J. Membrane Biol. 77, 33–44 (1984)

The Journal of
Membrane Biology

Electrical Activity of an Intestinal Epithelial Cell Line: Hyperpolarizing Responses to Intestinal Secretagogues

Toshihiko Yada* and Yasunobu Okada
Department of Physiology, Faculty of Medicine, Kyoto University, Kyoto 606, Japan

Summary. Cultured epithelial cells (Intestine 407) derived from fetal human small intestine exhibited spontaneous oscillations of membrane potential between the resting level of about −20 mV and the activated level of about −75 mV. The cells were hyperpolarized to the latter level in response to mechanical or electrical stimuli. The hyperpolarizing responses were also elicited by the application of intestinal secretagogues: acetylcholine, histamine, serotonin and vasoactive intestinal polypeptide (VIP). The spontaneous oscillation of membrane potential became prominent and long-lasting in the presence of acetylcholine, histamine, serotonin or VIP. These secretagogue-induced responses were mediated by individual independent receptors on the cell membrane. Muscarinic receptors were responsible for the acetylcholine response, and H_1-receptors for the histamine response. The cells also responded with a slow hyperpolarization to calcium ionophore A23187, which is known to induce intestinal secretion. The spontaneously occurring hyperpolarizing responses and those induced by stimuli were both due to an increase in the K^+ conductance of the cell membrane. Since acetylcholine, histamine, serotonin and A23187 are known to promote mobilization of cellular Ca^{2+} ions in intestinal secretory cells, it is hypothesized that these electrical activities of the cell are closely related to the receptor stimulation which leads to the Ca^{2+}-mediated intestinal secretion.

Key Words: intestinal epithelial cell · secretagogue · receptor · hyperpolarizing response · calcium · secretion

Introduction

The small intestine has a well-developed epithelium with two opposite functions concerning the transport of ions and water: secretion and absorption. A number of circumstantial evidences have suggested that intestinal secretion is carried out by undifferentiated crypt epithelial cells, whereas absorption is performed by villous epithelial cells (Field, 1981; Welsh, Smith, Fromm & Frizzell, 1982). The Intestine 407 cell is an established cell line of the epithelial cells derived from jejunum and ileum of a human embryo of about 2 months' gestation (Henle & Deinhardt, 1957). To date, there have been no attempts to determine the origin or identity of the cells. However, the cells are assumed to be derived from undifferentiated epithelial cells in the small intestine, for the following facts: (1) The villus structure is not developed yet at this gestational stage (Grand, Watkins & Torti, 1976). (2) Only the undifferentiated (crypt) cells are mitotically active (Leblond & Messier, 1958). (3) The origin of another cell line of intestinal epithelial cells, IEC-6, has been determined as undifferentiated crypt epithelial cells (Quaroni, Wands, Trelstad & Isselbacher, 1979). Thus, some characteristics of crypt secretory cells are expected to be retained in the Intestine 407 cells.

In the present study, we attempted to characterize the electrical membrane properties in these cells. The results show that the Intestine 407 cells respond with membrane hyperpolarizations to mechanical or electrical stimuli and to several intestinal secretagogues, but not to glucose (which is absorbed by villous epithelial cells with membrane potential changes). Some of the results have been reported in abstract form (Yada & Okada, 1980; 1982).

Materials and Methods

Cells

Uncloned Intestine 407 cells (purchased from Flow Labs. Inc.) were cultured in Fischer medium supplemented with 10% newborn calf serum (Flow Labs. Inc.). For electrophysiological studies, attempts were made to obtain multinucleate giant cells by cell fusion. For this purpose, the monolayer cells were treated for 10 to 30 sec with a 50% polyethyleneglycol (PEG) 1540 or 6000 (Nakarai Chemical Co.) in the Fischer medium without

* *Present address:* Department of Physiology, Tokyo Medical and Dental University School of Medicine, Tokyo 113, Japan.

adding the serum. The cells were washed with a phosphate-buffered saline to remove PEG. Multinucleate cells thus obtained ranged in diameter from approximately 30 μm (a few nuclei) to 100 μm (tens of nuclei). The giant cells attached to the substrate were then maintained in the culture medium with serum for 20 to 48 hr.

Morphology

Morphological studies were made on the cells cultured on coverslips. The cells were observed in a living state under a phase contrast or an interference microscope (Nikon Optiphoto NT).

Electrophysiology

Electrophysiological studies were made on either the confluent or sparse monolayer cells cultured in plastic dishes (6 cm in diameter) under an inverted phase-contrast microscope (Chiyoda T-2). The temperature of the cell chamber was maintained at 35 to 37°C by circulating warm water. The procedures for intracellular recording were essentially the same as those described previously (Okada et al., 1977*a*). The glass microelectrodes were filled with 3 M KCl; their resistances were about 20 MΩ, and their tip potentials were less than 5 mV. The electrode was connected to a high-input impedance electrometer (WPI M-701 or KS-700). The bathing medium was grounded via a 3 M KCl-agar bridge and an Ag-AgCl half-cell. The membrane resistance was measured by passing 0.3 nA through the recording microelectrode after compensating for the electrode resistance, using a bridge circuit. For electrical stimulation, an outward or inward current of 14 nA (pulse duration, 0.5 to 3.0 sec) was applied. Mechanical stimuli were applied by touching the cell surface with an additional microelectrode. A variety of bioactive substances including intestinal secretagogues, gastrointestinal hormones and putative neurotransmitters were added directly to the bathing medium ("bulk application") or administered focally to the cell surface by the pressure application through a blunted micropipette which was placed about 200 μm distant from the cell ("local application"). Reversal potentials of the responses were determined by applying currents through additional intracellular electrode impaled simultaneously.

Media and Chemicals

A bathing solution used in the electrophysiological and morphological experiments was a Tris-buffered saline (TBS) which was composed (in mM) of 4.2 K^+, 143.0 Na^+, 163.5 Cl^-, 0.5 Mg^{2+}, 0.9 Ca^{2+} and 13.5 $Tris^+$ (pH 7.3). A 3 mM Na^+-TBS was prepared by replacing Na^+ with $Tris^+$, and a 30 mM Cl^--TBS by replacing Cl^- with SO_4^{2-}. Osmolarity was adjusted with mannitol. Changes in K^+ concentrations of the bathing medium were accomplished by replacing all or a part of NaCl with equimolar amounts of KCl.

The substances applied by the local or bulk application to test the membrane response were acetylcholine chloride (Nakarai), carbamylcholine chloride (Sigma Chemical Co.), DL-norepinephrine hydrochloride (Nakarai), L-isoproterenol D-bitartrate (Nakarai), 5-hydroxytryptamine hydrochloride (Sigma), histamine (Nakarai), porcine vasoactive intestinal polypeptide (Peninsula Labs. Inc.), A23187 (gift from Eli Lilly), prostaglandin E_1 (Sigma), cholera toxin (Chemo-Sero-Therapeutic Research Foundation), pentagastrin (Sumitomo Chemicals), eel calcitonin (gift from Dr. H. Ito, the Tokyo Metropolitan Geriatrics Hospital), D-glucose (Nakarai), D-galactose (Nakarai), dibutyryl cyclic AMP (Sigma), 8-bromo cyclic AMP (Sigma), theophylline (Nakarai), 3-iso-butyl-1-methylxanthine (Nakarai), atropine sulfate (E. Merck AG.), curare (Nakarai), diphenhydramine hydrochloride (Kongo Chemicals) and cimetidine (Fujisawa Pharmaceuticals). A23187 was dissolved in ethanol, and the other substances were in TBS, NaCl solution, or distilled water. For the bulk application, these substances were given by adding an aliquot (usually 1/100 volume of the bathing solution) of these solutions into the bathing solution.

