Endogenous Arachidonic Acid Inhibits Hypotonically-Activated Cl^- Channels in Isolated Rat Hepatocytes

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Abstract: Properties of hypotonically-activated Cl^- channels in isolated rat hepatocytes were studied by the patch-clamp whole-cell technique. Hypotonic stress ($140\text{--}150\text{ mosmol kg}^{-1}\text{ H}_2\text{O}$) induced a hyperpolarization of the membrane of hepatocytes in the presence of an inwardly oriented Cl^- gradient, but had no effect on the membrane potential in the absence of Cl^- . An increase in the hypotonically-induced conductance was significantly inhibited by 4-acetamido-4'-isothiocyanatostilbene-2,2'-disulfonic acid (SITS; $50\text{ }\mu\text{M}$), but not by Ba^{2+} (1 mM). Pre-incubation with arachidonic acid ($20\text{ }\mu\text{M}$) significantly inhibited the hypotonically-activated conductance. The combined application of a cyclo-oxygenase inhibitor, indomethacin ($50\text{ }\mu\text{M}$) and a lipoxygenase

inhibitor, esculetin ($100\text{ }\mu\text{M}$) also inhibited the conductance, whereas quinacrine ($200\text{ }\mu\text{M}$), a phospholipase A_2 inhibitor, significantly induced a large steady conductance. Outward Cl^- currents, but not cationic currents, were elicited by the hypotonic stress. The current did not show any rapid time-dependent inactivation during the voltage clamp of 0.1 s . The combined application of arachidonic acid, indomethacin and esculetin inhibited the hypotonically-activated Cl^- currents. The present study has shown that the Cl^- channel is activated by exposure to hypotonic stress and closed by an increase in arachidonic acid concentration in isolated rat hepatocytes. [Japanese Journal of Physiology, 46, 311–318, 1996]

Key words: rat hepatocyte, Cl^- channel, arachidonic acid.

Cell swelling of hepatocytes elicits a mechanism of regulatory volume decrease (RVD) which is associated with activation of both K^+ and Cl^- conductances. Activation of the K^+ conductance hyperpolarizes the membrane potential of hepatocytes, which was followed by activation of a Cl^- conductance (for reviews see [1–3]). Effects of several channel blockers on these conductances in rat hepatocytes were reported; the K^+ conductance was inhibited by Ba^{2+} [4–6], quinine [4, 6] and quinidine [5], and the Cl^- conductance by 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS [7]) and 4-acetamido-4'-isothiocyanatostilbene-2,2'-disulfonic acid (SITS [5]). Physiological regulators for these hepatocyte conductances, however, are unknown at present. Although volume-sensi-

tive whole-cell K^+ currents with a linear current-voltage relationship were recorded in guinea-pig hepatocytes [8], electrophysiological properties of the swelling-induced Cl^- channel in mammalian hepatocytes have not been reported so far. In the present study, we therefore investigated the hypotonically-activated Cl^- channel of rat hepatocytes under the patch-clamp whole-cell configuration.

In Ehrlich ascites tumor cells, Lambert [9] found that arachidonic acid inhibited RVD and leukotriene D_4 accelerated RVD. Subsequently, arachidonic acid was shown to inhibit swelling-induced Cl^- channels in cultured Intestine 407 cells derived from human small intestine epithelia [10] and the HSG cell line cloned from human submandibular glands [11]. In ad-

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dition, leukotriene D₄ was shown to activate the Cl⁻ channels in isolated crypts from the rat distal colon [12, 13]. Therefore, we tested effects of arachidonic acid-related compounds on the hypotonically-activated Cl⁻ channel in rat hepatocytes.

METHODS

Chemicals. Quinacrine dihydrochloride was obtained from Sigma (St. Louis, MO, USA). O,O'-bis(2-aminophenyl)ethyleneglycol-N,N,N',N'-tetraacetic acid (BAPTA) was from Dojindo Laboratories Co. (Kumamoto, Japan). 4-Acetamido-4'-isothiocyanato-stilbene-2,2'-disulfonic acid (SITS; Sigma) and esculetin (Extrasynthèse, Genay, France) were dissolved in dimethyl sulfoxide. Arachidonic acid (Funakoshi Co., Tokyo, Japan) and leukotriene D₄ (Cayman Chemical Co., Ann Arbor, MI, USA) were kept in ethanol at -20 and -85°C, respectively. Indomethacin (Wako Pure Chemical Industries, Osaka, Japan) was kept at +4°C, and dissolved in ethanol before use. All these chemicals in either dimethyl sulfoxide or ethanol were diluted to the required concentrations with the appropriate bathing solution just before use. Dimethyl sulfoxide and ethanol concentrations in the bathing solutions never exceeded 0.5%. At this concentration the vehicles per se did not affect cell membrane potentials and whole-cell Cl⁻ currents of hepatocytes.

