

Does Acetylcholine Change the Electrical Resistance of the Basal Membrane of Secretory Cells in Eccrine Sweat Glands?

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Summary. The present experiment was intended to study whether or not acetylcholine decreases the electrical resistance of the basal membrane of secretory cells in stimulating eccrine secretion of fluid and electrolytes. An isolated segment of the secretory coil of the monkey palm eccrine sweat gland was dissected out *in vitro* and immobilized in the tip of a constriction pipette. Using a bridge-balanced single glass microelectrode, input impedance of the secretory cell was compared before and after local superfusion of acetylcholine in each cell. The mean input impedance was 27 M Ω , which did not significantly change after application of acetylcholine. Between 15 and 30 sec after cessation of acetylcholine superfusion, input impedance increased by 42% and then returned to normal within 60 sec. The current-induced voltage deflection due to intraluminally injected current pulse was measured across both the basal membrane (ΔV_b) and the epithelial wall (ΔV_l) as qualitative measures of the respective membrane resistances. Both ΔV_b and ΔV_l increased by about 10%, but their ratio remained unchanged after stimulation with acetylcholine. A Ca⁺⁺ ionophore, A23187, which is as potent a stimulant of eccrine sweat secretion as acetylcholine *in vitro*, also failed to change the above two parameters. It was concluded that the decrease in the electrical resistance of the basal membrane of the secretory cells could not be detected in the sweat gland after stimulation with acetylcholine or A23187. The possibility was discussed that the action of acetylcholine at the basal membrane is one of enhancing the activity of the nonconductive pathway rather than the conductive pathway in this exocrine gland.

In exocrine glands such as the eccrine sweat glands and the salivary glands, acetylcholine is the most efficient stimulant of fluid and electrolyte secretion. Despite numerous studies, the mechanism by which acetylcholine interacts with and modifies the secretory cell membrane leading to secretion of fluid and electrolytes has not yet been fully

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elucidated. According to the most widely accepted theory (Petersen, 1970; Nishiyama & Petersen, 1974*a-b*), the sequence of events from the time of interaction of acetylcholine with the secretory cell membrane until the commencement of secretion consists of the following: Acetylcholine interacts with the cholinergic receptor in the basal membrane of the secretory cell. Na^+ and K^+ permeability of the basal cell membrane increases, presumably mediated by (acetylcholine-induced) influx of Ca^{++} into the cell. Now Na^+ can enter the cell according to the electrochemical potential gradient. Simultaneously some cellular K^+ leaves the cell and Cl^- enters the cell passively (Iwatsuki & Petersen, 1977).

The signals that turn on the active Na^+ (or Cl^-) transport system at the luminal cell membrane may be an increase in Na^+ and a decrease in K^+ concentrations in the cell. The influxed Na^+ is then pumped out from the cell into the tubular lumen. This hypothesis, which is simple and attractive, is in fact an application of Ussing's pump and leak model (Ussing & Zerahn, 1951) for frog skin to the exocrine secretory epithelium. This exocrine version of the pump and leak model has thus far withstood the trial of experimental examination, especially at its first step in the overall process, namely, the effect of acetylcholine on the membrane permeability to Na^+ and K^+ (Nishiyama & Petersen, 1974*b*). For example, in the salivary glands (Nishiyama & Petersen, 1974*a*, 1974*b*) and the pancreas (Nishiyama & Petersen, 1975; Petersen & Ueda, 1975), a drastic decrease in input impedance, which is an indirect measure of the total cell membrane resistance, has been observed in acinar cells after stimulation with acetylcholine; this has been interpreted as indicating that the basal membrane conductance and thus the ionic permeability of the basal membrane increased during stimulation with acetylcholine. Such observations are of interest since they conform to the action of acetylcholine in the nervous system, where this and other neurotransmitters react with their receptors at synapses to produce striking changes in Na^+ , K^+ , or Cl^- permeability across the postsynaptic membrane (Weight, 1974).

In fact, in a previous publication (Sato & Dobson, 1973), we have also adopted a similar pump and leak model in explaining the acetylcholine-induced stimulation of energy metabolism by an isolated eccrine sweat gland. In the present communication we have focused on the validity of the first step of the modified pump and leak model, namely, the effect of acetylcholine on the electrical resistance of the basal secretory cell membrane. The technical difficulties in studying the struc-

turally and functionally complex salivary glands or the exocrine pancreas has prompted us to employ another exocrine gland, the secretory coil of the monkey eccrine sweat glands, where the secretory epithelium is a simple tubule and no known exocytotic mechanism of secretion is present.

Materials and Methods

We have used two approaches for estimating the change in the electrical resistance of the basal membrane of the secretory cell during stimulation with acetylcholine: Measurement of the input impedance of the secretory cell membrane in one series of studies and measurement of voltage deflection across the basal cell membrane arising from the intraluminally injected electric current pulses. Skin biopsy specimens were obtained from the palms of rhesus monkeys tranquilized with phencyclidine·HCl (Parke, Davis & Co.). The specimens were quickly sliced freehand with a razor blade, rinsed in several changes of ice-cold Ringer's solution (Krebs Ringer's bicarbonate containing 5.5 mM glucose and 50% bovine serum albumin), gassed (with 95% O₂ + 5% CO₂), and placed in a dissection chamber (Sato, 1973). The Ringer's solution in the dissection chamber was kept cold by introducing cold water, approximately 8°C, into the water jacket of the chamber, which was stirred constantly and oxygenated by gas lift with a mixture of 95% O₂ + 5% CO₂. The eccrine sweat glands were dissected out with a pair of sharp forceps under a stereomicroscope (40 to 160×) from a small slice of the palm skin. The secretory coil segment was further isolated from the whole gland and was transferred to the incubation chamber, which is a duplicate of the dissection chamber. The open end of the secretory coil segment was drawn by suction into a constriction pipette. In some experiments the tissue-glass junction was sealed with Sylgard 184 (Dow Corning Co., Midland, Mich.), in others, a length of the tubule, 400 to 500 μm (more than 4 times the length constant) (Sato, 1977a), was simply drawn into the constriction pipette. The segment of the secretory coil hanging from the tip of the constriction pipette was placed on a small square block, 2-mm cube, which was made of cured Sylgard 184 and glued to the bottom of the incubation chamber. The sweat gland was further immobilized by means of two micro-hooks constructed on the tips of tapered glass capillaries. The entire preparative procedure was performed at 10°C. The incubation chamber was then warmed to 37°C. For recording the cell PD, a semi-floating and self-filling electrode was used (Sato, 1977b) unless otherwise described. The semifloating microelectrode was constructed simply by replacing the shaft of the conventional Ling-Gerard type glass microelectrode with a length (5 to 6 cm) of 3 M KCl-filled silicon rubber tubing. The tip resistance of microelectrodes ranged from 10 to 20 MΩ and the tip potential was smaller than -2 mV.

Impalement of the Secretory Cell

As compared with such tissues as the kidney, exocrine pancreas, and the salivary glands which the author has thus far experienced, the eccrine sweat gland proved the most difficult tissue to impale with a glass microelectrode. Only a few percent of the entire impalements yielded a relatively stable membrane PD. Two types of cell PD's were observed. The first type was seen infrequently but tended to be observed when the electrode was advanced along the surface of the secretory coil. This type of potential

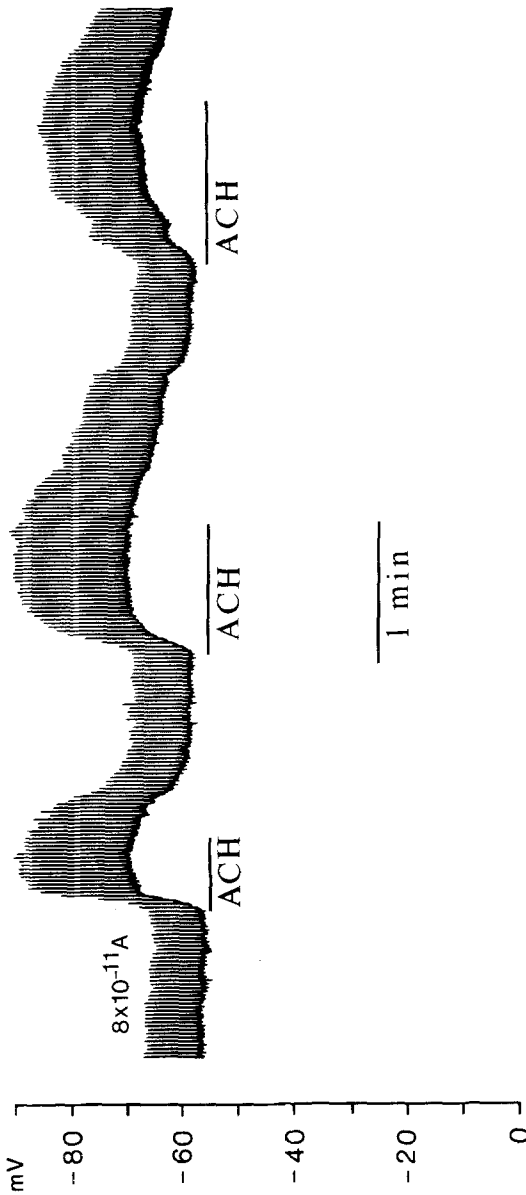


Fig. 1. A rare type of cell PD, possibly myoepithelial cell PD. Note the slowly rising hyperpolarization on exposure to locally superfused acetylcholine. The superimposed potential pulses are due to injection into the cell of square current pulses (8×10^{-11} A) through a bridge-balanced glass microelectrode. Input impedance is defined as the height of the potential pulse divided by the injected current

pattern was characterized by a stable, noiseless baseline (without the so-called superimposed miniature potentials) (Dean & Matthews, 1972), high input impedance (from 50 to 100 M Ω), and a slow hyperpolarization on exposure to acetylcholine (Fig. 1). In contrast, the other type of commonly seen cell PD's showed a noisy baseline with superimposed miniature depolarization potentials. The input impedance of these cells was much smaller than that of the first group of cells, ranging usually from 10 to 40 M Ω . Because this latter type of cell PD could be seen consecutively by moving the electrode along the tubular wall (Fig. 2) and because it tended to show a transient depolarization or