Results

Morphology

Intestine 407 cells grown in a monolayer culture were mononuclear and typically epithelioid, as shown in Figs. 1*A* and *B*. Dense nucleoli were observed by either phase-contrast (Fig. 1*A*) or interference microscopy (Fig. 1*B*). Giant cells obtained by cell fusion with PEG were multinucleated and also have dense nucleoli (Figs. 1*C* and *D*). Well-developed cytoplasmic granularity was often observed in both mononuclear (Fig. 1*B*) and multinucleate cells (Fig. 1D). No alterations in cell morphology were observed under a phase-contrast microscope by the application of 10 mM glucose, 10 mM galactose, 0.5 mM acetylcholine (ACh), 0.1 mM histamine, 0.3 μM vasoactive intestinal polypeptide (VIP) or 1 mM 5-hydroxytryptamine (5-HT: serotonin).

Membrane Potential Oscillation

Stable intracellular recordings from the Intestine 407 cells were difficult presumably because of the small cell size. To overcome this difficulty, electrophysiological studies were made in the giant cells obtained by cell fusion with PEG (Figs. 1*C* and *D*). Almost all the giant cells exhibited consistent profiles of membrane potential, as exemplified in Fig. 2*B*. Upon penetration with the microelectrode, the resting potential was initially about −20 mV; the membrane was then rapidly hyperpolarized up to about −80 mV and followed by an oscillation of membrane potential. The pattern of the oscillations was classified into three types: (1) A transient type (Fig. 2*B*); the oscillation ceased within a few minutes, and the potential was stabilized at the resting level. (2) A burst type (Fig. 2*C*); the oscillations were repeated intermittently. (3) A continuous type (Fig. 2*D*); the oscillation continued (for more than 1 hr, in some cases) without interruption. Cell populations of the three types were about 55, 25 and 20%, respectively. The most hyperpolarized and depolarized (resting) levels of these oscillations

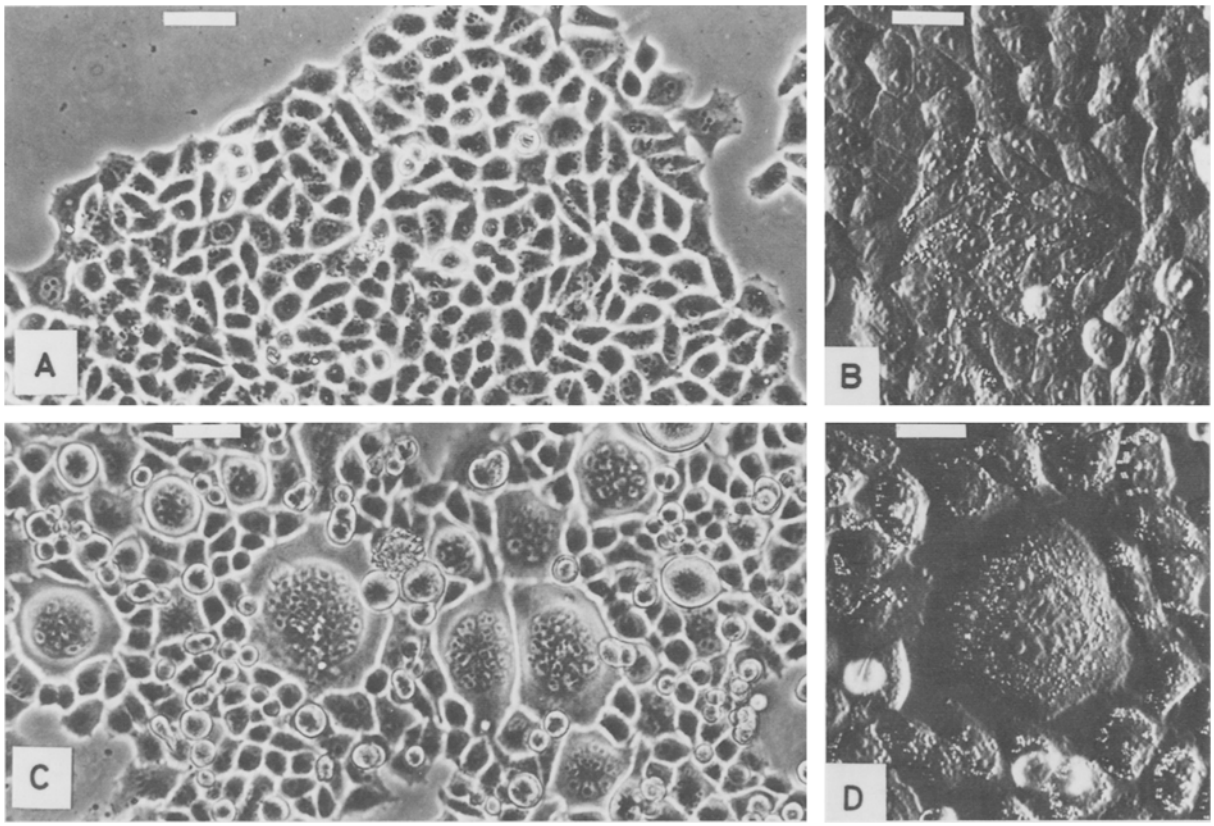

Fig. 1. Morphology of the Intestine 407 cells in monolayer culture. *A*) cells without PEG-treatment; phase contrast (bar: 50 μm); *B*) cells without PEG-treatment; interference contrast (bar: 25 μm); *C*) giant cells after PEG-treatment; phase contrast (bar: 50 μm); *D*) giant cells after PEG-treatment; interference contrast (bar: 25 μm)

were about −74 and −22 mV, respectively (Table 1). During the oscillation of membrane potential, the membrane resistance also oscillated between about 22 and 3 MΩ (Table 1). A distinct reduction of the membrane resistance associated with the hyperpolarizing response suggests an increment in the ionic conductance. The frequency of oscillation varied from cell to cell between 3 and 20 cycles/min (mean ± SE = 7.0 ± 0.3, n = 89), but was stable in a given cell. The oscillations were obtained not only from the cells in the confluent monolayer but also from the solitary cells isolated from others.

Similar oscillations of membrane potential and membrane resistance were also observed in the cells without PEG treatment, in which stable intracellular recordings were successfully made. One of the examples is shown in Fig. 2*A*.

Hyperpolarizing Responses to Mechanical and Electrical Stimuli

When a mechanical stimulus was applied to the giant cell at the resting state, a hyperpolarizing response (HR) was induced. In most cells, a mechanical stimulus produced a single HR (Fig. 3*A*, open asterisk), but, in some cases, elicited a potential oscillation composed of several HRs (Fig. 3*B*, open asterisk). The peak of the HR induced by mechanical stimuli was about −74 mV (Table 1), and the membrane resistance reduced remarkably during the hyperpolarization (Table 1). The initial hyperpolarization observed immediately after penetration of a microelectrode (Figs. 2*A* and *B*) might be due, at least in part, to the mechanical stimuli by the electrode.

The response could also be elicited in the giant cells by the intracellular current injection either with inward current (Figs. 2*B* and 3*D*, closed asterisks) or with outward current (Fig. 3*C*, closed asterisk), although the former was more effective. An electrical stimulation induced, in an all-or-none fashion, HR in the majority of cells (more than 90%). The threshold current was around 10 nA. The response induced by a current pulse was usually a single hyperpolarization (Figs. 2*B* and 3*C*), but occasionally was an oscillation composed of several hyperpolarizations (Fig. 3*D*). The peak of the HR

