Prostaglandin E₂-Activated Housekeeping Cl⁻ Channels in the Basolateral Membrane of Rat Gastric Parietal Cells

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Abstract: Cl⁻ channels in the basolateral membrane of parietal cells within isolated rat gastric glands were studied by the whole-cell patch-clamp technique. The membrane potential (E_m) of non-stimulated parietal cells changed as a function of the basolateral extracellular Cl⁻ concentration (46 mV per decade), but E_m did not change significantly as a function of the K⁺ concentration. The extracellular addition of prostaglandin E₂ (PGE₂; 10 μ M) increased the whole-cell Cl⁻ current. A bifunctional prostaglandin EP3 agonist/EP1 antagonist, 5(Z)-7-[(1S,2S,3S,5R)-3-(*trans*- β -styren)sulfonamido-6,6-dimethylbicyclo-(3.1.1)hept-2-yl]-5-heptenoic acid (ONO-NT-012; 10 μ M), also increased the

Cl⁻ current. Basal Cl⁻ currents and the PGE₂- and ONO-NT-012-increased Cl⁻ currents were voltage-independent and inhibited by a Cl⁻ channel blocker, 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB), at 500 μ M. The single Cl⁻ channel conductance was estimated to be 0.29 pS by variance noise analysis. Both PGE₂ and ONO-NT-012 increased intracellular free Ca²⁺ concentration in the fura-2-loaded parietal cell transiently. The present study has shown that housekeeping sub-pS Cl⁻ channels are present in the basolateral membrane of rat parietal cell, and that the channels are regulated positively by PGE₂ via the EP3 receptor. [Japanese Journal of Physiology, 49, 365–372, 1999]

Key words: rat stomach, parietal cell, Cl⁻ channel, prostaglandin E₂, calcium.

Prostaglandin E₂ (PGE₂) is one of the major prostaglandins produced by arachidonic acid metabolism. PGE₂ has been reported to activate Cl⁻ channels through the adenylate cyclase/cAMP pathway in a number of epithelial cells such as T84 human colonic carcinoma cells [1], human skin fibroblasts [2], porcine thyroid epithelial cells [3], A6 frog distal nephron cells [4] and porcine endometrial epithelial cells [5]. So far, 4 subtypes of prostaglandin E receptor, EP1, EP2, EP3 and EP4, have been cloned and characterized (for review see Coleman *et al.* [6] and Ichikawa *et al.* [7]). Among them, EP2 and EP4 receptors stimulate adenylate cyclase, resulting in increases in cellular cAMP levels [8,9].

We have recently found that PGE₂ activates basolateral Cl⁻ channels (0.3–0.4 pS) via the EP3 receptor/

Ca²⁺/nitric oxide (NO)/cGMP pathway in rabbit gastric parietal cells: the activation of EP3 receptor stimulates Ca²⁺-dependent NO synthase, inducing the subsequent activation of guanylate cyclase [10,11]. This Cl⁻ channel has two important physiological roles. One is a housekeeping role through dominating the cell membrane potential [12,13], and the other is a cytoprotective role (against ethanol-induced injury) via NO/cGMP-dependent channel activation [14]. We do not yet know whether the presence of this gastric Cl⁻ channel is restricted only to rabbits or if it is present widely in a number of mammals.

The recent finding that EP3 receptor mRNA was enriched in rat gastric parietal cells [15] promoted us to examine the presence of PGE₂-activated gastric Cl⁻ channels in the rat. In the present study with rat pari-

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etal cells, we found basolateral Cl^- channels activated by PGE_2 via the EP3 receptor.

