experiments in the standard medium (6 mM K^+) yielded a reversal potential of −78.4 ± 4.3 mV (n = 7). The reversal potential in 3 mM K^+ medium was −97.2 ± 6.4 mV (n = 5) (Fig. 4*C*). The value of the negative shift of the reversal potential in 3 mM K^+ medium from that in 6 mM K^+ medium was almost identical to that expected from the Nernst equation. We, therefore, conclude that the membrane hyperpolarization by somatostatin was caused by an increase in the permeability to K^+.

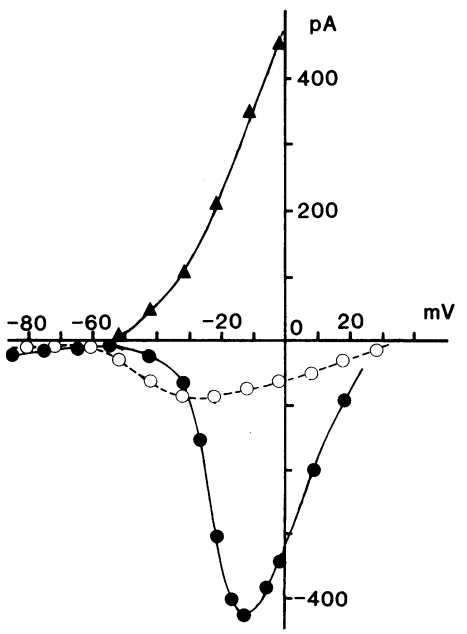

FIG. 3. *I–V* curves of various membrane currents. ▲, The records in Fig. 2*A*; ●, the records in Fig. 2*B*; ○, the records in Fig. 2*C* (Ca^{2+} channel current).

Among three adenomas, the membrane hyperpolarization induced by somatostatin was observed in 92.7% (n = 41, adenoma no. 1), 80% (n = 10, adenoma no. 2), and 81.3% (n = 16, adenoma no. 3) of the cells examined. The hyperpolarized membrane potential in the cells during somatostatin responses was −63.6 ± 5.9 mV in the standard medium. Considering the *I–V* curve of Ca^{2+} and Na^+ currents, this level was sufficient to inhibit spontaneous action potentials.

It is known in the pituitary cells that the membrane hyperpolarization can be elicited by an increase in intracellular Ca^{2+} through Ca^{2+}-activated K^+ conductance (26, 27). In the present study, similar membrane hyperpolarization was induced by the application of Ca^{2+} ionophore A23187 (10 μM), and this response was completely prevented when the