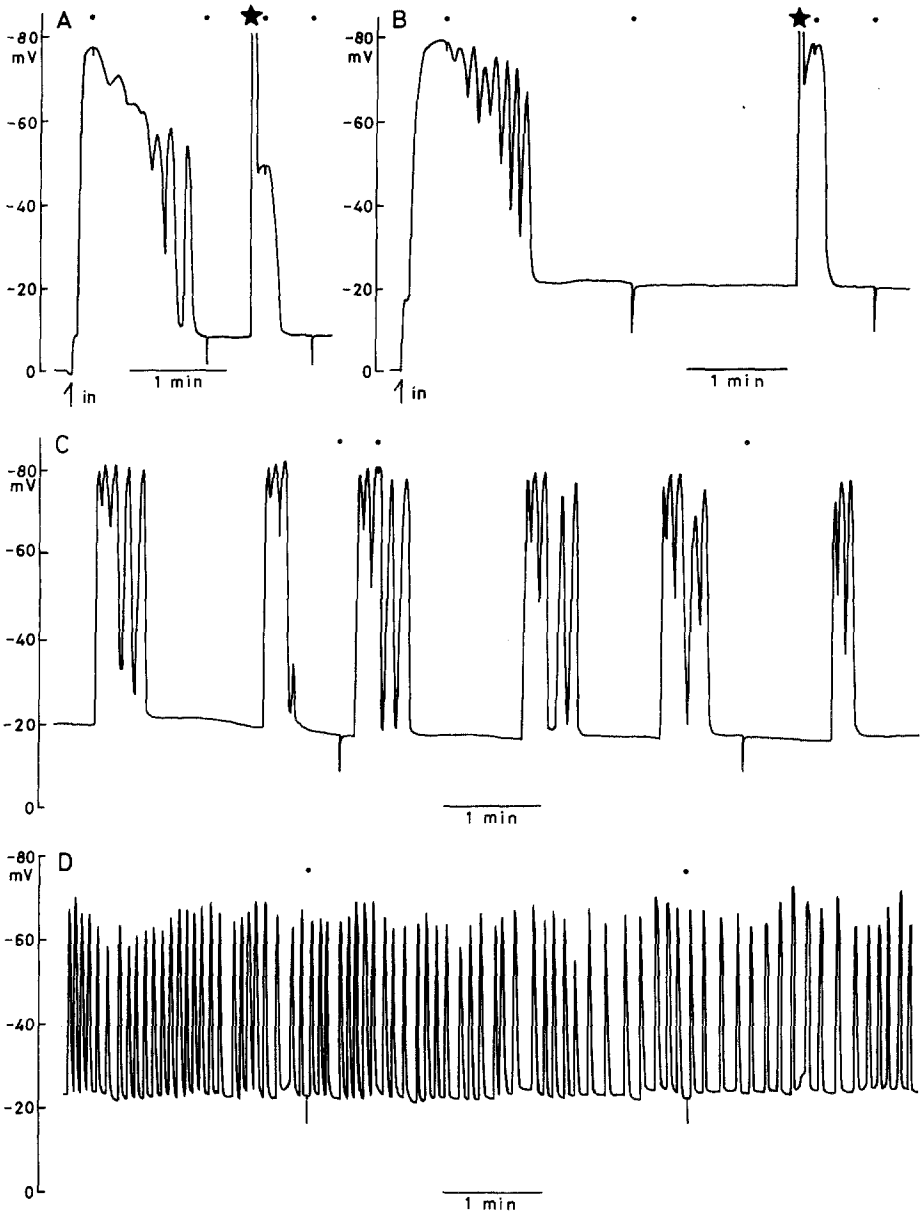

Fig. 2. Membrane potential profiles recorded from an Intestine 407 cell without PEG-treatment (*A*) and from giant Intestine 407 cells after PEG-treatment (*B-D*). The time of entry of the recording microelectrode is indicated by *in* (↑). Dots and closed asterisks indicate the application of outward current (0.3 nA) to measure membrane resistance and that of inward current (14 nA) to stimulate the cell, respectively

induced by electrical stimulation was about −73 mV (Table 1), and at the peak, the membrane resistance decreased down to about 3 MΩ (Table 1).

The cells without PEG treatment also responded with similar HRs to mechanical or electrical stimuli (Fig. 2*A*).

Hyperpolarizing Responses to Several Secretagogues

Acetylcholine (ACh) applied onto the cell surface through a micropipette produced oscillatory HRs in the giant cells (Fig. 4*A*). These HRs were obtained only when the micropipette was filled with ACh over 5×10^{-6} M. More than 80% of the cells exhibited the HRs with a latency of several seconds, when the concentration in the micropipette was 10^{-1} M. This latency seemed to be mainly dependent on the time of diffusion of ACh, since the closer the ACh-containing micropipette to the cell, the shorter was the latency. The most hyperpolarized level of the ACh response was about −84 mV (Table 1) at which the membrane resistance reduced to about 3 MΩ (Table 1). The ACh-induced changes in the membrane potential and resistance were observed not only in the cells in confluent monolayer but in the solitary cells isolated from others. Repeated applications of ACh caused progressive decrements in the amplitude of the HRs. Thus, the cell membrane

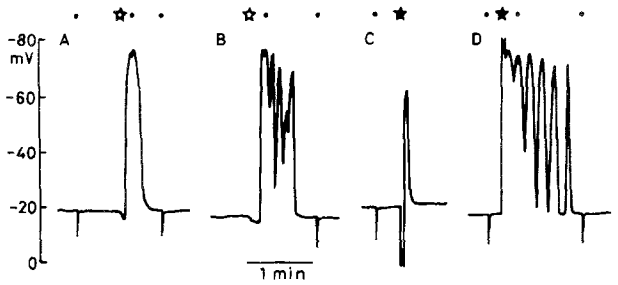

Fig. 3. Hyperpolarizing responses induced by mechanical (*A*, *B*) and electrical stimuli (*C*, *D*) in giant Intestine 407 cells. Open asterisks indicate the time of the application of mechanical stimuli. Closed asterisks indicate the time of the application of outward (*C*) or inward current (*D*) of 14 nA. Dots are the same as in Fig. 2

Table 1. Membrane potential and membrane resistance of giant Intestine 407 cells in the resting and activated states

	Number of observations	Membrane potential (mV)	Membrane resistance (MΩ)
Resting state:	112	−22.1 ± 0.5	21.7 ± 0.9
Activated state:			
oscillation	89	−74.3 ± 0.9	2.6 ± 0.2
mechanical stimulus	9	−73.6 ± 3.4	3.2 ± 0.7
electrical stimulus	51	−73.0 ± 1.2	2.9 ± 0.3
ACh	24	−83.6 ± 1.5	3.0 ± 0.5
histamine	42	−88.4 ± 0.7	2.2 ± 0.2
5-HT	14	−82.9 ± 2.7	3.0 ± 0.4
VIP	11	−83.2 ± 2.8	3.1 ± 0.7

The data presented are the mean ± SE. Mechanical or electrical stimulation was made as described in the text. ACh, histamine, 5-HT, or VIP was administered to the cell surface by the local application described in the text.

became virtually unresponsive (desensitized) after successive 5 to 10 administrations of ACh at an interval of 1 to 2 min (Fig. 4*A*). Carbamylcholine (CCh), a cholinergic agonist, also elicited similar HRs (Fig. 4*C*). A muscarinic receptor antagonist (atropine) completely blocked the ACh or CCh response, while a nicotinic receptor antagonist (curare) did not affect the ACh or CCh response (Figs. 4*B* and *C*). Since the membrane still responded to electrical stimulation (Fig. 4*C*, closed asterisk), the blocking effect of atropine could not be ascribed to nonspecific cell damage. Histamine receptor antagonists (cimetidine and diphenhydramine) did not affect the ACh response (*data not shown*). The bulk application of ACh or CCh produced a rather irregular oscillation which lasted for a long time (usually more than 10 min and sometimes over 1 hr) (Fig. 4*D*), despite the receptor desensitization expected from the results shown in Fig. 4*A*. This fact suggests that continuous receptor stimulation is not responsible for the long-lasting oscillation.

The local application of histamine also induced HR in the giant cells. The pattern of the histamine response was different from that of ACh. The response was usually a single HR with a longer (10 to 60 sec) duration (Fig. 5*A*). Effective concentrations of histamine filled in the micropipette were over 10^{-7} M. More than 90% of cells responded to histamine when the concentration in the micropipette was 10^{-2} M. At the peak of the response, the membrane potential and resistance were about −88 mV and 2 MΩ, respectively (Table 1). The histamine-induced response still occurred after the membrane desensitization to ACh (*data not shown*). Repeated local applications of histamine also made the membrane gradually desensitized to histamine but not to ACh (*data not shown*). It is widely accepted that there are two independent receptors for histamine: H_1 and H_2 receptors (Douglas, 1980). As shown in Fig. 5*A*, diphenhydramine (H_1-antagonist) blocked the HR induced by the local application of histamine (but not that by electrical stimulation), while cimetidine (H_2-antagonist) did not block the histamine response. The bulk application of histamine evoked a sustained hyperpolarization followed by regular long-lasting oscillations of a burst type (Fig. 5*B*). Neither atropine nor curare affected the histamine response (Fig. 4*B*).