Solution. Modified Krebs-Henseleit solution for cell isolation contained (in mM): 120 NaCl, 24 NaHCO₃, 4.8 KCl, 1.2 KH₂PO₄, 1.3 CaCl₂, 1.2 MgSO₄, and 5 glucose. The solution was gassed with carbogen (95% O₂-5% CO₂). Whole-cell recordings were carried out with the following solutions. The 133 K⁺-13 Cl⁻ or 133 K⁺-0 Cl⁻ pipette solution contained (in mM): 133 potassium aspartate, 7 NaCl (or sodium aspartate), and 3 MgCl₂ (or MgSO₄). The 0 K⁺-15 Cl⁻ pipette solution contained (in mM): 87 Cs₂SO₄, 9 CsCl, and 3 MgCl₂. All pipette solutions contained 10 mM HEPES, 0.1 mM EGTA, 62 μM CaCl₂, and 2 mM ATP (Oriental Yeast Co., Tokyo, Japan) and were adjusted to pH 7.3 with KOH or Tris. The free Ca²⁺ concentration of the pipette solutions was calculated to be 0.1 μM. The 5 K⁺-80 Cl⁻ or 5 K⁺-0 Cl⁻ isotonic bathing solution contained (in mM): 70 NaCl (or sodium aspartate), 5.4 KCl (or potassium aspartate), 1.25 CaCl₂ (or CaSO₄), 1 MgCl₂ (or MgSO₄), and 140 mannitol. The 0 K⁺-80 Cl⁻ isotonic solution contained (in mM): 75.4 CsCl, 1.25 CaCl₂, 1 MgCl₂, and 140 mannitol. When hypotonic bathing solutions were required, mannitol was omitted from the isotonic solutions. All bathing solutions were supplemented with

10 mM HEPES and 0.1% bovine serum albumin, and adjusted to pH 7.4 with NaOH or Tris. Osmolality of the isotonic and hypotonic bathing solutions were measured with a SMO-1 semi-microosmometer (Dr. Ing. Herbert Knauer GmbH., Berlin, Germany) and determined to be 275–285 and 140–150 mosmol kg⁻¹ H₂O, respectively.

Preparation of isolated hepatocytes. Isolated hepatocytes were prepared from male Sprague-Dawley rats (Japan SLC, Shizuoka, Japan) weighing 180–260 g, as described elsewhere [14]. Briefly, animals were anesthetized by intraperitoneal administration of pentobarbital sodium (50 mg kg⁻¹, Abbott Lab., North Chicago, IL, USA). The liver was digested by portal infusion of the modified Krebs-Henseleit solution supplemented with 100 U ml⁻¹ of collagenase (Wako Pure Chemical Industries) and 1% bovine serum albumin for 7–9 min at 37°C. The perfusing rate was 25–30 ml min⁻¹. The cell viability was determined to be >90% by the trypan blue exclusion test. Isolated cells (1.5 × 10⁵ cells ml⁻¹), which were suspended in modified Leibovitz L-15 medium (ICN Biomedicals, Costa Mesa, CA, USA) supplemented with 3 mM glutamine, 50 U ml⁻¹ penicillin, 50 μg ml⁻¹ streptomycin, and 10% fetal bovine serum were placed on glass coverslips. Then, the cells were incubated for 2–6 h at 37°C.

Whole-cell recordings. The incubated hepatocytes attached to a glass coverslip were gently rinsed with a NaCl-rich solution containing 140 mM NaCl, 5.4 mM KCl, 1.25 mM CaCl₂, 1 mM MgCl₂, and 10 mM HEPES (pH 7.4). The coverslip was mounted in an experimental chamber (designed in this laboratory), and the volume of the chamber was 680 μl. Then the NaCl-rich solution was perfused in the chamber at the rate of 800 μl min⁻¹. Whole-cell recordings of isolated hepatocytes were performed using an EPC-9 patch clamp system (HEKA elektronik Dr.-Ing. P. Schulze GmbH., Lambrecht, Germany) as previously described [15–17]. The resistance of the patch electrode filled with pipette solutions was about 6 MΩ. The liquid junction potential between the pipette and bathing solutions was experimentally determined, and corrected. After the whole-cell configuration was achieved, the NaCl-rich solution was switched to the appropriate bathing solution. The cell resistance was 0.2–0.5 GΩ in the configuration. Series resistance and capacitance were compensated electronically as best as possible. The membrane potential was recorded at the current-clamp mode. To obtain the current-voltage relationship, the cell was clamped at -120 mV and command pulses (-110 to 60 mV, voltage jump by 10 mV, duration of 100 ms) were delivered using

