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The Electrical Properties of Cell Membranes in Granular or Agranular Anterior Pituitary Clonal Cells (40437)

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An electrical feature of excitable cells is the generation of an action potential in response to a stimulus. A similar response has recently been observed in some endocrine cells such as adrenocortical cells (1), pancreatic islet cells (2), adrenal chromaffin cells (3, 4), anterior pituitary cells (5, 6), and tumor cells (7, 8). It is not clear whether or not this electrical change in the membrane is necessary for secretory activity in these cells. The present study was undertaken to examine the electrical properties of normal functional granular (SR7E8) and agranular (SR7F2) subclonal cells which are known to continuously release growth hormone (GH) into the medium. These two subclonal cell lines (SR7E8 and SR7F2) were derived from the normal GH-producing clonal cells (R7) which were established from normal anterior pituitary cells of adult rats. In order to stimulate growth hormone secretion a median eminence extract (MEE) prepared from the brains of adult rats was added to the culture medium.

Materials and methods. SR7E8 and SR7F2 subclonal cells were derived from our R7 clonal strain by using the cell plating technique with a MicroTest II tissue culture plate (Falcon, #3040) and by adding 2 μg/ml of rat median eminence extract (MEE) to Ham's F10 medium (Ham's F10 90% + fresh rat serum 10%). All the cells were grown routinely in Falcon dishes (60 × 10 mm, #3002) in a humidified atmosphere of 5% CO_2, 5% O_2 and 90% air at 37°. The cell growth (cell proliferation) and organ-specific function (production of growth hormone) was continuous in this culture system. Prior to the experiment, these subclonal strains were cultured in growth medium with fresh serum from hypophysectomized rats (30 days after hypophysectomy). The radioimmunoassay for GH was performed by the method of Birge *et al.* (9) using the NIAMDD-rat GH RIA kit. The parent clone, R7, and two subclones, SR7E8 and SR7F2, were cultured with or without MEE in the growth medium for 3 days. The concentration of MEE was 2 μg/ml. A lyophilized powder from MEE of adult rats was obtained by the method of Kuroshima *et al.* (10). Somatostatin (Tanabe Seiyaku, Japan), α-Isopropyl-α[(*N*-methyl-*N*-homoveratryl)-γ-aminopropyl]-3,4-dimethoxyphenylacetonitrile · HCl (Verapamil) and α-Isopropyl-α[(*N*-methyl-*N*-homoveratryl)-γ-aminopropyl]-3,4,5-trimethoxyphenylacetonitrile · HCl (D-600; both were obtained from Knoll AG, Ludwigshafen am Rhein) and tetrodotoxin (Sankyo Seiyaku, Japan) were added to the culture medium in acute experiments at concentrations given elsewhere in the text.

The electrical properties of the membranes of these cells were examined using the intracellular micro-electrode technique. Intracellular potential recording and current injection were carried out by the bridge balance method. All experiments were conducted at 34–37°. For each cell studied the resting membrane potential was measured after stability and a steady state had been established for 3 min.

Results. When the clonal or subclonal cells were cultured in medium lacking MEE and fresh serum, secretory granules were not observed within the cells. However, when the cells were cultured in a medium containing MEE and fresh rat serum secretory granules were observed in SR7E8 subclonal cells but not in SR7F2 subclonal cells (Fig. 1A and 1a).