The cells without PEG treatment also responded with similar HRs to ACh and histamine (*data not shown*).

The giant cell responded to 5-HT and to VIP administered by the local (Figs. 6*A*, *B*, and *C*) or bulk application (Fig. 6*D*). The membrane resistances decreased markedly during the hyperpolarizations induced by 5-HT and VIP (Table 1). After repeated local applications of 5-HT or VIP the membrane became desensitized (Figs. 6*A* and *B*). The desensitization to 5-HT or VIP did not prevent the VIP-induced or 5-HT-induced response, respectively (Figs. 6*A* and *B*). The 5-HT or VIP response was not affected by a muscarinic antagonist or histamine (H_1) antagonist (Fig. 6*C*). The bulk application of VIP (Fig. 6*D*) or 5-HT brought about a long-lasting oscillation of membrane potential.

The application of calcium ionophore A23187 (10 μg/ml) also induced a slow hyperpolarizing response accompanied by a decrease in the membrane resistance (Fig. 5*C*).

Other intestinal secretagogues such as cholera toxin (1 to 5 μg/ml) and prostaglandin E_1 (100 μM) did not provoke any responses. Dibutyryl cyclic AMP (1 to 3 mM), 8-bromo cyclic AMP (0.1 to 0.2

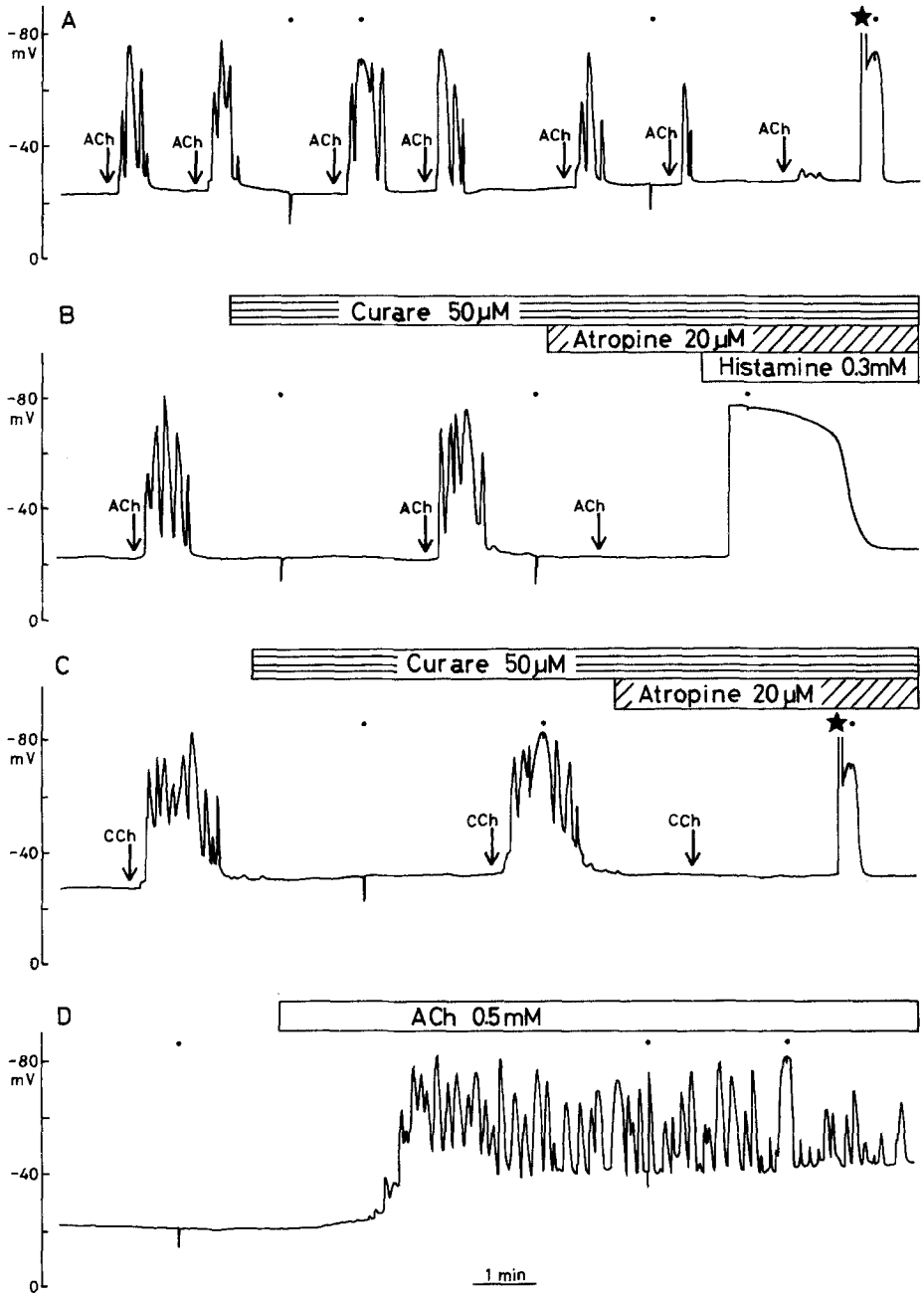

Fig. 4. Hyperpolarizing responses induced by acetylcholine (ACh) or carbamylcholine (CCh) in giant Intestine 407 cells. Arrows and rectangular bars indicate the local application and the bulk application of chemicals, respectively. Dots and closed asterisks are the same as in Fig. 2. *A*) Responses induced by repeated local applications of ACh. Note that the amplitude of the ACh responses became gradually decreased (desensitized), while a full response could still be evoked by electrical stimulation. *B*) Inhibition of the ACh response by atropine (not by curare). Note that neither atropine nor curare blocked the histamine response. *C*) Inhibition of the CCh response by atropine (not by curare). Note that the response induced by electrical stimulation was not impaired by curare and atropine. *D*) Long-lasting oscillation of membrane potential induced by the bulk application of ACh

mM), theophylline (1 to 10 mM) and isobutylmethylxanthine (1 mM) were also ineffective in the generation of electrical responses.

Norepinephrine (1 to 500 μM), isoproterenol (1 to 20 μM), pentagastrin (3 μM) and calcitonin (0.3 μg/ml) did not affect the membrane potentials and resistances.

Neither D-glucose (10 mM) nor D-galactose (10 mM) induced any electrical responses.

Effects of Changes in the External K^+, Na^+ and Cl^- Concentrations

In order to explore the ionic mechanism involved in the HR generation, the extracellular ionic concentrations were changed. A reduction of the external Na^+ concentration to 3 mM had little effect on the membrane potentials in the giant cells (Table 2). The resting potential, the peak level of spontaneous oscillatory HRs, and that of the HR induced by electrical or chemical stimulation were not statistically different from those obtained in the control TBS ($P > 0.05$). On the other hand, as indicated in Table 2, a reduction of the external Cl^- concentration to 30 mM significantly diminished the resting potential to about −12 mV without affecting the peak levels of HRs ($P > 0.05$). This depolarization continued for more than 30 min after reducing the external Cl^-. These results suggest that the cell membrane is, to a certain extent, permeable to Cl^-