the PULSE+PULSEFIT program (HEKA, installed on a Macintosh Quadra 700); resulting currents were stored on a computer. Experiments were performed at room temperature ($25 \pm 2^\circ\text{C}$) using an upright microscope (Axioskop FS, Carl Zeiss, Oberkochen, Germany) with Nomarski optics ($\times 40$).

Statistics. Statistical significance was analyzed with Student's *t*-test or a paired *t*-test. The *p* value below 0.05 was considered to be significant. Data were expressed as mean $\pm$ SEM.

RESULTS

First, the cell membrane potential of hepatocytes (V_m) was recorded at the current clamp mode with the $133\text{K}^+ - 13\text{Cl}^-$ pipette and the $5\text{K}^+ - 80\text{Cl}^-$ bathing solutions. Replacement of the bathing solution with the mannitol-free bathing solution caused a transient hyperpolarization, i.e. the hypotonic stress induced a transient hyperpolarization of $7.0 \pm 0.6\text{ mV}$ ($n=16$; Fig. 1A). Return to the isotonic bathing condition

Fig. 1. Effects of Cl^- concentrations on the change of the rat hepatocyte membrane potential induced by hypotonic stress. A,B: Typical traces of the membrane potential (V_m). Whole-cell configuration with a pipette containing the $133\text{K}^+ - 13\text{Cl}^-$ (A) or $133\text{K}^+ - 0\text{Cl}^-$ (B) solution was achieved in isolated hepatocytes bathed in the $5\text{K}^+ - 80\text{Cl}^-$ (A) or $5\text{K}^+ - 0\text{Cl}^-$ (B) isotonic solution. V_m was continuously recorded at the current-clamp mode. When indicated "isotonic," the isotonic bathing solution was perfused. During the period indicated "hypotonic," the corresponding hypotonic bathing solution (see METHODS) was perfused. The data represent a typical example from 4 (B) or 5 (A) similar experiments.

Fig. 2. Effects of K^+ and Cl^- channel blockers on the hypotonically-induced change of the membrane potential. A: Representative traces of membrane potential (V_m) of hepatocytes recorded with the $133\text{K}^+ - 13\text{Cl}^-$ pipette solution and the $5\text{K}^+ - 80\text{Cl}^-$ isotonic or hypotonic bathing solution. When indicated "hypotonic," the hypotonic solution was perfused. The bathing solution contained $50\text{ }\mu\text{M}$ SITS (b) or 1 mM Ba^{2+} (c). B: Maximal effects of the hyperpolarization (ΔV_m) obtained 60–70 s after the hypotonic challenge were measured in the absence (a) and presence of $50\text{ }\mu\text{M}$ SITS (b) or 1 mM Ba^{2+} (c). Experimental conditions were the same as those in A. The data are expressed as mean $\pm$ SEM from 6–8 experiments. ** Significantly different from the value of a ($p < 0.01$). NS, not significantly different from a ($p > 0.05$).

after the hypotonic stress caused a transient depolarization of 9.4 ± 1.8 mV ($n=5$; Fig. 1A). Thereafter, the V_m returned to the same level before the hypotonic stress (Fig. 1A). These changes of V_m disappeared when the Cl^- in solutions was replaced by aspartate ($n=4$; Fig. 1B). The V_m values before the hypotonic stress under both conditions were -30.8 ± 1.3 ($n=16$) and -22.8 ± 1.2 mV ($n=6$), respectively. These values almost agreed with the values of -30.1 and -23.8 mV, respectively, calculated based on the Hodgkin-Katz-Goldman equation [18] assuming that the ratio of permeability coefficients for K^+ , Na^+ , and Cl^- ($P_{\text{K}^+}:P_{\text{Na}^+}:P_{\text{Cl}^-}$) was 1:0.7:1. In the present experiments, Na^+ probably permeated through cation selective channels which discriminated poorly between Na^+ and K^+ as previously reported in isolated rat hepatocytes [19].