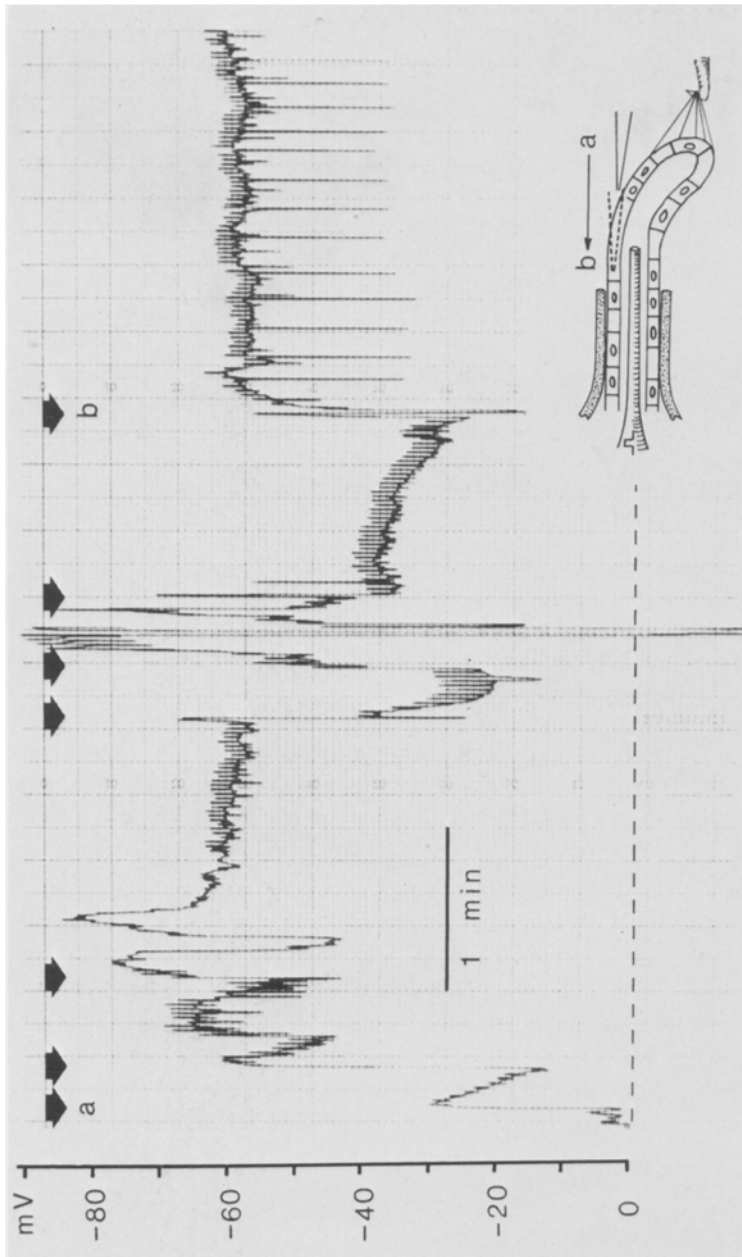


Fig. 2. Original trace of cell PD's when the electrode is advanced from position *a* to *b* as shown in the insert. In this particular experiment, current pulses were injected through a luminal electrode into the lumen and the cell PD, and the voltage pulses were measured differentially across the basal membrane (see also Figs. 3 and 4). The segment of a secretory coil was immobilized by stretching the attached collagen fibers by means of a glass hook. Note the change in height of the voltage pulses as the electrode crosses the area of differing current density. Some of the low cell PD's probably result from leaky impalement of the electrode. Irregular downward pulses represent the so-called miniature depolarization potentials. The electrode was advanced in microsteps as indicated by dark arrows

hyperpolarization due to acetylcholine, we reasoned that it represents the PD of the secretory cells. Furthermore, since the myoepithelial cells are discontinuous cells with slim and spindle-shaped cell bodies and the time course of the hyperpolarizing response to acetylcholine roughly coincides with the myoepithelial contraction (Sato, 1977c and unpublished), this type of cell PD probably belongs to the myoepithelial cells.

Measurement of Input Impedance

Square pulses, 1 to 10×10^{-10} A and 0.2 to 0.4 sec duration, were injected through a semi-floating glass microelectrode, and the potential drop arising from the tip resistance of the microelectrode was bridge-balanced before impaling the cell by means of a W-P (New Haven, Conn.) 701 Microprobe System. The maintenance of balancing during the cell impalement was continuously monitored with a Tektronix (Beaverton, Oregon) 565 oscilloscope and was checked again after withdrawing the electrode from the cell.

Estimation of the Change in the Basal Membrane Resistance

An outline of the methodology is illustrated in Figs. 3 and 4. Square current pulses, 1 to 5×10^{-6} A and 0.4 sec duration, were injected through a luminal electrode, and the current induced voltage deflection across the basal cell membrane was recorded differentially using a Keithly (Cleveland, Ohio) 604 differential amplifier between the impaled electrode and the reference electrode placed immediately above the impaled cell. Since a long segment of the secretory coil, i.e., longer than 5 times the length constant (Sato, 1977a), was aspirated into the outer pipette, no attempt was made to seal the tissue glass junction with Sylgard 184. In the absence of changes in the transepithelial voltage deflection, the voltage deflection across the basal cell membrane should represent a qualitative measure of the electrical resistance of that side of the cell membrane (Fig. 4) under the assumptions to be discussed in the *Discussion*.

Effect of A23187 on Input Impedance

In a limited number of cells, the effect of a calcium ionophore A23187 was studied as in acetylcholine for two reasons. Firstly, A23187 is as potent a stimulant of eccrine sweat secretion as acetylcholine *in vitro* (Sato, 1977a). Secondly, unlike acetylcholine, it

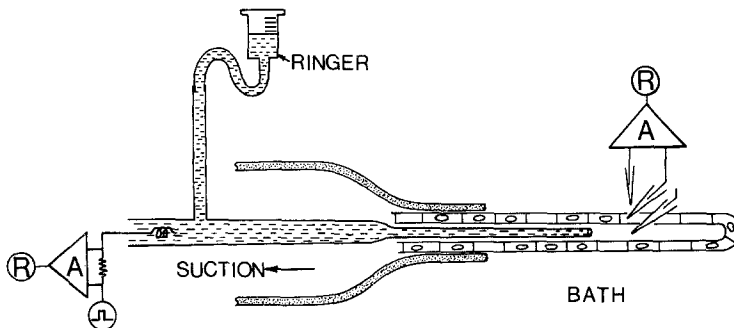


Fig. 3. Schematic illustration of the method for measuring the change in the basal membrane resistance and the transepithelial resistance of the secretory coil. A small hydrostatic pressure was continuously applied to the lumen to prevent the collapse of the secretory coil lumen. Instead of sealing the tissue-glass junction, a long segment of the tubule, more than 5 times the length constant ($83 \mu\text{m}$) (Sato, 1977a), was drawn by suction into the pipette. A: differential amplifier. R: recorder

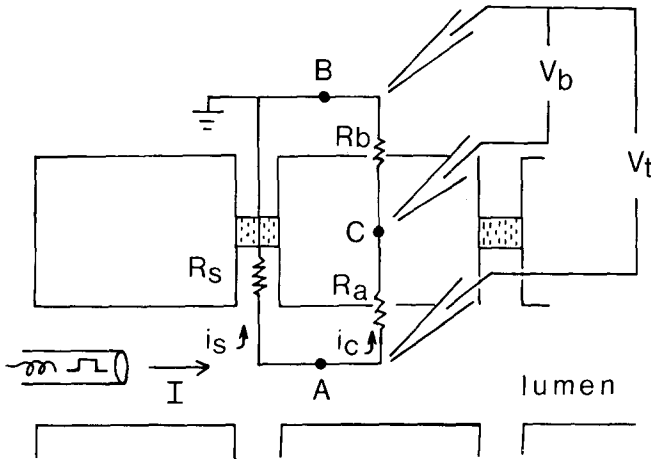


Fig. 4. Schematic illustration of the equivalent circuit of the secretory cells. C , secretory cell cytoplasm; A , lumen; B , bath; I , (intraluminally injected) current at the impaled site ($=i_c+i_s$); R_a and R_b , electrical resistance of the luminal and basal membranes, respectively; R_s , paracellular shunt resistance; V_b and V_t , PD across the basal membrane and epithelial wall, respectively

does not contract the myoepithelium (Sato, 1977c) so that the possible artifactual changes in potential profile or the leaky sealing of the electrode tip can be effectively avoided.

All the salts used for Ringer's solution were of reagent grade. Albumin was the purest form available from Sigma. A23187 was a gift from Eli-Lilly & Co. (Indianapolis, Ind.).