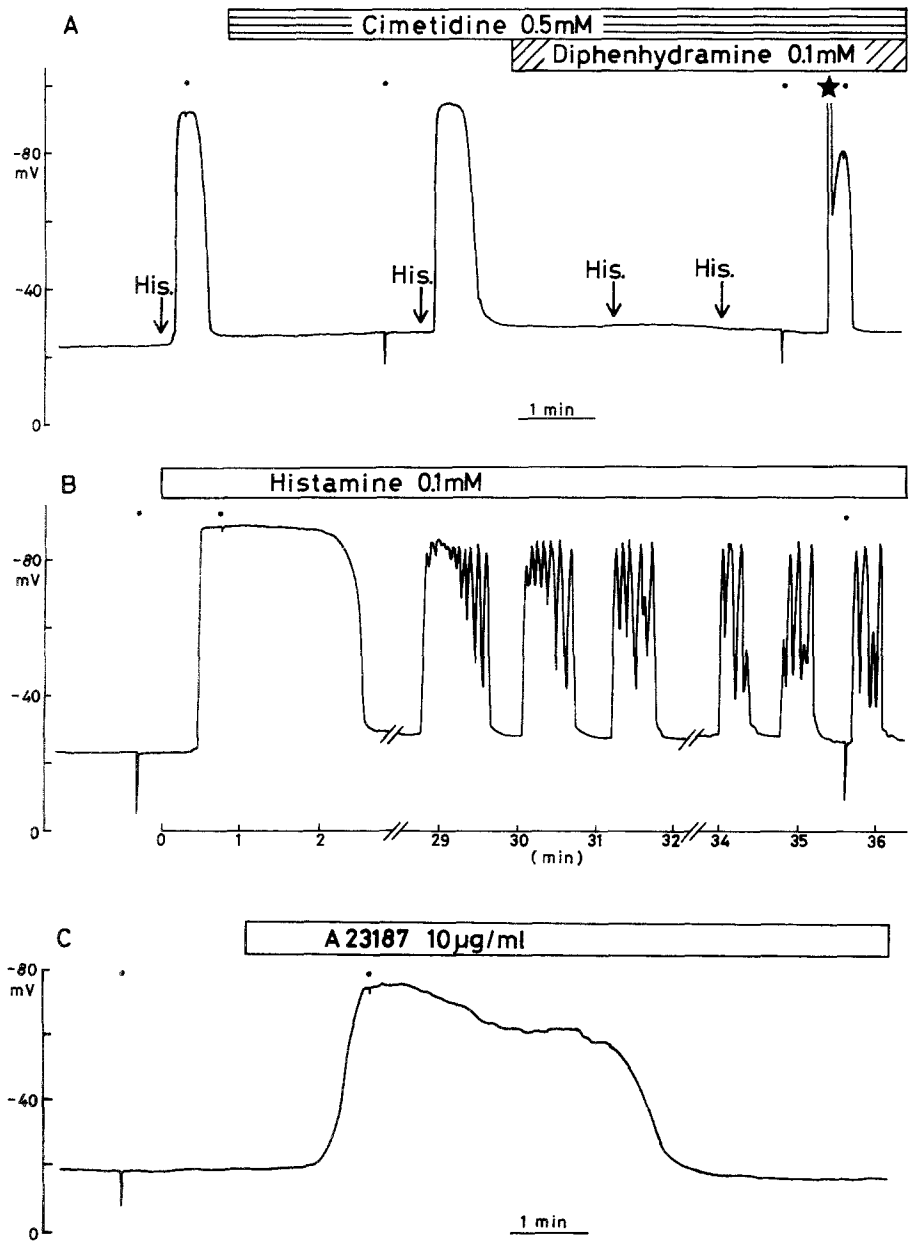

Fig. 5. Hyperpolarizing responses induced by histamine and A23187 in giant Intestine 407 cells. Dots and a closed asterisk are the same as in Fig. 2. Arrows and rectangular bars are the same as in Fig. 4. *A*) Inhibition of the histamine (His.) response by an H_1-antagonist diphenhydramine (not by an H_2-antagonist, cimetidine). Note that the response induced by electrical stimulation was not influenced by the H_1- and H_2-antagonists. *B*) Long-lasting potential oscillation (a burst type) induced by the bulk application of histamine. *C*) Response induced by the bulk application of A23187

Table 2. Effects of external low Na^+ and low Cl^- on the membrane potential in giant Intestine 407 cells

	Control (143.0 mM Na^+, 163.5 mM Cl^-)	3 mM Na^+	30 mM Cl^-
Resting potential (mV):	−22.7 ± 0.7 (36)	−24.8 ± 0.8 (26)	−12.4 ± 0.7[a] (28)
Activated potential (mV):			
oscillation	−71.7 ± 1.3 (30)	−70.9 ± 1.7 (21)	−73.8 ± 1.4 (25)
electrical stimulus	−75.1 ± 1.3 (34)	−74.3 ± 1.5 (26)	−70.5 ± 2.3 (22)
histamine	−86.4 ± 1.1 (32)	−84.5 ± 0.9 (22)	−83.9 ± 0.7 (24)

The data presented are the mean ± SE. Numbers in parentheses indicate number of observations. The electrical stimulation and the local application of histamine to the cell were made as described in the text.

[a] Significantly different from the control value ($P < 0.05$).