The hypotonically-induced hyperpolarization was significantly inhibited by $50 \mu\text{M}$ SITS (Fig. 2A-b and B), a blocker of Cl^- conductance in rat hepatocytes [5], whereas a significant effect of Ba^{2+} (1 mM), a blocker of K^+ conductance in hepatocytes [4–6], was not observed (Fig. 2A-c and B). These results suggest that the hypotonic stress increased the Cl^- conductance under the present experimental conditions. Both SITS and Ba^{2+} did not significantly affect the resting membrane potential (Fig. 3).

Fig. 3. Effects of K^+ and Cl^- channel blockers and arachidonic acid-related compounds on the resting membrane potential. The membrane potential of hepatocytes (V_m) was recorded with the $133\text{K}^+-13\text{Cl}^-$ pipette solution and the $5\text{K}^+-80\text{Cl}^-$ isotonic bathing solution in the absence (control) and presence of $50 \mu\text{M}$ SITS, 1 mM Ba^{2+} , $20 \mu\text{M}$ arachidonic acid, $50 \mu\text{M}$ indomethacin plus $100 \mu\text{M}$ esculetin, or $200 \mu\text{M}$ quinacrine. The data are expressed as mean $\pm$ SEM from 4–6 experiments. NS, not significantly different from control ($p>0.05$).

We therefore checked whether the hypotonic stress increased whole-cell Cl^- currents in rat hepatocytes by using the $0\text{K}^+-15\text{Cl}^-$ pipette and the $0\text{K}^+-80\text{Cl}^-$ bathing solutions. K^+ and Na^+ were replaced by Cs^+ to avoid an interference with cationic currents. In Fig. 4, outward Cl^- currents were significantly elicited by the hypotonic stress, while the increase of inward currents was not significant. The reversal potential of the increased current was -46.1 ± 5.0 mV (Fig. 4B, inset), which was not significantly different ($p>0.05$) from the theoretical Nernst potential for Cl^- at 25°C (-42.9 mV). In contrast, the hypotonic stress did not increase whole-cell cationic currents recorded under the Cl^- -free conditions, i.e. with the $133\text{K}^+-0\text{Cl}^-$ pipette and the $5\text{K}^+-0\text{Cl}^-$ bathing solutions (Fig. 5).

Fig. 4. Whole-cell Cl^- currents elicited by hypotonic stress. **A:** Whole-cell Cl^- currents were recorded from a hepatocyte equilibrated with the $0\text{K}^+-15\text{Cl}^-$ pipette solution and the $0\text{K}^+-80\text{Cl}^-$ isotonic or hypotonic bathing solution. Typical current traces with the isotonic (left) and hypotonic bathing solutions (70 s after the perfusion, right) are shown. Membrane potential (V_m) was changed by a step of 10 mV from the holding potential (at -120 mV). **B:** The relationship between the Cl^- current (I) and V_m . The experimental protocol was the same as that in A. The currents were measured 70 ms after application of voltage steps. The I - V_m relations with the isotonic ($\bullet$) and hypotonic ($\circ$) bathing solutions are shown. The data are mean $\pm$ SEM from 5 experiments. * and ** Significantly different from the value of the isotonic solution ($p<0.05$ and 0.01 , respectively). **Inset:** The hypotonically-induced current ($\blacksquare$), determined as the difference between currents with the isotonic and hypotonic bathing solutions.

Fig. 5. Effect of the hypotonic stress on whole-cell cationic currents. **A:** Whole-cell cationic currents were recorded from a hepatocyte equilibrated with the $133\text{K}^+-0\text{Cl}^-$ pipette solution and the $5\text{K}^+-0\text{Cl}^-$ isotonic or hypotonic bathing solution. Typical current traces with the isotonic (left) and hypotonic bathing solutions (70 s after the perfusion, right) are shown. Membrane potential (V_m) was changed by a step of 10 mV from the holding potential (at -120mV). **B:** The relationship between the cationic current (I) and V_m . The experimental protocol was the same as that in A. The currents were measured 70 ms after application of voltage steps. The I - V_m relations with the isotonic ($\bullet$) and hypotonic ($\circ$) bathing solutions are shown. The data are mean $\pm$ SEM from 3 experiments.