Results

Figure 5 is a frequency distribution of the cell PD's in 67 successfully impaled secretory cells. Only those PD's lasting longer than 2 min were included in this figure. The highest frequency occurred between 60 to 65 mV. The mean input impedance calculated from 27 secretory cells was 27 ± 7.5 (SD, range 14-46) $M\Omega$ in the resting state. The linearity of the current-voltage relationship was demonstrated over a wide range of current pulses used throughout the present study, namely from 1 to 10×10^{-10} A (Fig. 6). The oscilloscope pictures of the voltage pulses observed in the same cell are presented in Fig. 7. Some of the most critical precautions that need to be taken in the measurement of input impedance are to secure the electrode tip-resistance compensation by means of a bridge circuit and to distinguish the artifactual changes from the true ones. In the eccrine sweat gland, the myoepithelial contraction (Sato,

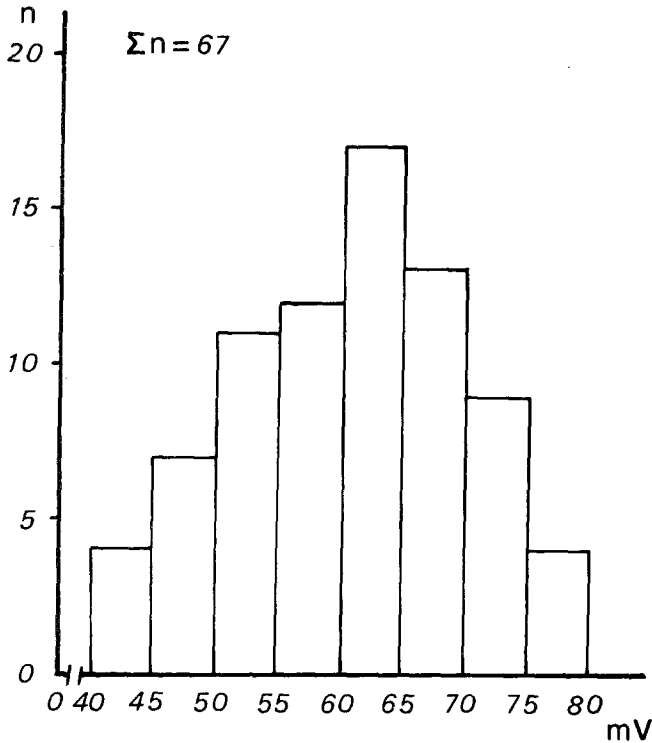


Fig. 5. Frequency distribution of cell PD's from 67 successfully impaled secretory cells

1977c) poses the strongest technical problem. When a stiff-shafted Ling-Gerard electrode was used, the electrode-tip was almost always dislocated when the sweat gland was stimulated with acetylcholine (Sato, 1977b). Even with the use of a semifloating electrode, the cell PD as well as voltage pulses were very often decayed if the impaled site of the secretory tubule was not sufficiently immobilized by means of micro-glass hooks. Figure 8 is a typical example of one such unsuccessful experiment where the strong myoepithelial contraction due to the addition of acetylcholine to the bath eliminated the cell PD as well as the voltage pulses arising from intracellularly injected current pulses. The subsequent impalement of adjacent cells by advancing the electrode in small steps, however, picked up both the cell PD and the voltage pulses. Experiments such as the one shown in Fig. 8 also indicate the adequacy of the tip-resistance compensation.

Upon local superfusion of acetylcholine ($5 \times 10^{-6} M$), we observed three types of change in membrane PD. Figure 9 is the monophasic type

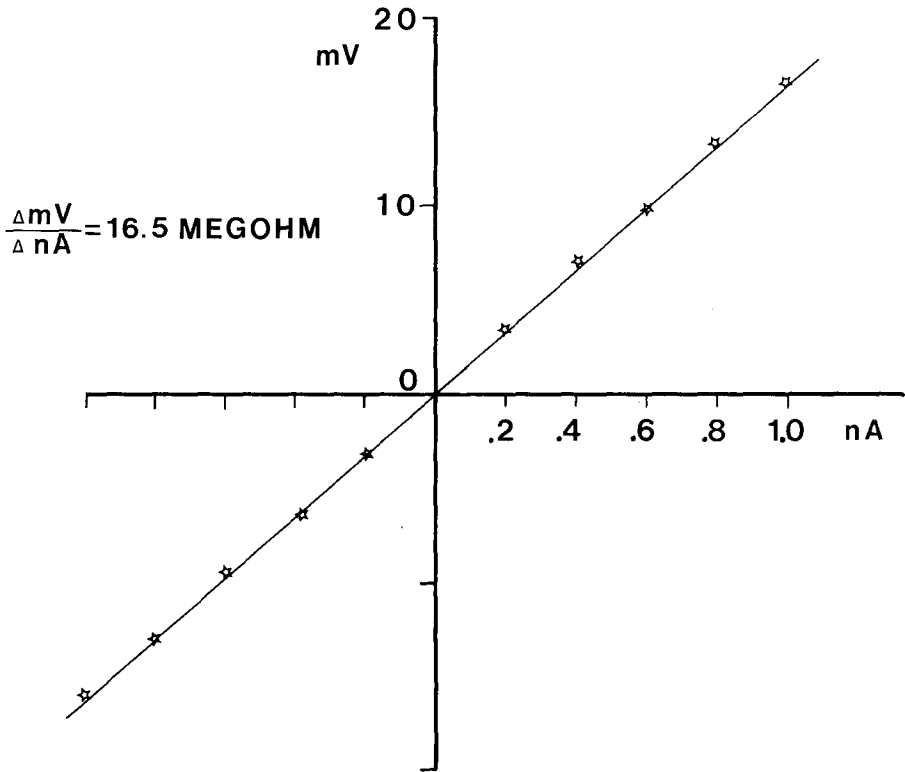


Fig. 6. Current voltage relationship in a secretory cell. Current pulse was injected through a bridge-balanced glass microelectrode into the impaled cell. Input impedance of this cell is calculated as $16.5 \text{ M}\Omega$

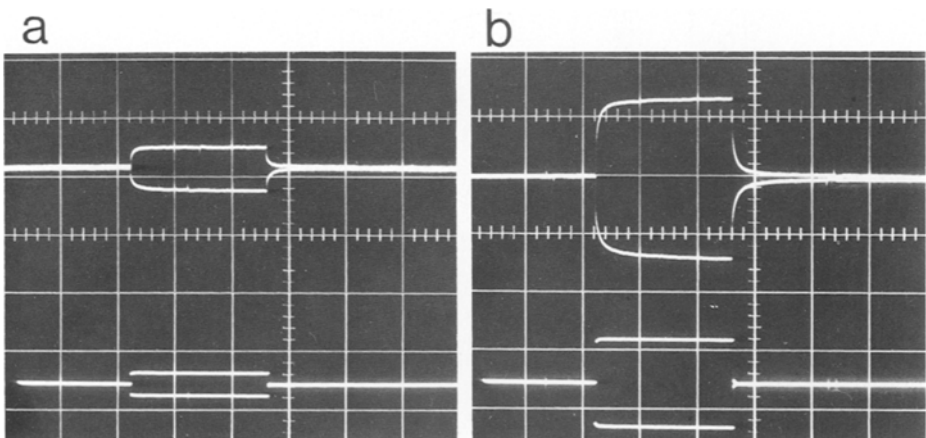


Fig. 7. Oscilloscope pictures showing the shape of voltage pulses induced by intracellularly injected current pulses. The same cell as in Fig. 6. Calibration: vertical, 10 mV ; horizontal, 2 msec . Current pulse (the lower trace in each photograph): *a*, $\pm 0.2 \text{ nA}$, *b*, $\pm 0.8 \text{ nA}$