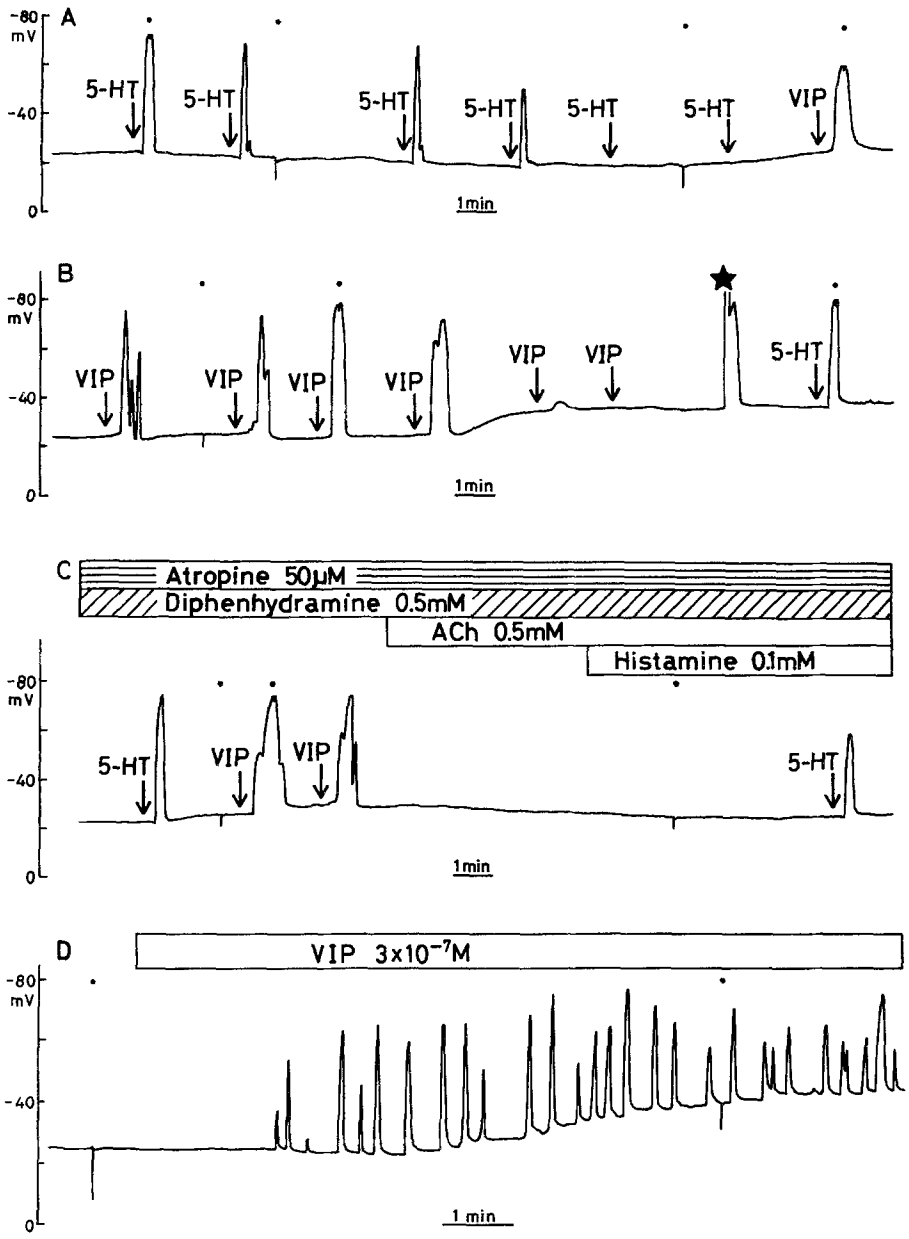

Fig. 6. Hyperpolarizing responses induced by serotonin (5-HT) and by vasoactive intestinal polypeptide (VIP) in giant Intestine 407 cells. Dots, a closed asterisk and arrows as well as rectangular bars are the same as in Fig. 2 and in Fig. 4. *A*) Responses induced by repeated local applications of 5-HT (through a micropipette filled with 0.08 to 0.2 M 5-HT). Note that the membrane still responded to VIP after the complete desensitization to 5-HT. *B*) Responses induced by repeated local applications of VIP (through a micropipette filled with 10^{-4} to 10^{-3} M VIP and 0.15 M NaCl). Note that the membrane fully responded to 5-HT or electrical stimulation after the desensitization to VIP. *C*) Responses induced by local application of 5-HT or VIP in the presence of a muscarinic antagonist and an H_1 antagonist. Note that the 5-HT or VIP response was not affected by these antagonists, whlie the ACh or histamine response was completely inhibited. *D*) Long-lasting oscillation of membrane potential induced by the bulk application of VIP

(but not to Na^+) at the resting state, while the relative permeabilities to both Cl^- and Na^+ are negligible in the activated state. Thus, neither Na^+ nor Cl^- ions are involved in the generation of hyperpolarizing activation.

In contrast, increased K^+ concentrations in the bathing solution affected not only the resting potential but also the peak level of HR. As shown in Fig. 7, the cell was depolarized by increasing the external K^+ concentration ($[K]_o$). The polarity of the responses was reversed at 147 mM $[K]_o$ (Fig. 7, *insets*). The peak potentials of HRs were markedly dependent on the $[K]_o$, varying linearly with the $\log[K]_o$ with a slope of 46 to 57 mV/decade (Fig. 7). Such quasi-Nernst behavior to K^+ of the activated membrane as well as dependence of the polarity on $[K]_o$ strongly suggest that the hyperpolarizing responses are caused solely by an increase in the K^+ conductance.

Reversal Potentials

To test the above hypothesis further, the membrane potential was current-clamped by injecting currents through an additional intracellular electrode. The amplitudes of both the spontaneous oscillatory HRs and HRs evoked by various stimuli were dependent

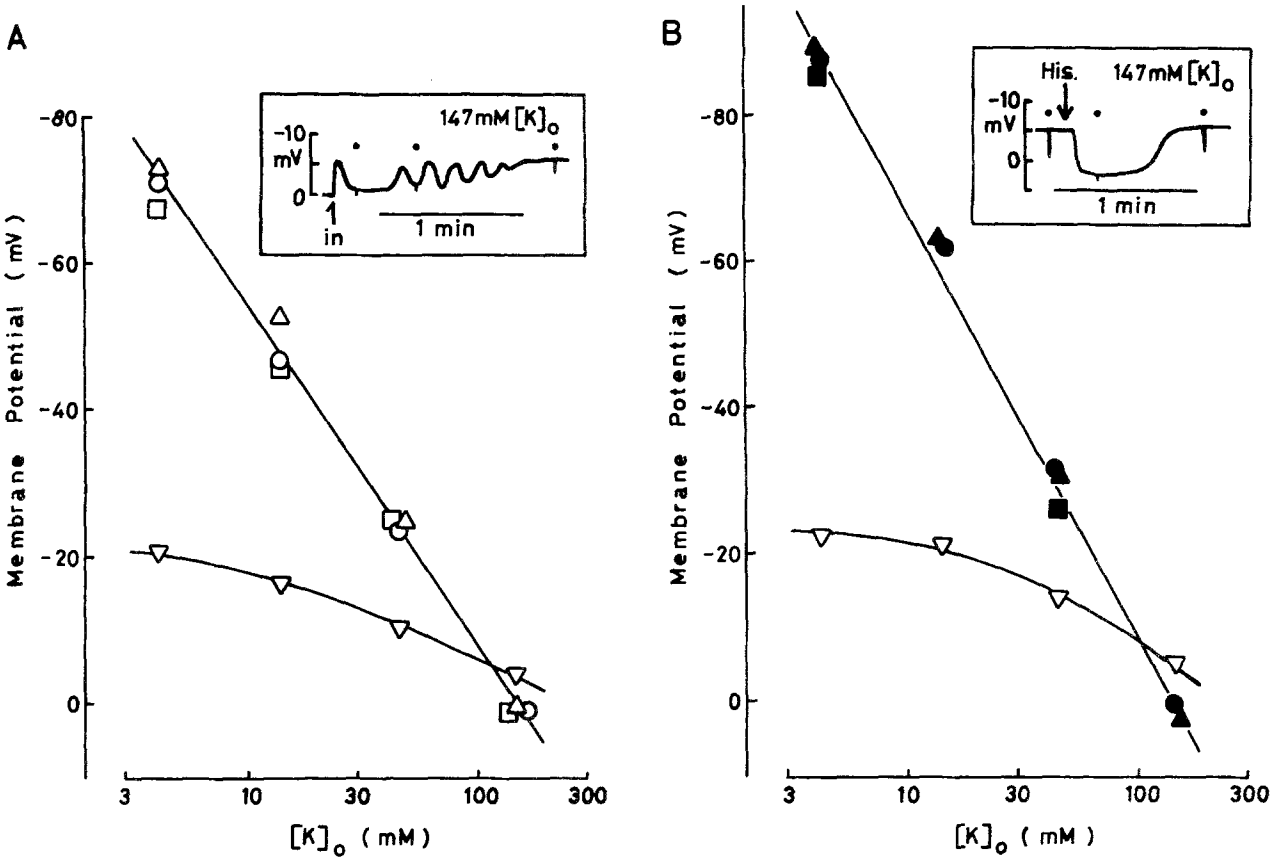

Fig. 7. Effects of changes in the external K^+ concentration ($[K]_o$) on the resting potential and the hyperpolarizing response in giant Intestine 407 cells. ▽, the resting potential; ○, the spontaneously occurring response; △, the response induced by mechanical stimuli; □, the response induced by electrical stimuli; ●, the ACh response; ▲, the histamine response; ■, the VIP response. The administration of secretagogues to the cells was made by the local application. Each point represents the mean value of 3 to 28 observations with its standard error of less than 3.8 mV. *Insets:* Reversed responses at 147 mM $[K]_o$. Dots, *in* (↑) and an arrow are the same as in Fig. 2 and in Fig. 4. *A*) the potential oscillation. *B*) the response induced by the local application of histamine (His.)

on the membrane potential level. The responses were increased by depolarization and decreased by hyperpolarization (Fig. 8). The reversal potentials of HRs were −80 to −90 mV for spontaneous oscillations (Fig. 8*A*) and about −90 mV for the ACh or histamine responses (Fig. 8*B*).

Discussion

In the present study, it was found that the cells responded to acetylcholine, histamine, 5-HT, VIP and A23187 which are all known to be effective secretagogues for the intestinal epithelium (Field, 1981), but not to glucose and galactose which are known to evoke the membrane depolarization in the absorptive intestinal epithelial cells (Rose & Schultz, 1971; White & Armstrong, 1971; Okada, Tsuchiya, Irimajiri & Inouye, 1977*b*). In the light of these observations, it is strongly suggested that the Intestine 407 cells are originated from crypt secretory cells of the intestinal epithelium. Based on the karyologic and enzymologic analysis, it has been suggested that a variety of established epithelial cell lines (including the Intestine 407) may be cross-contaminated by the HeLa cells (Nelson-Rees & Flandermeyer, 1976; Lavappa, 1978). In our studies, however, the HeLa cells (supplied by Dr. K. Izutsu, Mie University) failed to respond to intestinal secretagogues (ACh, 5-HT and VIP) under the same experimental conditions as those employed herein (T. Yada & Y. Okada, *unpublished observations*).