The amount of GH released from these clonal cells cultured for 3 days in control

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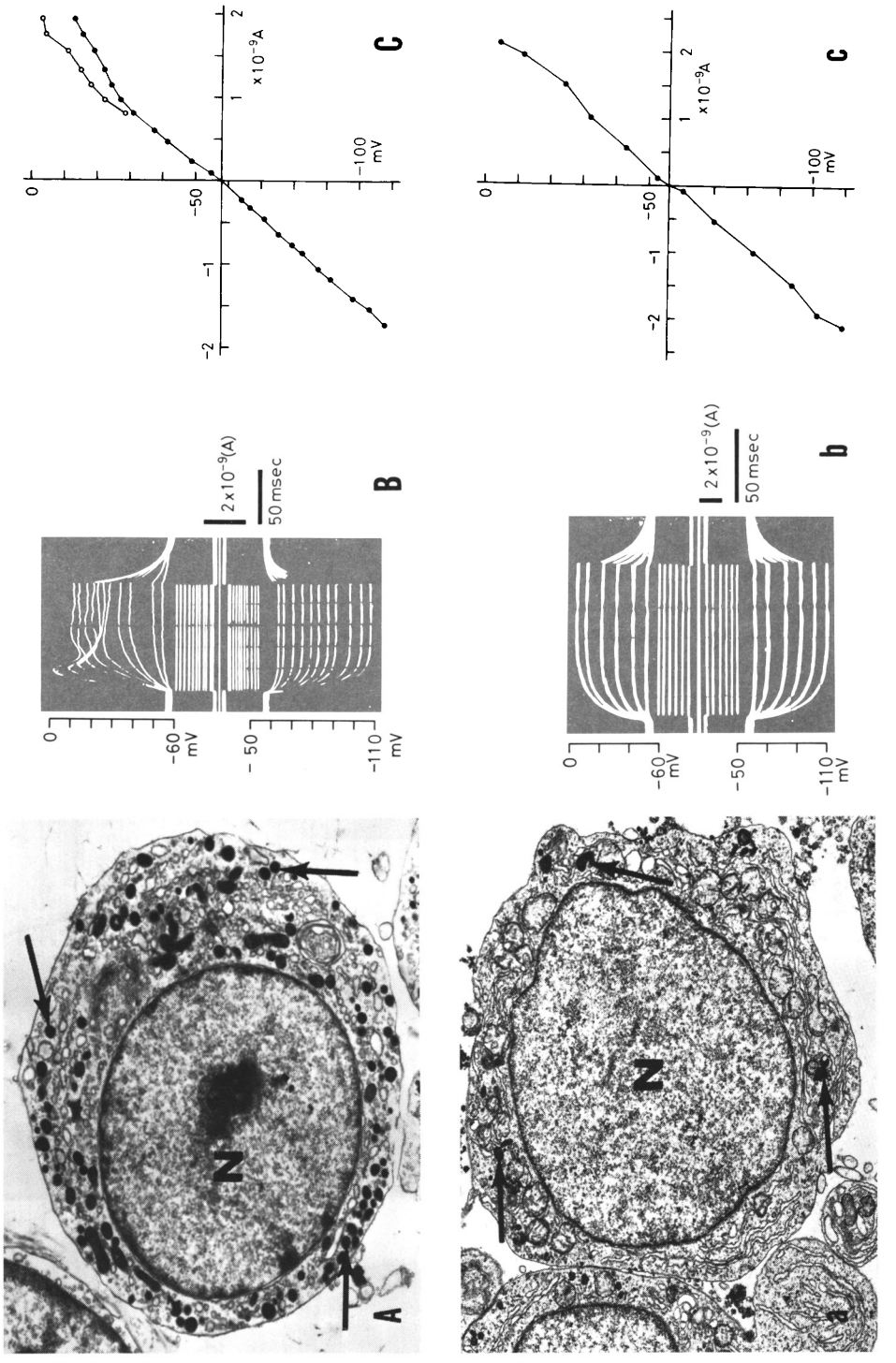
C
x10⁻⁹A
2
1
-1
-2
0
-50
-100 mV
B
2x10⁻⁹(A)
50 msec
0
-60 mV
- 50
-110 mV
A
N
c
x10⁻⁹A
b
2x10⁻⁹(A)
50 msec
-50
N

medium or in MEE-containing medium is shown in Table I. The amount of GH released into the medium was not significantly different when these clonal and subclonal strain cells were cultured for 3 days in the MEE-free medium. The MEE greatly elevated the amount of GH released from all three strains in our culture system (Table I). Then a significant difference in the amount of GH released was found between the R7 parent clonal cells and the SR7E8 subclonal cells ($p < 0.01$). Also, the granular subclone released significantly more GH than the agranular subclone ($p < 0.02$).