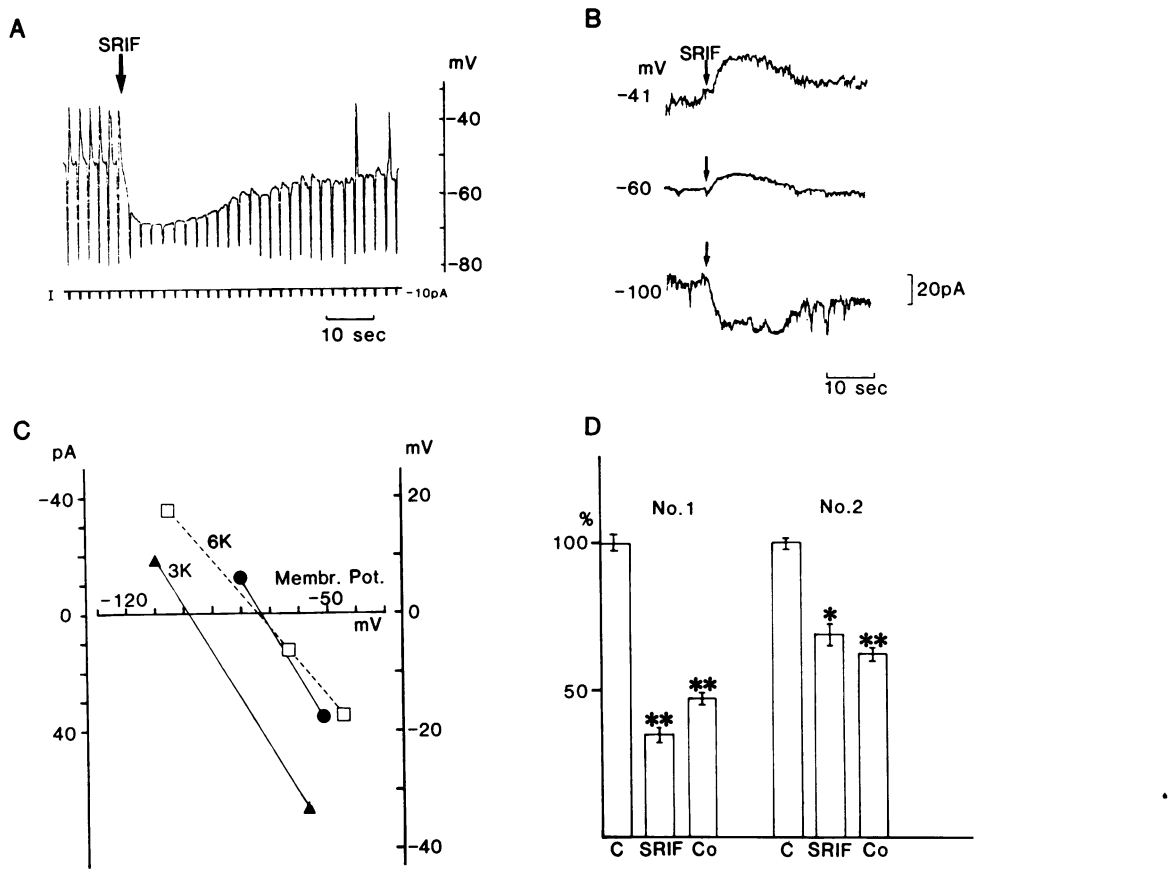

FIG. 4. (*A*) Somatostatin (SRIF)-induced membrane hyperpolarization. The timing of SRIF administration for 500 msec is indicated by an arrow. The cell, in the standard medium, was under the current clamp with the patch electrode containing 0.5 mM EGTA and K^+. Hyperpolarizing current pulses of 10 pA were applied for 500 msec. Temperature, 32°C. (*B*) Somatostatin-induced membrane current under the voltage clamp, same cell as in *A*. The holding potentials are indicated and the timing of somatostatin administration is indicated by arrows. (*C*) Relationship between membrane potential and somatostatin-induced response. The ordinate on the left indicates the amplitude of somatostatin-induced current under the voltage clamp. The ordinate on the right indicates the amplitude of somatostatin-induced membrane potential change under the current clamp. The abscissa indicates the membrane potential. ●, Record in *A* (6 mM K^+); □, record in *B* (6 mM K^+); ▲, obtained in 3 mM K^+ medium under the current clamp. The intersect of the solid or broken line with the abscissa indicates the reversal potential. (*D*) Effects of somatostatin (0.1 μM) and Co^{2+} (5 mM) on GH release. Hormone release is expressed as percent of control (*C*); 100% represents GH release of 496.5 ng/ml per 2 hr in adenoma no. 1, 545.5 ng/ml per 2 hr in adenoma no. 2 ($n = 4$). *, $P < 0.02$ compared with control; **, $P < 0.001$.

patch electrode contained 20 mM EGTA (data not shown). However, the somatostatin-induced hyperpolarization was consistently observed irrespective of EGTA concentration in the patch electrode. Therefore it is concluded that the somatostatin-induced hyperpolarization is not due to an increase in intracellular Ca^{2+}.

Hormone Release. GH secretion from the cells is shown in Fig. 4*D* (adenomas 1 and 2 were examined). GH release was significantly suppressed by 0.1 μM somatostatin. It was also significantly suppressed by the addition of 5 mM Co^{2+} in the external medium, in parallel with the result that Co^{2+} suppressed the voltage-gated Ca^{2+} current.

DISCUSSION

The resting potential of human GH-producing cells was -52.4 ± 8.0 mV. To determine the resting potential under the whole cell clamp condition, it is necessary to obtain much higher seal resistance than the input resistance of the cell membrane. In the present experiment the seal resistance was more than several tens of GΩ and the input resistance was about a few GΩ with the patch electrode containing the standard internal solution. The error of the recorded resting potential was considered to be less than 10%. Spontaneous action potentials were observed in 58% of the cells examined. However, spontaneous action potentials may have existed in all cells. We may not have observed spontaneous action potentials in some cells as a result of cell injury or some other experimental conditions.

Somatostatin and Co^{2+} in the medium inhibited GH release. However, in both instances the inhibition was partial. It has been reported that basal hormone release in rat GH_3 cells is independent of external Ca^{2+} (26). The uninhibited portion of basal GH secretion in our human GH-producing cells could be attributed to such external Ca^{2+}-independent hormone release.

Somatostatin-induced membrane hyperpolarization has been reported in purified rat somatotrophs with the glass microelectrode technique (28) and in clonal rat GH_3 cells with the fluorescence technique (29). However, in both reports the underlying ionic mechanism and the relation to hormone secretion were not investigated. In purified rat somatotrophs, the somatostatin-induced hyperpolarization was associated with a decrease in the membrane conductance, which is opposite to the present result. This discrepancy is difficult to resolve at this moment. In rat $GH_3/6$ cells somatostatin-induced K^+ conductance has been reported (30). However the inhibition of Ca^{2+} current by membrane hyperpolarization has not been clarified.

The present study revealed the membrane hyperpolarization caused by somatostatin, which was considered to suppress GH secretion by inhibiting Ca^{2+} currents. However,

there appear to exist other mechanisms of somatostatin action for the following reasons: (*i*) It has been reported in rat GH_4Cl cells and in purified rat somatotrophs that somatostatin reduces basal hormone release without a detectable change in cAMP (7, 8, 31). In addition, basal hormone release is independent of external Ca^{2+} (8, 32). (*ii*) Ca^{2+} ionophore- or high K^+-induced GH release is inhibited by somatostatin (8, 33). These phenomena cannot be explained by the reduction of intracellular cAMP or Ca^{2+} influx. For clarification of other mechanisms of somatostatin action, further experiments are required.

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