The membrane became gradually desensitized after repeated applications of ACh, histamine, 5-HT and VIP. The membrane desensitization to one of these secretagogues did not prevent the responses to other secretagogues. A muscarinic antagonist selectively inhibited the ACh or CCh response but not the responses induced by the other three secreta-

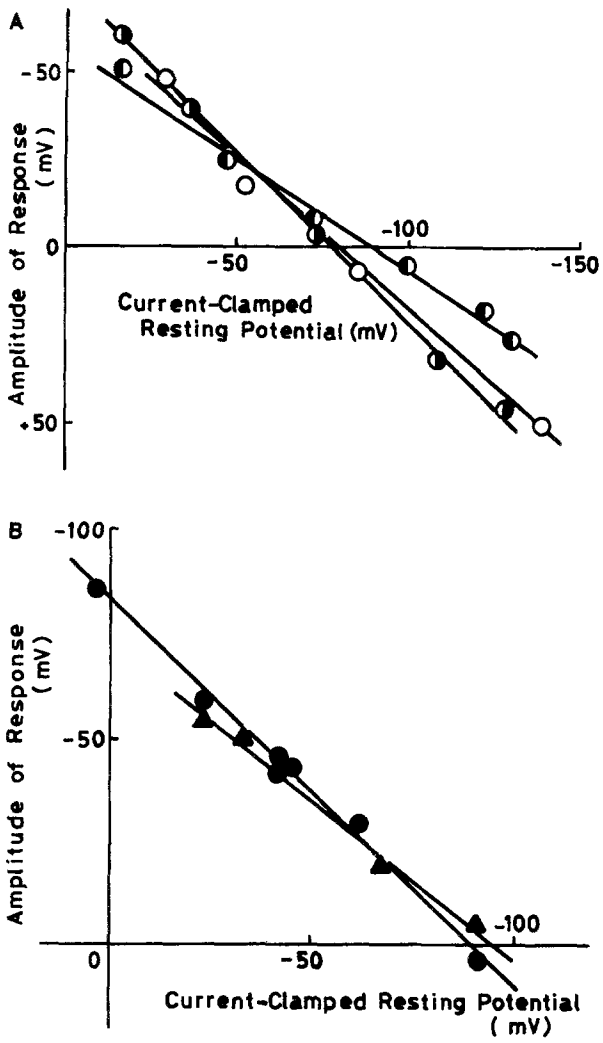

Fig. 8. Reversal potentials of spontaneous oscillations (*A*) and the ACh or histamine response (*B*). ○, ◑ and ◐, oscillations in three different cells; ●, the ACh response; ▲, the histamine response. The secretagogues were administered to the cells by the local application

gogues. Similarly, an H_1-receptor antagonist blocked specifically the histamine response. From these results it can be concluded that the electrical membrane activation in response to ACh, histamine, 5-HT or VIP is mediated by its own specific receptors. The receptors for these secretagogues normally exist only in the basolateral surface of the cells. The cells cultured on the plastic substrate would have lost their polarity, thereby providing the accessibility of the agents to the receptors. At present, there is no evidence for the actual secretion of ions or fluid by this cell. No morphological alterations were observed upon exposure to these secretagogues under a light microscope. However, in the small intestine *in vivo*, no alterations in cell morphology have been observed in secretagogue-treated cells (Donowitz, Charney & Heffernan, 1977; Krejs, Barkley, Read & Fordtran, 1978). At least, the present results indicate that Intestine 407 cells retain receptors to several intestinal secretagogues.

The secretory effect of acetylcholine has been reported to be mediated by the elevated level of intracellular Ca^{2+} (Bolton & Field, 1977; Field, 1981). It is generally accepted that the stimulation of H_1-receptor is coupled to an increased mobilization of intracellular Ca^{2+} ions, while the activation of adenylate cyclase is associated with the H_2-receptor stimulation (Schwartz, 1979; Douglas, 1980). The H_1-receptor (but not H_2-receptor) is known to be involved in the intestinal ion secretion evoked by histamine (Linaker, McKay, Higgs & Turnberg, 1981). Intestine 407 cells were, in fact, found to respond to stimulation of H_1- (not H_2-) receptor in the present study. In addition, it has been reported that the 5-HT-induced stimulation of intestinal chloride secretion is mediated by the increased Ca^{2+} ions within the cells (Donowitz, Asarkof & Pike, 1980). Taken together, it is conceivable that the intestinal secretagogues mediated by cellular Ca^{2+} mobilization can evoke the hyperpolarizing responses in Intestine 407 cells. Supporting this concept, the application of A23187, which is also a Ca^{2+}-mediated intestinal secretagogue (Bolton & Field, 1977; Frizzell, 1977; Ilundain & Naftalin, 1979), induced the hyperpolarizing response (Fig. 5*C*).

Contrary to our initial expectation, the cells did not respond to cholera toxin and prostaglandin E_1. It is inferred that these secretagogues exert their action in the intestinal mucosa via an increment of intracellular cyclic AMP level (Kimberg et al., 1971; Kimberg, Field, Gershon & Henderson, 1974). In contrast, the cells responded to VIP which has also long been thought to be mediated via cyclic AMP production in the intestinal mucosa (Schwartz et al., 1974; Gaginella, Phillips, Dozois & Go, 1978). However, no increase in cyclic AMP level by the VIP administration was observed in the rabbit small intestine *in vivo*, while the fluid secretion was significantly stimulated (Camilleri, Cooper, Bloom & Chadwick, 1979). At any rate, it appears that the increase in intracellular cyclic AMP is, if anything, not a causal factor for the VIP-induced responses in Intestine 407 cells, because the application of cyclic AMP analogs (dibutyryl cyclic AMP and 8-bromo cyclic AMP) or phosphodiesterase inhibitors (theophylline and isobutylmethylxanthine) failed to evoke the electrical membrane responses.

The hyperpolarizing responses were also evoked by mechanical or electrical stimuli in Intes-

tine 407 cells. The physiological significance of these responses remains unclear. However, those evoked mechanically are presumably related to some secretory processes in intestinal epithelial cells, since mechanical irritation of the intestinal mucosa or movement of the luminal contents is one of the regulatory factors for the intestinal secretion (Florey, Wright & Jennings, 1941).

Another important aspect of the electrophysiological properties of Intestine 407 cells is oscillations of membrane potential. Since mechanical stimulation evoked a single or only several HRs, it is unlikely that the long-lasting potential oscillation is artifactually caused by the impalement of a microelectrode. Since Intestine 407 cells showed electrical cell-to-cell coupling (T. Yada & Y. Okada, *unpublished observations*), it is possible that mutual transfer of hyperpolarizing responses among the coupled cells is responsible for the oscillation. However, the oscillation was obtained even in a solitary cell which had no contact with neighboring cells. Therefore, it is suggested that the potential oscillation is an event intrinsic to the cell. Incubation of the cells with relatively high doses of intestinal secretagogues (e.g., acetylcholine, Fig. 4*D;* histamine, Fig. 5*B;* VIP, Fig. 6*D*) prominently enhanced the spontaneous electrical activities or induced a long-lasting oscillation of membrane potential. Thus, there is a possibility that the spontaneous potential oscillation is associated with the basal secretory activity of the cell.

The marked $[K]_o$ dependency of the activated potential (Fig. 7) suggested that these hyperpolarizing responses are due to an increase in the K^+ conductance. The measurements of the reversal potential (Fig. 8) supported this inference, since those values (−80 to −90 mV) were very close to the equilibrium K^+ potential obtained in the Intestine 407 cells by means of K^+-selective microelectrodes (−89 mV: S. Oiki, S. Ueda, T. Yada & Y. Okada, *unpublished observations*). The functional significance of the K^+ conductance increase *per se* has not been elucidated. However, it is worthwhile to note that Ca^{2+}-mediated secretagogues have similarly been found to cause the K^+ permeability increase in gastric parietal cells in primary culture (Ueda, Miyake, Uchino & Okada, 1982; Ueda et al., 1983) and in the salivary gland cells (Roberts, Iwatsuki & Petersen, 1978; Ginsborg, House & Mitchell, 1980; Petersen, 1981; Putney & Weiss, 1981).