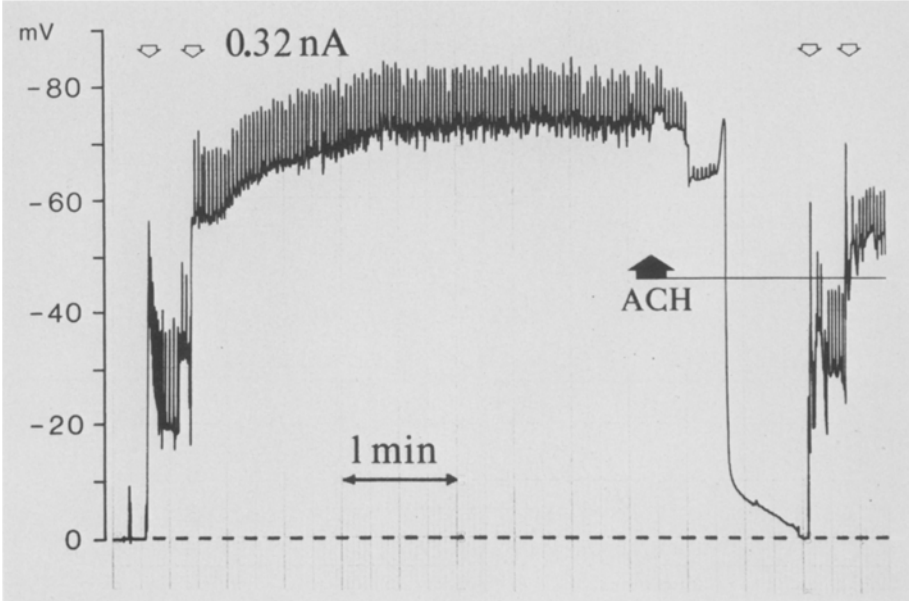


Fig. 8

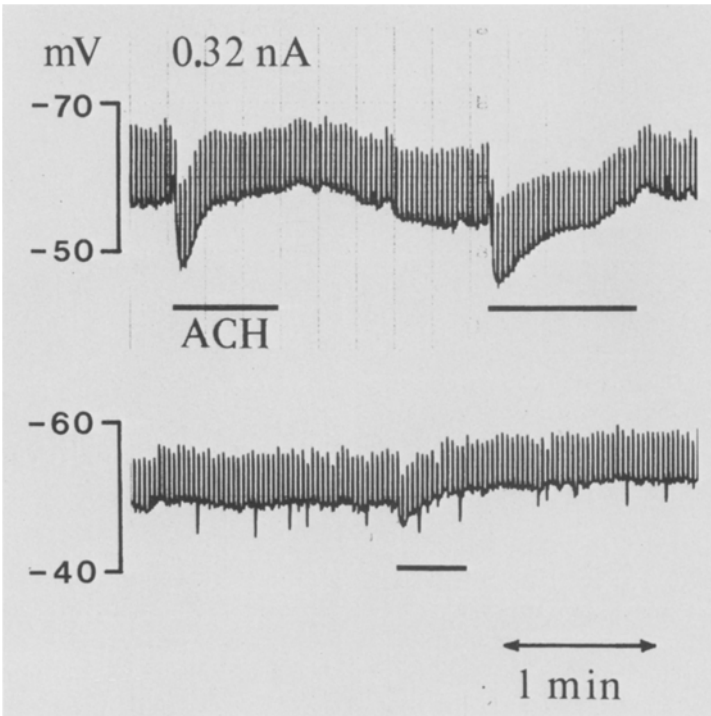


Fig. 9

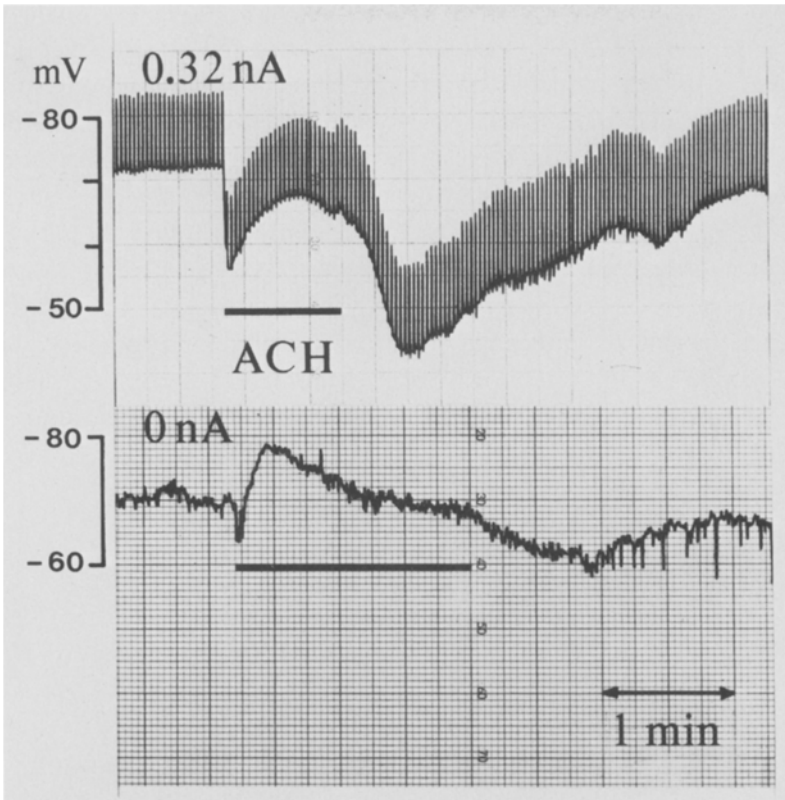


Fig. 10

Fig. 8. A typical example of artifactual decrease in input impedance (the height of the voltage pulse divided by the injected current) in the secretory coil inadequately immobilized for potential recording despite the use of semi-floating electrode. The secretory coil contracted strongly upon addition of acetylcholine (ACH, 5×10^{-6} M) to the incubation medium, which caused the decay of cell PD as well as voltage pulses. Advancing the electrode by small steps, as indicated by open arrows, picked up both the PD as well as voltage pulses from adjacent secretory cells. Note that the bridge-balance of the electrode-resistance was maintained after the cell-impalement. Unless otherwise noted, acetylcholine concentration used was 5×10^{-6} M throughout the study

Fig. 9. Illustrative example of PD's showing transient monophasic depolarization due to local superfusion of acetylcholine (ACH). The superimposed voltage pulse is a measure of input impedance. The current injected was 0.32 nA

Fig. 10. Illustrative example of PD's showing a biphasic depolarization pattern due to local superfusion of acetylcholine. The voltage pulse in the upper trace is a measure of input impedance. No current pulse was injected in the lower trace. The lower trace was retouched by an artist to darken the original pen-recorded PD trace

in which stimulation with acetylcholine brings about a transient depolarization and the PD subsequently returns to the resting level despite continued stimulation with the drug. The height of the superimposed voltage pulses in Figs.9 through 11 is the measure of input impedance, Figure 10 is the biphasic type in which the initial depolarization is followed by repolarization or hyperpolarization for a short period of time. The PD subsequently begins to depolarize or stays at the resting level. On cessation of acetylcholine superfusion, the cell PD transiently depolarizes before returning to the prestimulation level. The third type shows a transient or continued hyperpolarization due to acetylcholine stimulation (Fig. 11). There was no correlation between the level of resting PD and the type of response to acetylcholine, at least among the cells thus far studied by us.

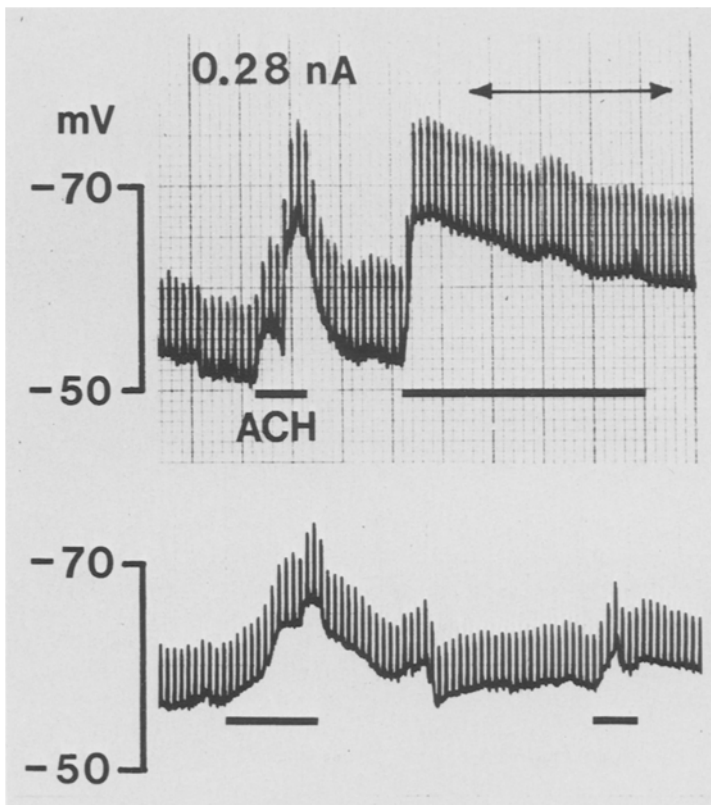


Fig. 11. Illustrative example of PD's showing hyperpolarization due to local superfusion of acetylcholine. The height of voltage pulse is a measure of input impedance. The horizontal arrow indicates 1 min

Table 1. Percentile change in input impedance during acetylcholine (ACH) application

sec after start of ACH application	0-15	16-30	31-60	post ACH ^a
<i>n</i>	12	12	8	12
Input impedance ^b in % of before ACH	104.3	109.8	114.5	142.3
SEM	±4.1	±7.5	±4.6	±12.5
<i>P</i>	0.4 > <i>P</i> > 0.3	0.3 > <i>P</i> > 0.2	0.005 > <i>P</i> > 0.001	0.005 > <i>P</i> > 0.001

^a 15 to 30 sec after cessation of ACH application.

^b For each impaled cell, an arithmetic mean of five typical voltage pulses carefully selected for each period was compared with its own control.

n: number of secretory cells studied.

P: probability as calculated by Student *t*-test.

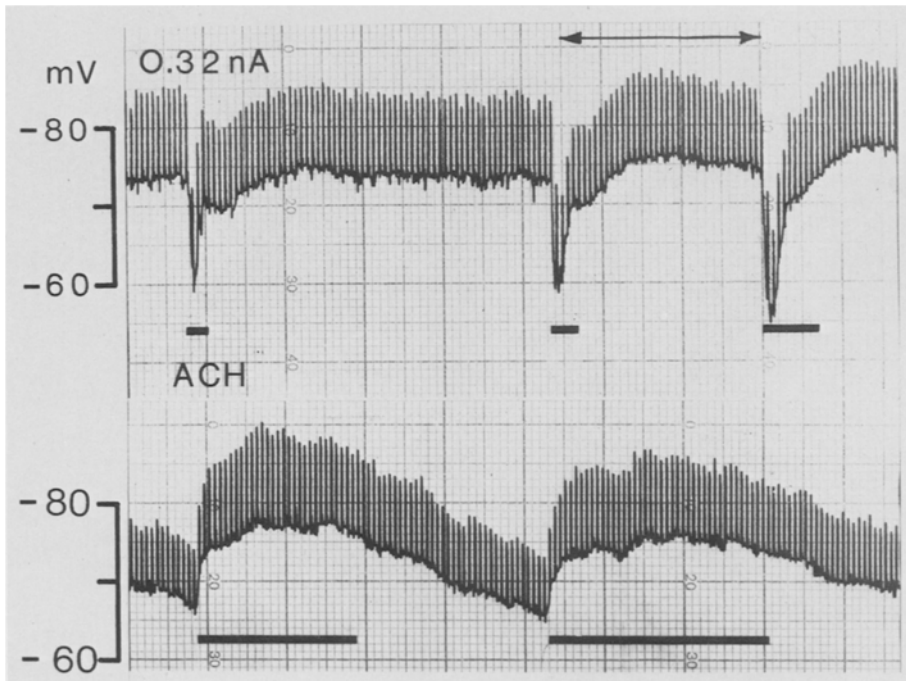


Fig. 12. Effect of repeated superfusion of acetylcholine on input impedance of the secretory cells showing (upper) and hyperpolarizing (lower trace) responses to the drug. Note the complete absence of a decrease in input impedance during the transient period of acetylcholine superfusion. The horizontal arrow indicates 1 min

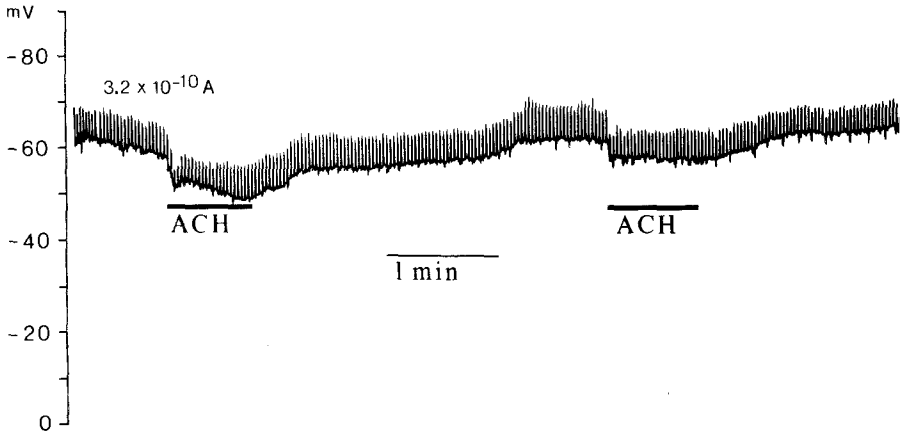


Fig. 13. Effect of locally superfused acetylcholine on input impedance of the secretory cell. An illustrative example. A small decrease in the height of the first two pulses in the initial stimulation period may be an artifact of leaky sealing of the electrode due to myoepithelial contraction (Sato, 1977*b*). Similar notable changes of input impedance did not occur after the second stimulation periods. See text for more discussion