Fig. 6. Effects of arachidonic acid-related compounds on the hypotonically-induced Cl^- conductance. **A:** Typical traces of membrane potential (V_m) of hepatocytes recorded with the $133\text{K}^+-13\text{Cl}^-$ pipette and the $5\text{K}^+-80\text{Cl}^-$ isotonic or hypotonic bathing solution. When indicated "hypotonic," the hypotonic solution was perfused. The bathing solution contained 20 μM arachidonic acid (b), 50 μM indomethacin plus 100 μM esculetin (c), or 200 μM quinacrine (d). (a) represents absence of arachidonic acid. **B:** Maximal effects of the hyperpolarization (ΔV_m) obtained 60–70 s (a–c) or 180 s (d) after the hypotonic challenge was measured in the absence (a) and presence of 20 μM arachidonic acid (b), 50 μM indomethacin plus 100 μM esculetin (c), or 200 μM quinacrine (d). Experimental conditions were the same as those in A. The data are expressed as mean $\pm$ SEM from 5–8 experiments. ** Significantly different from the value of a ($p < 0.01$).

It is noted that the basal current in Fig. 5 is larger than that in Fig. 4, because the solutions used in Fig. 5 contained K^+ and Na^+ instead of Cs^+ .

Next, we tested effects of arachidonic acid-related compounds on the hypotonically-induced hyperpolarization with the $133 K^+-13 Cl^-$ pipette and $5 K^+-80 Cl^-$ bathing solutions. Arachidonic acid ($20 \mu M$) significantly inhibited the membrane hyperpolarization induced by hypotonic stress (Fig. 6A-b and B). The combined application of indomethacin ($50 \mu M$) and esculetin ($100 \mu M$), that is, the combination of the cyclo-oxygenase inhibitor and the lipoxygenase inhibitor [20] also inhibited the hyperpolarization (Fig. 6A-c and B). In contrast, quinacrine ($200 \mu M$), a phospholipase A_2 inhibitor [21], significantly increased the amplitude of the hypotonically-induced hyperpolarization (Fig. 6A-d and B) and prevented the time-dependent inactivation, which was ob-

served under control conditions. All chemicals used here did not significantly affect the resting membrane potential (Fig. 3). This hyperpolarization, however, was not affected by $2 mM$ BAPTA, a Ca^{2+} chelator, in the intracellular solution; the cells hyperpolarized by -6.3 ± 0.8 and $-6.0 \pm 0.4 mV$ in the absence and presence of BAPTA, respectively ($n=4$, $p>0.05$). These results suggest that a turnover of arachidonic acid takes place in hepatocytes and that the increase in the concentration of arachidonic acid inhibits the opening of hypotonicity-inducible Cl^- channels via a Ca^{2+} -independent pathway. An arachidonic acid metabolite, leukotriene D_4 , has been shown to act as a mediator for the activation of Cl^- channels in rat colonic epithelium during the RVD [12, 13]. In the present experiments, however, $1 \mu M$ leukotriene D_4 did not affect the membrane potential of hepatocytes (4 observations, data not shown).

Consistent with the results in Fig. 6, the hypotonic stress did not stimulate outward whole-cell Cl^- currents, when arachidonic acid, indomethacin, and esculetin were present in the bathing solution (Fig. 7). The amplitudes of basal currents in Fig. 7 ($319 \pm 73 pA$ at $+60 mV$ and $-228 \pm 72 pA$ at $-110 mV$) were not significantly different ($p>0.05$) from those in Fig. 4 ($316 \pm 88 pA$ at $+60 mV$ and $-182 \pm 47 pA$ at $-110 mV$), indicating that arachidonic acid did not affect the basal Cl^- current.

DISCUSSION

In this study, we investigated a hypotonically-induced Cl^- conductance in isolated rat hepatocytes. The activation of this Cl^- conductance actually facilitates RVD in the hepatocyte, because RVD was accelerated by a step decrease in the extracellular Cl^- concentration and abolished by Cl^- depletion of the cells [22]. In accordance with a previous finding that the RVD in rat hepatocytes was Ca^{2+} -independent and sensitive to DIDS [7], the present hypotonically-induced Cl^- conductance was observed even in the presence of BAPTA, a Ca^{2+} chelator, and was significantly inhibited by SITS (Fig. 2). Although the K^+ conductance, which is inhibited by Ba^{2+} , was also reported to be activated during the RVD in rat hepatocytes [4, 6, 22], we did not observe a significant activation of such Ba^{2+} -sensitive K^+ conductance (Fig. 2). Under our experimental conditions, only the Cl^- conductance could be activated (Figs. 1, 2, and 6). This discrepancy is probably due to different experimental temperatures; our experiments were performed at $25 \pm 2^\circ C$ while others were performed at $36-37^\circ C$ [4, 6, 22]. In fact, the swelling-induced K^+ conductance in mouse