The authors are grateful to Professor M. Kuno for critical reading of this manuscript. Thanks are due to Dr. S. Ueda for discussion. This work was supported by Grant-in-Aid (nos. 5670029 and 57880038) from the Ministry of Education, Science and Culture.

References

Bolton, J.E., Field, M. 1977. Ca ionophore-stimulated ion secretion in rabbit ileal mucosa: Relation to actions of cyclic 3',5'-AMP and carbamylcholine. *J. Membrane Biol.* **35:**159–173

Camilleri, M., Cooper, B.T., Bloom, S.R., Chadwick, V.S. 1979. Effects of vasoactive intestinal peptide on jejunal, ileal, and colonic fluid transport and cAMP levels in the rabbit. *Gut* **20:**A439

Donowitz, M., Asarkof, N., Pike, G. 1980. Calcium dependence of serotonin-induced changes in rabbit ileal electrolyte transport. *J. Clin. Invest.* **66:**341–352

Donowitz, M., Charney, A.N., Heffernan, J.M. 1977. Effect of serotonin treatment on intestinal transport in the rabbit. *Am. J. Physiol.* **232:**E85–E94

Douglas, W.W. 1980. Histamine and 5-hydroxytryptamine (serotonin) and their antagonists. *In:* The Pharmacological Basis of Therapeutics. A.G. Gilman, L.S. Goodman and A. Gilman, editors. pp. 609–646. MacMillan, New York

Florey, H.W., Wright, R.D., Jennings, M.A. 1941. The secretions of the intestine. *Physiol. Rev.* **21:**36–69

Field, M. 1981. Secretion of electrolytes and water by mammalian small intestine. *In:* Physiology of the Gastrointestinal Tract. L.R. Johnson, editor. pp. 963–982. Raven, New York

Frizzell, R.A. 1977. Active chloride secretion by rabbit colon: Calcium-dependent stimulation by ionophore A23187. *J. Membrane Biol.* **35:**175–187

Gaginella, T.S., Phillips, S.F., Dozois, R.R., Go, V.L.W. 1978. Stimulation of adenylate cyclase in homogenates of isolated intestinal epithelial cells from hamsters. Effects of gastrointestinal hormones, prostaglandins, and deoxycholic and ricinoleic acids. *Gastroenterology* **74:**11–15

Ginsborg, B.L., House, C.R., Mitchell, M.R. 1980. On the role of calcium in the electrical responses of cockroach salivary gland cells to dopamine. *J. Physiol. (London)* **303:**325–335

Grand, R.J., Watkins, J.B., Torti, F.M. 1976. Development of the human gastrointestinal tract. *Gastroenterology* **70:**790–810

Henle, G., Deinhardt, F. 1957. The establishment of strains of human cells in tissue culture. *J. Immunol.* **79:**54–59

Ilundain, A., Naftalin, R.J. 1979. Role of Ca^{2+}-dependent regulator protein in intestinal secretion. *Nature (London)* **279:**446–448

Kimberg, D.V., Field, M., Gershon, E., Henderson, A. 1974. Effects of prostaglandins and cholera enterotoxin on intestinal mucosal cyclic AMP accumulation. *J. Clin. Invest.* **53:**941–949

Kimberg, D.V., Field, M., Johnson, J., Henderson, A., Gershon, E. 1971. Stimulation of intestinal mucosal adenyl cyclase by cholera enterotoxin and prostaglandins. *J. Clin. Invest.* **50:**1218–1230

Krejs, G.J., Barkley, R.M., Read, N.W., Fordtran, J.S. 1978. Intestinal secretion induced by vasoactive intestinal polypeptide. A comparison with cholera toxin in the canine jejunum in vivo. *J. Clin. Invest.* **61:**1337–1345

Lavappa, K.S. 1978. Survey of ATCC stocks of human cell lines for HeLa contamination. *In Vitro* **14:**469–475

Leblond, C.P., Messier, B. 1958. Renewal of chief cells and goblet cells in the small intestine as shown by radioautography after injection of thymidine-H^3 into mice. *Anat. Rec.* **132:**247–259

Linaker, B.D., McKay, J.S., Higgs, N.B., Turnberg, L.A. 1981. Mechanisms of histamine stimulated secretion in rabbit ileal mucosa. *Gut* **22:**964–970

Nelson-Rees, W.A., Flandermeyer, R.R. 1976. HeLa cultures defined. *Science* **191:**96–98

Okada, Y., Doida, Y., Roy, G., Tsuchiya, W., Inouye, K., Inouye, A. 1977*a*. Oscillations of membrane potential in L cells: I. Basic characteristics. *J. Membrane Biol.* **35:**319–335

Okada, Y., Tsuchiya, W., Irimajiri, A., Inouye, A. 1977*b*. Electrical properties and active solute transport in rat small intestine. I. Potential profile changes associated with sugar and amino acid transports. *J. Membrane Biol.* **31:**205–219

Petersen, O.H. 1981. Electrophysiology of exocrine gland cells. *In:* Physiology of the Gastrointestinal Tract. L.R. Johnson, editor. pp. 749–772. Raven, New York

Putney, J.W., Jr., Weiss, S.J. 1981. Relationship between receptors, calcium channels, and responses in exocrine gland cells. *In:* Methods in Cell Biology. A.R. Hand and C. Oliver, editors. Vol. 23, pp. 503–511. Academic, New York

Quaroni, A., Wands, J., Trelstad, R.L., Isselbacher, K.J. 1979. Epithelioid cell cultures from rat small intestine. Characterization by morphologic and immunologic criteria. *J. Cell Biol.* **80:**248–265

Roberts, M.L., Iwatsuki, N., Petersen, O.H. 1978. Parotid acinar cells: Ionic dependence of acetylcholine-evoked membrane potential changes. *Pfluegers Arch.* **376:**159–167

Rose, R.C., Schultz, S.G. 1971. Studies on the electrical potential profile across rabbit ileum. Effects of sugars and amino acids on transmural and transmucosal electrical potential differences. *J. Gen. Physiol.* **57:**639–663

Schwartz, J.-C. 1979. Histamine receptors in brain. *Life Sci.* **25:**895–912

Schwartz, C.J., Kimberg, D.V., Sheerin, H.E., Field, M., Said, S.I. 1974. Vasoactive intestinal peptide stimulation of adenylate cyclase and active electrolyte secretion in intestinal mucosa. *J. Clin. Invest.* **54:**536–544

Ueda, S., Miyake, T., Uchino, H., Okada, Y. 1982. Membrane potential profiles in cultured gastric parietal cell: Electrical responses to secretagogues. *Cell Struct. Funct.* **7:**390

Ueda, S., Miyake, T., Uchino, H., Yada, T., Okada, Y. 1983. Electrophysiological study of gastric epithelial cell in tissue culture. *In:* Gut Peptides. A. Miyoshi, editor. Biomedical Research Foundation, Tokyo (*in press*)

Welsh, M.J., Smith, P.L., Fromm, M., Frizzell, R.A. 1982. Crypts are the site of intestinal fluid and electrolyte secretion. *Science* **218:**1219–1221

White, J.F., Armstrong, W. McD. 1971. Effect of transported solutes on membrane potentials in bullfrog small intestine. *Am. J. Physiol.* **221:**194–201

Yada, T., Okada, Y. 1980. Hyperpolarizing responses and oscillations of membrane potential in cultured epithelial cells. *Cell Struct. Funct.* **5:**423 (*Abstr.*)

Yada, T., Okada, Y. 1982. Electrical membrane responses to acetylcholine in cultured epithelial cells (Intestine 407). *J. Physiol. Soc. Jpn.* **44:**346 (*Abstr.*)

Received 26 January 1983; revised 31 May 1983