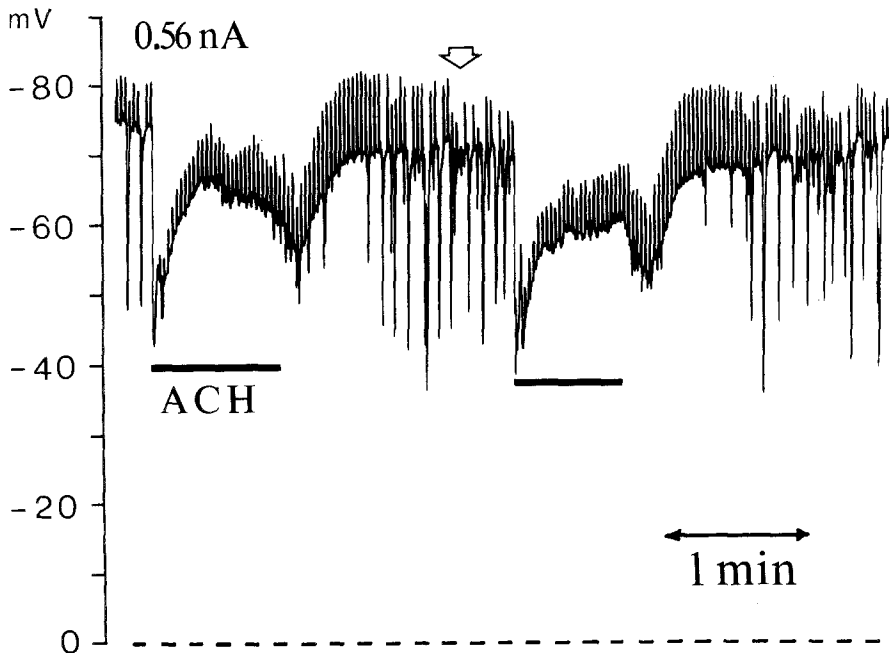


Fig. 14. Effect of local superfusion of acetylcholine on input impedance of a cell showing a marked activity for spontaneous miniature depolarization potentials. It is impossible to measure the height of voltage pulse during the transient period of acetylcholine stimulation because of a rapid shift in the baseline of the PD trace. The voltage pulse apparently disappears when it superimposes with miniature depolarization potentials, as indicated by the open arrow

Contrary to our prior expectation, input impedance failed to decrease during stimulation with acetylcholine in both depolarizing and hyperpolarizing types (Table 1, Figs. 9–12, and 15). Rather, it tended to increase with time. In 8 of 12 cells, input impedance showed a still further increase after cessation of the drug application, reaching its maximum of 42% increase between 15 and 30 sec and returning to the resting level by 60 to 90 sec. In a few cells, however, we did encounter a situation where the height of the voltage pulse appeared diminished during the transient period of acetylcholine stimulation of approximately 5 sec (Fig. 13) or could not be measured from a pen-recorded PD trace during this transient period because of a very rapid baseline potential shift (depolarization or repolarization) (Fig. 14) or because of a noisy baseline due to superimposition of tiny miniature depolarization potentials. As an interpretation of these observations, we considered the following three possibilities: (i) the membrane resistance was indeed decreased during this transient period; (ii) the transient decrease in the height of the

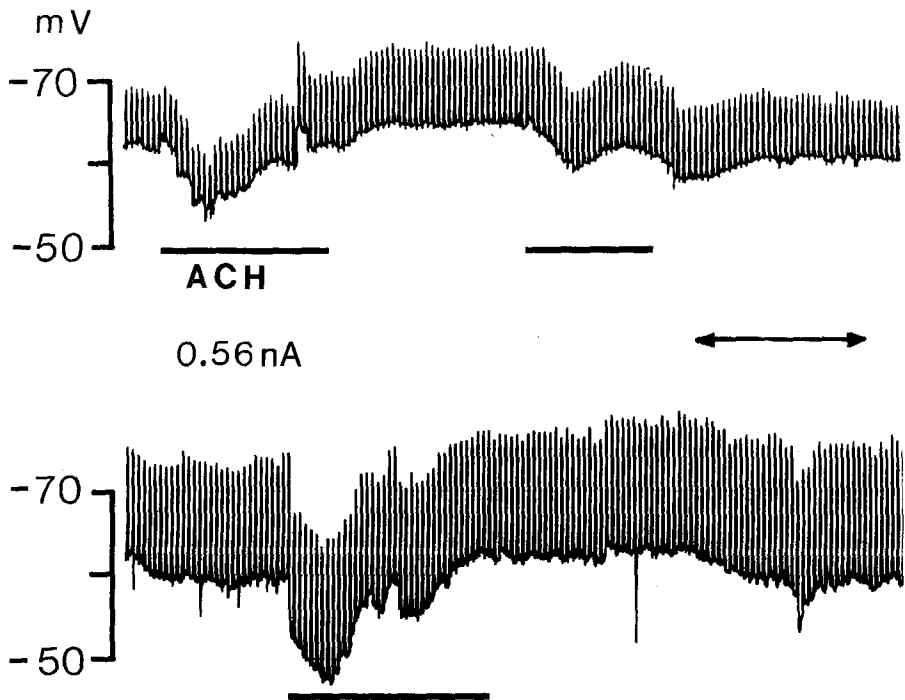


Fig. 15. Effect of local superfusion of acetylcholine on input impedance of two cells. The dose of superfused acetylcholine was gradually increased by gently moving the superfusion pipette from a distance toward the impaled site. Note the absence of any change in input impedance during acetylcholine stimulation. The horizontal arrow, 1 min

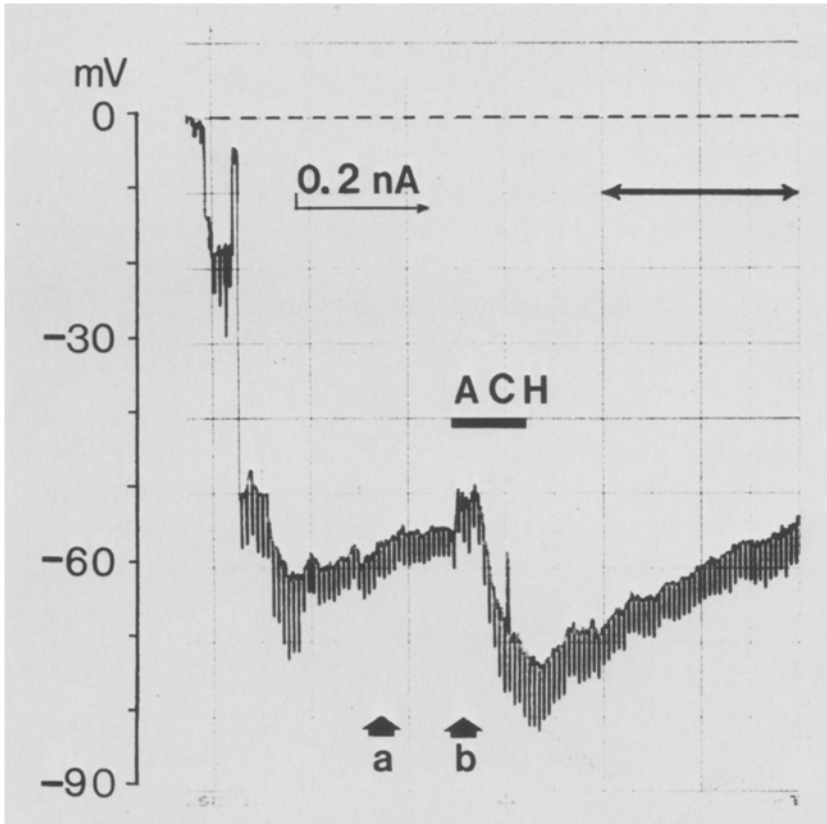


Fig. 16. Effect of local superfusion of acetylcholine on input impedance in a cell. The first pulse (arrow) in the transient period of acetylcholine superfusion was recorded by an oscilloscope camera and compared with the control (arrow *a*). See Fig. 17 for oscilloscope pictures. The horizontal arrow, 1 min

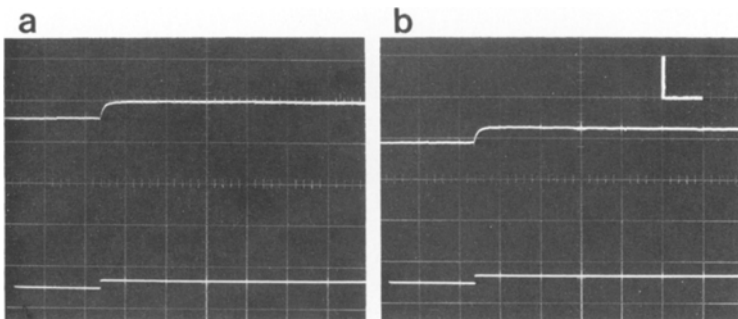


Fig. 17. Oscilloscope pictures of current-induced voltage pulses before (*a*) and immediately after (*b*) stimulation with acetylcholine. Calibration: Vertical, 10 mV; horizontal, 2 msec. Current injected was 0.2 nA and 200 msec duration. The long duration of the pulse was needed to enable the simultaneous pen-recording of voltage pulses

voltage pulse was an artifact of transient leaky sealing of the membrane around the electrode at the sudden onset of myoepithelial contraction (Fig. 8 and *also see* Fig. 6 of Sato, 1977*b*); and (iii) the superimposition of miniature depolarization potentials decreased or apparently decreased the height of the voltage pulses during this transient period (*see* the arrow in Fig. 14).

As regards possibility *ii*, we attempted to gradually stimulate the sweat gland (and thus also the myoepithelial cells) by superfusing acetylcholine from a site distant from the sweat gland in the beginning and slowly moving the perfusing pipette toward the impaled site. The velocity of myoepithelial contraction was much slower in this way and, as can be seen in Figs. 15, there was no decrease in input impedance in these cells. Figures 16 and 17 will partially clarify the problem of the superimposition of miniature depolarization potentials. Although the height of the voltage pulse was difficult to determine from the pen-recorded PD trace during the transient period of acetylcholine stimulation (arrow *b* in Fig. 16) because of the noisy baseline (due to a cluster of very tiny miniature depolarization potentials), the oscilloscope picture taken at this point (arrow *b* in Fig. 16) showed no significant change in the pulse height and the time constant (Fig. 17*b*) as compared with that of control (Fig. 17*a*).