Fig. 7. Inhibition of the hypotonically induced Cl^- current by arachidonic acid. **A:** Whole-cell Cl^- currents were recorded as described in the legend to Fig. 4. Typical current traces with the isotonic (left) and hypotonic bathing solutions (70 s after the perfusion, right) are shown. The bathing solutions contained $20 \mu M$ arachidonic acid, $50 \mu M$ indomethacin, and $100 \mu M$ esculetin. **B:** The relationship between the Cl^- current (I) and V_m . Experimental protocol was the same as that in A. The currents were measured 70 ms after application of voltage steps. The $I-V_m$ relations with the isotonic (●) and hypotonic (○) bathing solutions are shown. The data are mean $\pm$ SEM from 5 experiments. **Inset:** The hypotonically-induced current (■), determined from the difference between currents with the isotonic and hypotonic bathing solutions.

hepatocytes were reported to be blocked at 27°C [23]. The membrane potential-dominating K^+ conductance in non-stimulated mouse hepatocytes was also decreased by lowering the temperature from 37 to 25°C [24], suggesting that the relatively small contribution of K^+ -selective conductance to V_m in Figs. 1 and 5 was due to the experimental temperature.

Corresponding to the activation of a Cl^- conductance but not of a K^+ conductance (Figs. 1 and 2), the hypotonic stress stimulated an outward whole-cell Cl^- current (Fig. 4), but it did not increase cationic currents (Fig. 5). The increased Cl^- current did not inactivate rapidly during the voltage clamp of 0.1 s in the range of -110 to $+60$ mV (Fig. 4). Swelling-induced Cl^- currents in epithelial cells such as human airway cells [25], human small intestine cells [10], and rat epididymal cells [26] showed very rapid time-dependent inactivation at most highly depolarized potentials. In contrast, the currents in apolar cells such as canine cardiac cells [27], human neutrophils [28], murine T lymphocytes [29], and rabbit osteoclasts [30] did not show rapid decay. Although hepatocytes are polarized cells, the present non-decaying property of the Cl^- current resembles that of apolar cells. The non-decaying Cl^- currents in human neutrophils [28] and rabbit osteoclasts [30] were Ca^{2+} -independent and SITS-sensitive as found here in rat hepatocytes (Fig. 2).

The activation of the hypotonically-induced Cl^- conductance reached a maximum at 60–70 s after the hypotonic challenge and subsequently was inactivated (Figs. 1 and 2). Quinacrine, a phospholipase A_2 inhibitor, sustained this activation (Fig. 6A-d), suggesting that the inactivation of the Cl^- conductance was not due to a rundown of the Cl^- channels but due to the inhibition of the channels by endogenously-produced arachidonic acid. It is reported that production of arachidonic acid is increased by cell-swelling via activation of phospholipase A_2 in human platelets [31]. In cultured Intestine 407 cells derived from human small intestine epithelia [10] and HSG cells cloned from human submandibular glands [11], arachidonic acid was reported to inhibit swelling-induced Cl^- channels, which are Ca^{2+} -independent and SITS-sensitive.

When the bathing solution was switched from hypotonic to isotonic, an opposite electrophysiological response (transient depolarization) was observed (Fig. 1A). The same behavior was previously reported in isolated rat hepatocytes [22]. However, the mechanism of this behavior has not yet been identified in detail.

The plasma membrane of non-stimulated rat hepa-

tocytes has a relatively high Cl^- conductance [32]. Arachidonic acid has been shown not to inhibit the basal Cl^- current which was observed before the hypotonic challenge in hepatocytes (cf. Figs. 4 and 7), suggesting that the basal and hypotonically-induced currents are generated by opening of different types of Cl^- channels, and that arachidonic acid regulates only the opening of the hypotonically-induced Cl^- channels. Finally, the hypotonically-activated Cl^- current present in rat hepatocytes is apparently different from the Ca^{2+} -activated Cl^- current in guinea-pig hepatocytes [33]. Koumi *et al.* [34] recently found protein kinase C-activated Cl^- channels in guinea-pig hepatocytes; it is unknown, whether these channels are involved in RVD. At present, intracellular messengers in the opening of swelling-induced Cl^- channels in rat hepatocytes are not defined.

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