In the granular subclonal cells (SR7E8) a spike-potential-like change of membrane potential was elicited by a depolarizing current of over 4.6×10^{-10} A (Fig. 1B). Delayed rectification is seen in these membrane responses of the granular subclonal cell. The input resistance obtained from the current-voltage relation of the granular subclonal cells (Fig. 1C) was $35.7 \pm 1.8 \times 10^6$ ohm. The resting membrane potential was 55.3 ± 1.8 mV. The mean membrane time constant was 8.8 ± 0.3 msec. (Means $\pm$ SE, $N = 15$).

TABLE I. *In Vitro* Effect of Rat Median Eminence Extract on GH Release in R7 Clonal Strain and Its Subclonal Strains.

	GH release (ng/ml)	
	Control	MEE
R7	373 ± 11^a	$1{,}168 \pm 53^{bc}$
SR7E8 (granular)	436 ± 24	$1{,}977 \pm 76^{bd}$
SR7F2 (agranular)	425 ± 13	$1{,}428 \pm 62^{cd}$

[a] Mean ± SE. Each group had seven dishes. Each dish contained 8×10^6 cells. Three strains were cultured with or without MEE supplemented medium for 3 days. The MEE was added 2 μg/ml.

[b] $p < 0.01$.

[c] $p < 0.05$.

[d] $p < 0.02$. The values given are the means of duplicate determinations.

Tetrodotoxin (TTX), a specific inhibitor of Na-potential, was used to determine whether or not the evoked spike potential of granular subclonal cells is a Na-potential. TTX at the concentration of 1.0×10^{-7} g/ml was ineffective in suppressing the evoked spike potential triggered by intracellular current injection (Fig. 2b). The evoked spike potential of granular subclonal cells could be abolished by applying 1.0×10^{-7} g/ml somatostatin (Fig. 2c), 5.0×10^{-8} g/ml verapamil and 5.0×10^{-8} g/ml D-600. These observations suggest that the presence of Na-ions is probably not an important factor in the generation of an evoked potential in granular subclonal cells.

Figure 1b shows the transmembrane potential changes of the agranular GH subclonal cell (SR7F2), caused by intracellular injection of depolarizing and hyperpolarizing currents (From 0.4 to 2.2×10^{-9}A and 100 msec duration). Unlike the responses obtained with the granular cells, there were no significant differences between the depolarizing and hyperpolarizing responses, although the responses were opposite in the direction of deflection. The configuration of potential changes due to the applied current was similar to that of nonexcitable cells. The current-voltage relations of the agranular subclonal cells are shown in Fig. 1c. From the linearity of the current-voltage relation, the input resistance was calculated to be $28.7 \pm 2.5 \times 10^6$ ohm. The resting membrane potential of these cells was -44.9 ± 1.8 mV. The mean membrane time constant was 5.1 ± 0.3 msec. (Means ± SE, $N = 10$) A comparison (by

FIG. 1A. SR7E8 subclonal cells derived from an R7 clonal strain of adult male rat pituitary were cultured for 3 days in a medium supplemented by median eminence extract and fresh serum from hypex rats (10%). The cells contain secretory granules (350 nm in diameter). G: Golgi area; N: Nucleus; secretory granules (arrows), 1% OsO_4 fixed. × 3000. 1B. Current-voltage relation in a granular subclonal cell. The upper pair of traces show the membrane potential changes and the amplitude of applied depolarizing currents and the membrane potential changes. 1C. Current-voltage relation in a granular subclonal cell, obtained from Fig. 1B. Filled circle: steady state value. Open circle: maximal amplitude of spike-potential-like discharge. 1a. SR7F2 subclonal cells derived from an R7 clonal strain were cultured for 3 days in a medium supplemented by median eminence extract. The cells have a number of free ribosomes, polysomes and lysosomes; however, they contain no secretory granules. N: Nucleus; lysosome (arrow), 1% OsO_4 fixed. × 4200. 1b. Current-voltage relation in an agranular subclonal cell. The upper pair of traces show the membrane potential changes and the amplitude of applied depolarizing currents. The lower pair of traces show the amplitude of applied hyperpolarizing currents and the membrane potential changes. 1c. Current-voltage relation in an agranular subclonal cell, obtained from Fig. 1b.