Table 2. Percentile change in voltage deflection due to intraluminally injected current pulses across the epithelial wall and the basal membrane

	Voltage deflection	
	Transepithelial ^b	Across the basal membrane
<i>n</i>	8	13
% of control ^a	110.3	108.5
SEM	± 3.4	± 3.4
<i>P</i>	<i>P</i> > 0.7	

n: number of cells.

P: probability as calculated by Student *t*-test.

^a mean of 5 typical pulses carefully selected for the period 15 to 30 sec of ACH application was compared with its own control.

^b Since the semifloating electrode used to record the cell PD could not usually be advanced further into the lumen because of flexibility of the electrode shaft and the structural resilience of the epithelial wall, an ordinary Ling-Gerard type electrode with a short taper and a sharpened tip (by means of a Narishige Electrode Grinder) had to be used to impale the epithelial wall in a separate set of experiments. However, in three tubules in which the cell electrode could be further advanced into the lumen, the $\Delta V_b/\Delta V_t$ ratio was a mean of 0.72 in the resting state.

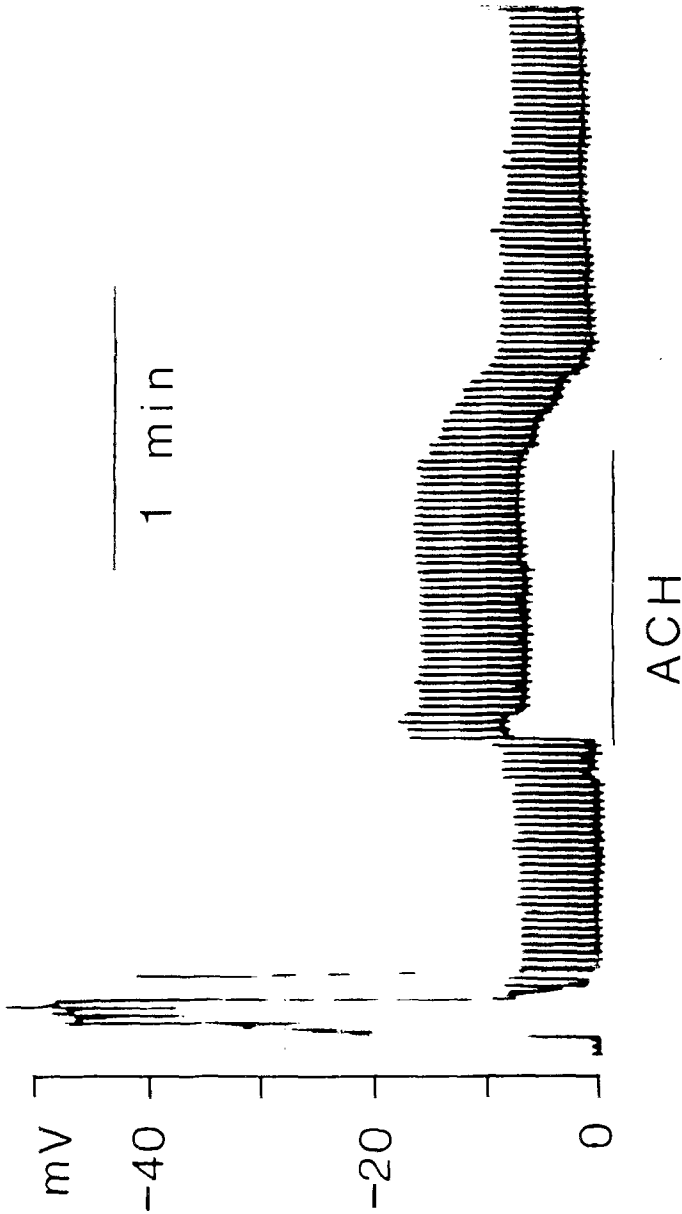


Fig. 18. Effect of acetylcholine on the voltage deflection due to intraluminally injected current pulses. The baseline shift during acetylcholine superfusion is the lumen negative transepithelial PD. The negative potential spike of -50 mV at the beginning of the trace is the cell PD picked up by the electrode when piercing the tubular wall

In Table 2, the changes in both transepithelial and trans-basal membranous voltage deflections due to intraluminally injected current pulses are listed and expressed in percentile terms. Since it was not usually successful to measure the voltage divider ratio by means of two microelectrodes or with a single electrode by a single successive advance-

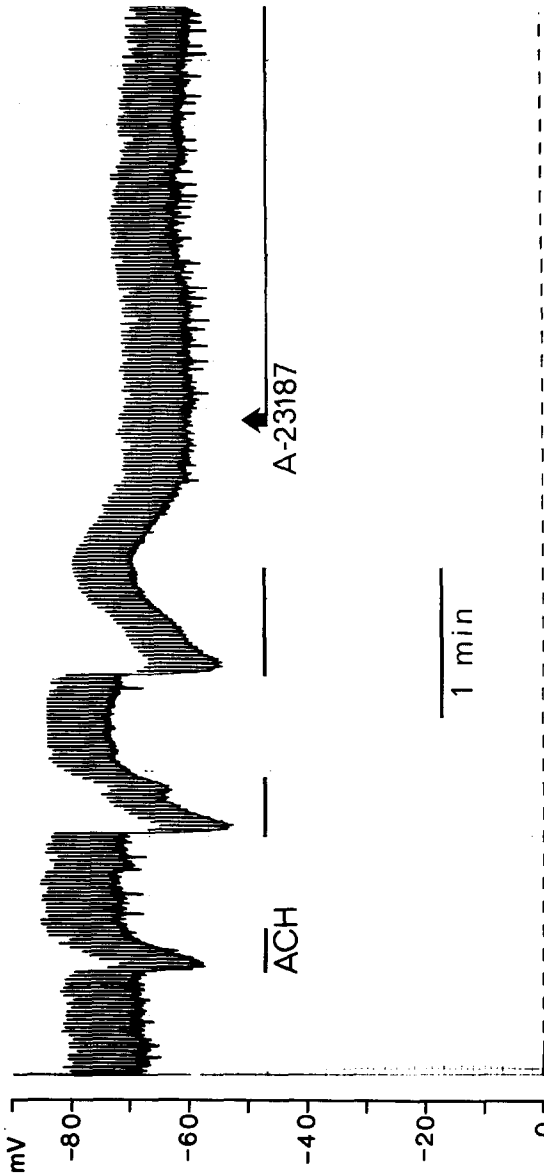


Fig. 19. Effect of acetylcholine and a Ca^{++} ionophore, A23187, on the current induced voltage deflection across the basal membrane. A23187 dissolved in 80% ethanol + 20% acetone at 5×10^{-2} M was added to the incubation bath to a final concentration of $1-4 \times 10^{-4}$ M. Acetone or ethanol *per se* at the final concentration used does not affect the secretory activity of the gland. A23187 at high concentration elicits secretion within 30 sec

ment of the electrode tip, it was necessary to measure them in two separate sets of experiments (*see also* the legend for Table 2). Figures 18–21 are illustrative examples of such experiments on the effect of acetylcholine on current-induced voltage deflections across the epithelial wall and the basal membrane, respectively. The change in voltage deflection

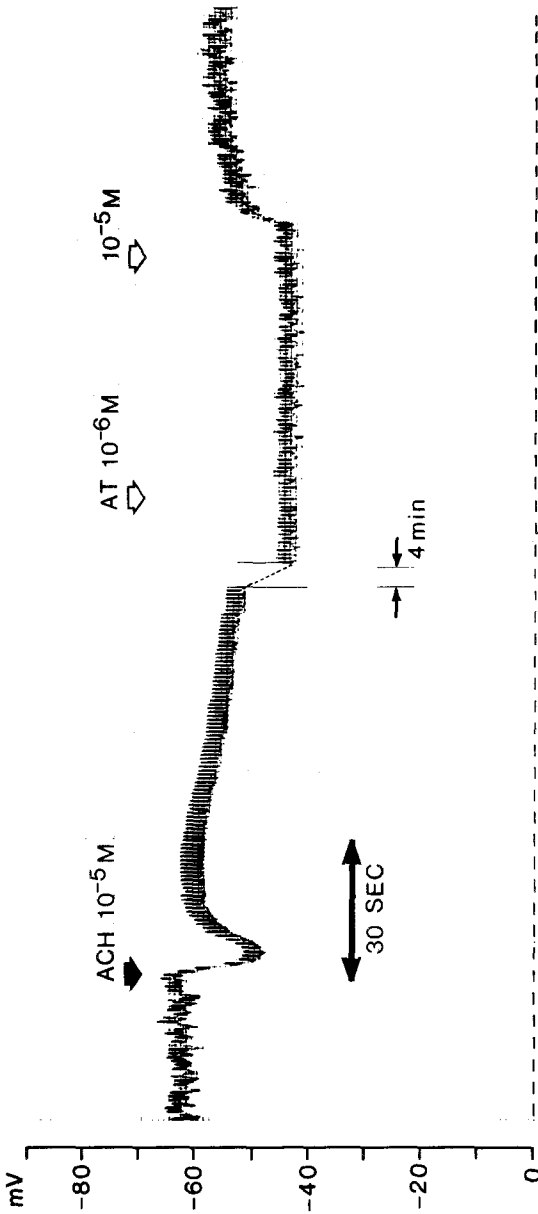


Fig. 20. Effect of high concentration of acetylcholine on the current-induced voltage deflection across the basal membrane. Acetylcholine was directly added to the incubation bath to a final concentration of 10^{-5} M. AT, atropine. Because of strong myoeptithelial contraction, a gradual decline of PD in the middle portion of the trace could be artifactual

was compared between the time immediately before (control) and 15 to 30 sec after start of local application of acetylcholine. The transepithelial voltage deflection increased by 10% during stimulation of the secretory tubule with acetylcholine (Table 2). Under the experimental conditions used, the tubule contracted by varying degrees due to its myoeptithelial

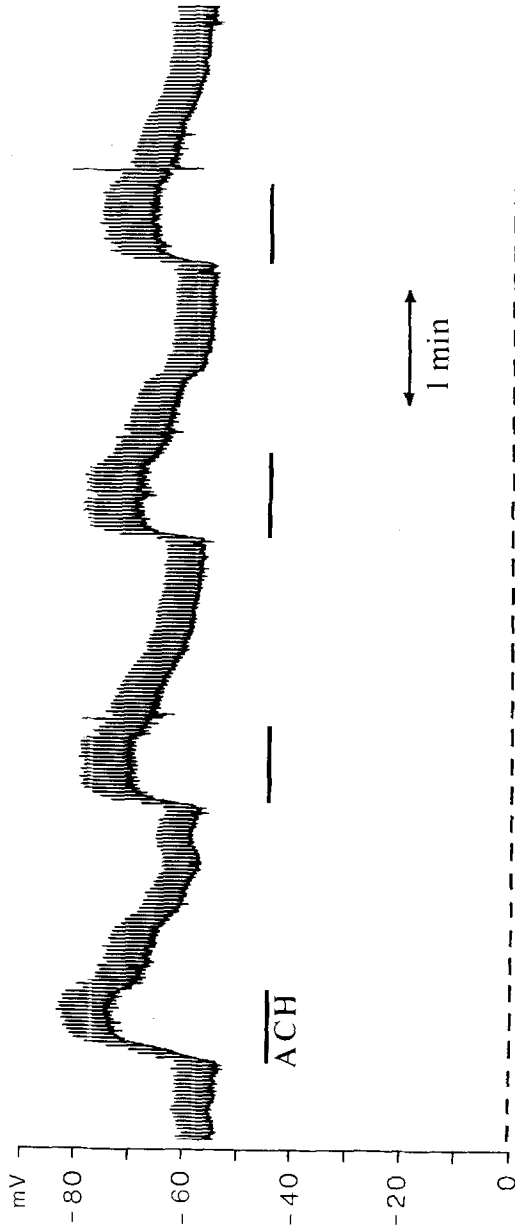


Fig. 21. Effect of acetylcholine on the voltage deflection across the basal membrane due to intraluminally injected current pulses in a cell repeatedly showing a hyperpolarizing response to locally superfused acetylcholine

action. Thus the shortening of the distance between the current injection site and the tip of the electrode, which causes an increase in current density at the measured site, may largely, if not entirely, explain the small increase in voltage deflection. The voltage deflection across the basal membrane also increased but to the same extent as that across the

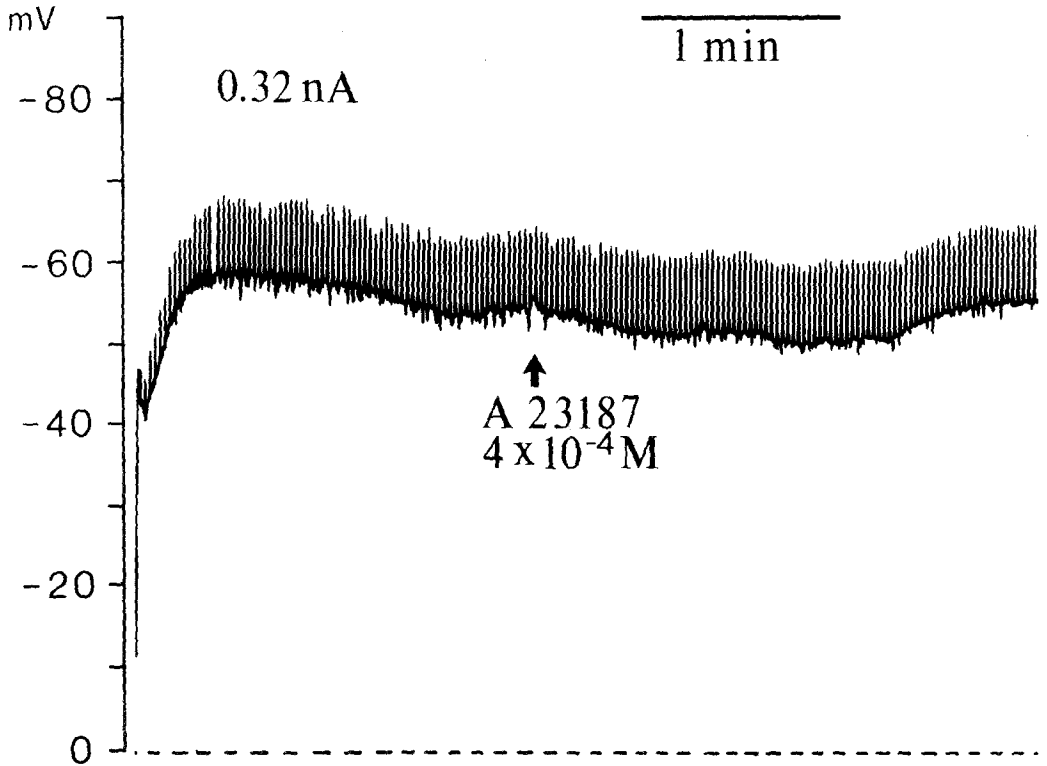


Fig. 22. Effect of A23187 on input impedance of the secretory cell. An illustrative example

epithelia wall, indicating that there was no significant relative change in voltage deflection across this membrane.

The simplified circuit in Fig. 4 gives,

$$R_t = \frac{R_s(R_a + R_b)}{R_a + R_b + R_s} \quad \text{or} \quad R_b = \frac{R_s \cdot R_t}{R_s - R_t} - R_a$$

$$V_t = I \cdot R_t = i_s \cdot R_s = i_c(R_a + R_b) = \Delta V_a + \Delta V_b.$$

The results in Table 2 can be rewritten as, $(\Delta V_b / \Delta V_t) = (i_c \cdot R_b / I \cdot R_t) =$ constant, before and after stimulation with acetylcholine. Thus, unless R_s and R_a (and therefore i_c) changes drastically due to acetylcholine, which is a less likely possibility, "no change in $i_c \cdot R_b / I \cdot R_t$ " most likely indicates that the electrical resistance of the basal membrane (R_b) did not change during stimulation with acetylcholine. As shown in Figs. 19, 22 and 23, A23187 ($1 - 4 \times 10^{-4}$ M) changed neither the input impedance nor

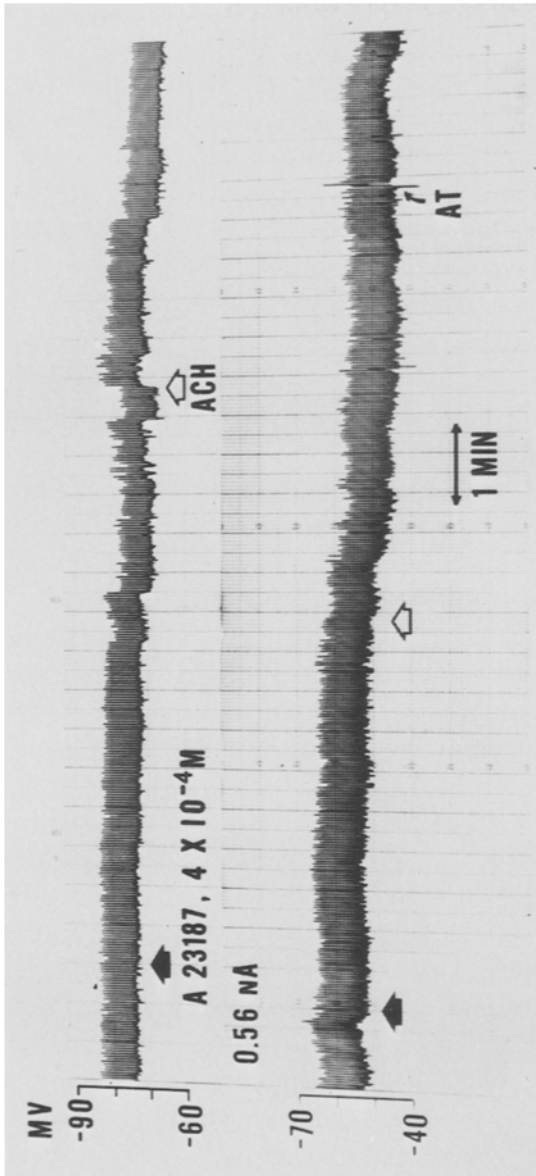


Fig. 23. Effect of A23187 and superimposed acetylcholine on input impedance in two secretory cells. The PD lasted for longer than 1.5 min in these cells. The current injected was 0.56 nA. The drugs were added directly to the incubation medium

the current-induced voltage deflection across the basal membrane ($n=4$) or across the epithelial wall ($n=3$) to a significant degree. The further addition of acetylcholine to the incubation media failed to change input impedance (Fig. 23). Puzzlingly enough, A23187 caused no change in the potential profile across the basal cell membrane (Figs. 22 and 23).

Discussion

The present investigation has indicated that the electrical resistance of the basal membrane does not change during stimulation of the sweat gland with acetylcholine *in vitro*. This is based on the observation that neither input impedance nor the voltage deflection across the basal membrane due to intraluminally injected current pulses was decreased by acetylcholine or by a calcium ionophore A23187.