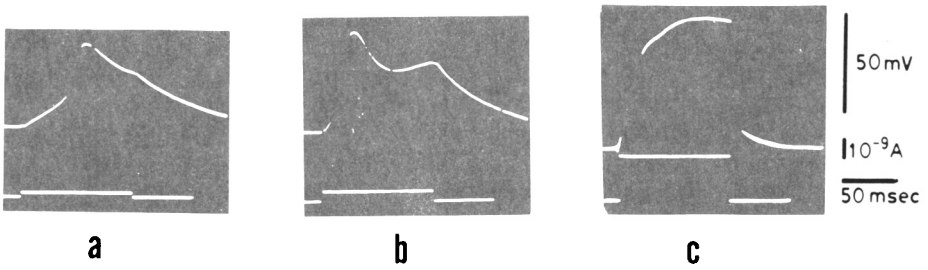

FIG. 2. Electrophysiological responses of a granular subclonal cell. a, b and c are recorded with the same cell. a. Control medium: The upper tracing shows the evoked spike potential change and the lower tracing shows the depolarizing current pulse. b. Treated with 1.0×10^{-7} g/ml TTX. The upper tracing shows the evoked spike potential change and the lower tracing shows the depolarizing current pulse. The evoked spike potential is similar to that seen in a. c. Depolarizing current pulse fails to evoke regenerative response in the granular subclonal cell in presence of 1.0×10^{-7} g/ml somatostatin. The upper tracing shows the spike potential change and the lower tracing shows the depolarizing current pulse.

Student's t test) of these mean values with those obtained from granular subclonal cells gave the following results: input resistance, $p = < .025$; resting membrane potential, $p = < .10$; membrane time constant, $p = < .001$.

Discussion. The results obtained in the present study pointed to a remarkable difference in the electrical properties between the membranes of granular and agranular subclonal cells, despite the fact that the two subclonal strains were derived from the same GH-producing clonal parent strain. This notable difference in electrical properties may relate to differences in the mechanism of normal secretion between the two subclonal strains. The evoked spike potential of the granular GH subclonal cells, unlike that of the squid axon, was not mediated by Na ions. Since the evoked spike potential was depressed by calcium blockers, such as somatostatin (11), Verapamil and D-600 (12), it seems likely that the evoked spike potential of granular subclonal cells results from an increase in membrane conductance for calcium ions. Further comparative studies of the differences between granular and agranular subclonal cells may help to clarify specific steps in the secretory process of endocrine cells. Recently, Shiino *et al.* (13) succeeded in producing secretory granule accumulation in 2A8 (multipotential clonal strain derived from rat Rathke's pouch epithelium) by using fresh rat serum and MEE supplemented medium *in vitro*. In this study, SR7F2 subclonal cells never demonstrated any granule accumulation even though they were cultured with fresh serum- and MEE-supplemented medium. Thus, it may be that specific mechanisms are involved in initiating granule accumulation in adult rat adenohypophysial cells *in vitro*. On the other hand, secretion of anterior pituitary cells has now been documented to be a microtubule dependent process (14, 15). The relationship between this evoked spike potential or hormonal secretion and the function of microtubules in the cells was not investigated in this study. Further investigations are required to resolve this problem.

Summary. The electrical properties of the membrane of granular and agranular growth hormone-secreting clonal cells derived from the same parent clone of rat anterior pituitary cells were studied by the intracellular microelectrode technique. An evoked spike potential was generated by a depolarizing current in the granular (SR7E8) but not in the agranular (SR7F2) clonal cells. The potential was not suppressed by tetrodotoxin (TTX), but could be abolished by somatostatin, verapamil and D-600. It, therefore, seems likely that it is a Ca-potential, rather than a Na-potential.

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