The input impedance method has been widely used in other exocrine glands for estimating the change in electrical resistance of the cell membrane. However, the input impedance reflects the changes in resistance of not only the basal membrane but also the luminal membrane as well as the cell to cell coupling. In spite of these methodological shortcomings, it is noteworthy that only in the salivary glands and the exocrine pancreas, but not in the eccrine sweat gland, a decrease in input impedance has been observed. The following three observations are pertinent to the validity of the present observation. Firstly, we used a semi-floating electrode (Sato, 1977b) to minimize the dislocation or leaky impalement of the electrode-tip during myoepithelial contraction due to the application of acetylcholine. Secondly, we failed to observe any significant change in input impedance in either the human or the rat eccrine sweat glands (*unpublished* and Sato, 1975). Lastly, using the same experimental set-ups as used for the sweat gland, we have been able to confirm the observations of others (Nishiyama & Petersen, 1974 & 1975; Petersen & Ueda, 1975) that acetylcholine, indeed, drastically decreases the input impedance of the acinar cells of the salivary glands (Fig. 24) and the exocrine pancreas *in vitro* (*unpublished*). The voltage deflection across the basal membrane due to intraluminally injected current pulses reflects a qualitative measure of the electrical resistance of the basal membrane unless R_s and R_a in Fig. 4 simultaneously change with R_b in such a way that $i_c \cdot R_b$ is always kept constant. However, this latter possibility is not likely because the secretory coil of the sweat gland is classified as a so-called "leaky epithelium" (Sato, 1977a) and thus R_a , $R_b \gg R_s$ (the preliminary estimate of R_a/R_b ratio being 1:2.5, see Table 2), and $R_t \simeq R_s$ (Sato & Ullrich, 1975), but T_t did not change significantly due to acetylcholine (Fig. 18 and Table 2). Furthermore, the cell-to-cell junction (terminal bar) is not where acetylcholine primarily acts, and the input impedance, which also reflects the change in the cell-to-cell coupling resistance, did not significantly change, at least during the first 30 sec of stimulation with acetylcholine.

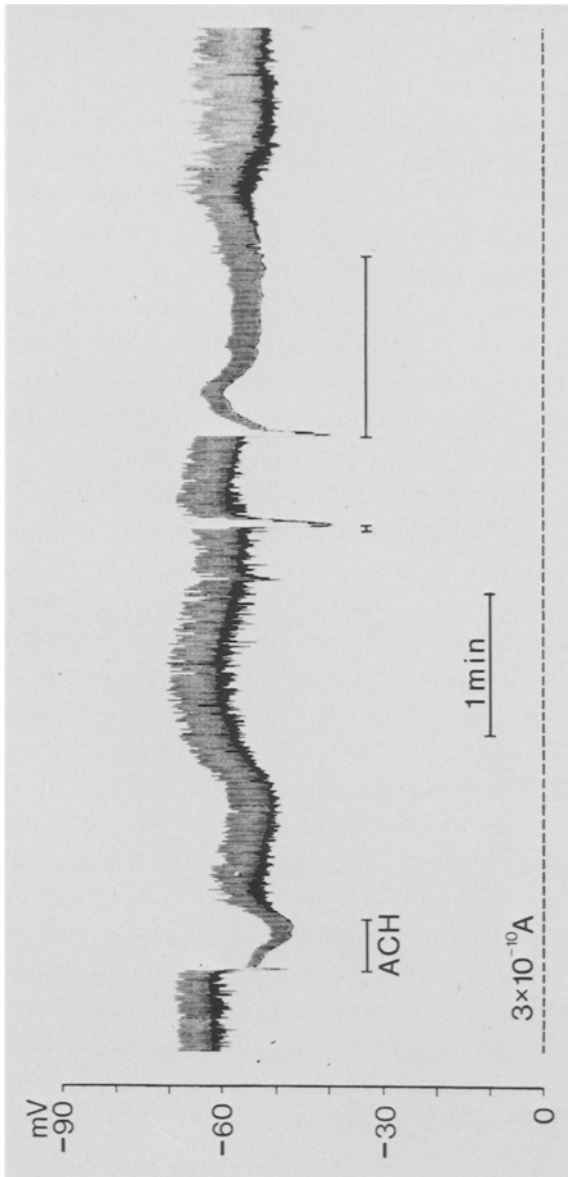


Fig. 24. Effect of acetylcholine on input impedance of an acinar cell of mouse submaxillary gland presented for the sake of comparison with that of the eccrine sweat gland. All the methodology is identical with that used for the eccrine sweat gland. A very tiny fragment of the lobule of the submaxillary gland was prepared, immobilized, and impaled with a semi-floating electrode *in vitro*. A drastic decrease in input impedance has been consistently observed in all the freshly prepared tissues from the salivary glands, as well as from the pancreas

The reason for a small increase in input impedance of the secretory cell during and after acetylcholine stimulation remains to be explained. However if Ca^{++} concentration in the cell, indeed, increases by acetylcholine (Sato, 1977a), then decoupling, if any, of cell-to-cell communication by an increase in cytoplasmic Ca^{++} concentration (Loewenstein,

1970) may account for the observed change in input impedance. However, we are still puzzled by the total absence of any change in input impedance, the current-induced voltage deflection across the basal membrane, and in the potential profile across the cell membranes even when a high concentration of a Ca^{++} ionophore, A23187 ($4 \times 10^{-4} \text{ M}$) was added to the incubation bath. This ionophore at this concentration induces copious sweat secretion within 30 to 60 sec (Sato, 1977a). If Ca^{++} influx into the cell indeed decouples the cell-to-cell communication also in the eccrine sweat gland, and if the increase in input impedance as observed in many cells after cholinergic stimulation is evidence for the occurrence of decoupling, then we should have observed a similar increase in input impedance also under stimulation with A23187, which, however, never happened. Thus, until otherwise proven, the increase in input impedance during the post-acetylcholine stimulation period in some cells should not be readily linked to decoupling of cell-to-cell communication in this eccrine sweat gland. One further possibility, among others, must also be considered: that myoepithelial contraction which is accompanied by cholinergic stimulations but not by stimulation with A23187 may in some unknown way hold the key to the problem. It should be noted, however, that even if decoupling does occur in the sweat gland, it may not affect the validity of the present contention that R_b does not change after stimulation with acetylcholine as long as i_c (and thus also i_e) remains unchanged. When acetylcholine is superfused or directly added to the incubation bath, the entire segment of the secretory coil is exposed to the drug, as evidenced by contraction of the entire sweat gland. Thus decoupling of cell-to-cell junction should occur at every cell junction, if it occurs at all. When current pulses are injected intraluminally as in the present study, some current may also travel through the cytoplasm and cell junctions of many cells until it reaches the impaled-cell interior, where a portion of this current may bifurcate and join i_c during the prestimulation period. However, with the development of decoupling at every cell junction, this communicating or coupling current diminishes and so does that part of the current having previously merged with i_c in the impaled cell. This results in the reduction of total i_c that passes through R_b . The fact that ΔV_b during acetylcholine stimulation remained unchanged should therefore indicate an "increase" rather than a "decrease" in R_b if significant decoupling indeed occurs. Furthermore, the decoupling of cell-to-cell junction may in theory decrease rather than increase i_c (by enhancing i_s), which tends to increase R_b given a constant ΔV_b . The effect of A23187 also supports

the validity of our contention. It should be noted, however, that these discussions should not be interpreted as supporting or refuting the presence of decoupling in the eccrine sweat gland during acetylcholine stimulation.

The present observation is significant because it is the first, to the best of our knowledge, to indicate that the stimulating effect of acetylcholine on the exocrine secretion of fluid and electrolytes can be achieved without any significant change in the electrical resistance of the basal membrane of the secretory cell, at least as detectable within the resolution of the methods used. At present we have no adequate explanation for the differing effects of acetylcholine on the membrane resistance between the eccrine sweat gland and the other exocrine glands such as the salivary glands and the pancreas. Several possibilities must be raised to account for this difference of response to acetylcholine for the completeness of discussion.

Firstly, the eccrine sweat gland may not be a miniature salivary gland or a miniature exocrine pancreas even through these secretory glands are collectively classified under the term "exocrine glands". Secondly, in the eccrine sweat gland no zymogen granules are present and no exocytotic mechanism (Jamieson & Palade, 1971) of secretion of fluid and electrolyte is known. Recently, Liebow and Rothman (1976) reported that the membrane of isolated zymogen granules from the pancreas is permeable to macromolecules *in vitro*, although it has not been proven that the same is also true *in vivo*. Thus, until shown otherwise, the possibility must also be considered that the electrical conductivity of the membrane of the zymogen granules that fuses with the plasma membrane when stimulated with acetylcholine or other secretagogues may also at least partially contribute to the change in membrane resistance in those exocrine glands which perform both exocytotic secretion of enzymes and secretion of fluid and electrolytes. Although it may appear at first glance to be unrealistic to tie together an enhanced fluid and electrolyte transport and the "no change" in the electrical resistance of the membrane during stimulation with acetylcholine in the eccrine sweat glands, the recent study by Frizzell, Ducas & Schultz (1975) on the gallbladder epithelium may be relevant to the possible mechanism of acetylcholine-induced eccrine sweat secretion. These authors suggested, based on theoretical reasoning as well as experimental observations in the gallbladder epithelium, that Na^+ and Cl^- enter the cell as a neutral complex through a nonconductive pathway and that such a neutral particle can easily cross a membrane possessing even an infinite electrical

resistivity. In other words, if Na^+ and Cl^- cross the basal membrane as a neutral complex through the nonconductive pathway also in the eccrine sweat gland, the measurement of the electrical membrane resistance of the conductive pathway alone should not provide relevant information on the permeability of ions. During sweat secretion most, if not all, of NaCl and fluid may traverse the secretory cell basal membrane, then traverse the cytoplasm to be ultimately actively transported across the luminal membrane (including the membrane of intercellular canaliculi) into the tubular lumen. If so, the theory of neutral NaCl entry seems most attractive in explaining how a massive influx of NaCl from the outer interstitial space into the cell can be achieved in the absence of any change in the electrically measured basal membrane resistance during acetylcholine or A23187-stimulated secretion in the eccrine sweat glands. Although it may be inappropriate to further speculate based on our present observation, the fact that amiloride, a known blocker of Na -channel, even at 0.1mM has no effect on sweat secretion but that replacement of Cl^- and HCO_3^- with SO_4^{--} plus mannitol in the incubation medium totally abolishes eccrine secretion *in vitro* (Sato, *unpublished*) appears to be in keeping with the neutral NaCl entry theory. Since the available evidence on the eccrine sweat gland suggests that the effect of acetylcholine is mediated by influx into the cell of Ca^{++} (Sato, 1977a and *unpublished*), it will be of importance to elucidate how Ca^{++} influx or intracellular Ca^{++} is related to the regulation of NaCl entry into the secretory cell, which may still be the first event in the overall steps of acetylcholine-mediated secretion of fluid and electrolytes.

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