MATERIALS AND METHODS

Chemicals. Collagenase (Wako Pure Chemical Industries, Osaka, Japan) and Actinase E (Kaken Pharmaceutical Co., Tokyo, Japan) were used for preparing isolated gastric glands. Prostaglandin E_2 (PGE_2 ; Toray Industries, Tokyo, Japan) and 5(Z)-7-[(1S,2S,3S,5R)-3-(*trans*- β -styren)sulfonamido-6,6-dimethylbicyclo-(3.1.1)hept-2-yl]-5-heptenoic acid (ONO-NT-012; ONO Pharmaceutical Co., Osaka, Japan) were generous gifts. They were dissolved in ethanol and diluted with appropriate solutions just before use. 5-Nitro-2-(3-phenylpropylamino)-benzoate (NPPB; Research Biochemicals International, Natick, MA, USA), 4-acetamido-4'-isothiocyanatostilbene-2,2'-disulfonic acid (SITS; Sigma Chemical Co., St. Louis, MO, USA), diphenylamine-2-carboxylate (DPC; Wako Pure Chemical Industries) and niflumic acid (Cayman Chemical Co., Ann Arbor, MI, USA) were dissolved in either ethanol or dimethyl sulfoxide and diluted to final concentrations just before use. Ethanol and dimethyl sulfoxide concentrations in the final solutions never exceeded 0.5%, at which concentrations the vehicles per se did not affect the whole-cell Cl^- current and intracellular free Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) of parietal cells. Fura-2/pentaacetoxymethyl ester and O,O'-bis(2-aminophenyl)ethyleneglycol-N,N,N',N'-tetraacetic acid (BAPTA) were obtained from Dojindo Laboratories Co. (Kumamoto, Japan), and Pluronic F127 was from Molecular Probes (Eugene, OR, USA).

Isolation of gastric glands. The following procedures were performed in accordance with the guidelines presented by the Animal Care and Use Committee of Toyama Medical and Pharmaceutical University. Gastric glands were prepared from male rats of the Wistar strain (weighing 140–280 g) following essentially the method reported for the isolation of rabbit gastric glands [16]. Rats were purchased from the Shizuoka Laboratory Animal Centre (Shizuoka, Japan) and had free access to water and food until the day of the experiment. Animals were sacrificed rapidly by stunning and cervical dislocation. The stomach was removed and the gastric mucosa in the fundic region was digested with collagenase (200 units ml^{-1}) at 35–37°C for 35 min. Isolated glands were further treated with 500 tyrosine units ml^{-1} of Actinase E at 25°C for 5 min. As a result of the additional digestion, parietal cells protruded from the bases of glands and had no leaky connection between

the intracellular canaliculi and the lumen [13]. The glands were suspended in a standard medium containing 132.4 mM NaCl, 5.4 mM KCl, 5 mM Na_2HPO_4 , 1 mM NaH_2PO_4 , 1.2 mM MgSO_4 , 1 mM CaCl_2 , 10 mM HEPES (pH 7.35), 2 mg ml^{-1} bovine serum albumin (BSA) and 2 mg ml^{-1} glucose.

Patch-clamp experiments. Isolated glands were attached to a glass coverslip coated with poly-L-lysine (Sigma Chemical Co.), and the coverslip was mounted in an experimental chamber designed in this laboratory. The whole-cell patch-clamp technique was applied to the basolateral membrane of parietal cells in isolated rat gastric glands. EPC-7 (List Electronic, Darmstadt, Germany) and EPC-9 patch-clamp systems (HEKA elektronik Dr.-Ing. P. Schulze GmbH., Lambrecht, Germany) were used for recording as previously described [13,17,18]. The resistance of patch electrodes filled with the pipette solution was about 5–8 M Ω . The cell resistance was 0.2–0.5 G Ω in the whole-cell configuration. Series resistance and capacitance were compensated electronically as best as possible. The liquid junction potential between the pipette and bathing solutions was experimentally determined and corrected. The following extracellular bathing and intracellular pipette solutions were used. The 133 K^+ -142 Cl^- bathing solution contained (in mM): 133 KCl, 7 NaCl, 1 sodium aspartate, 1 MgCl_2 , 1 CaSO_4 , 5 ouabain and 10 HEPES. When indicated, low Cl^- (20 or 50 mM Cl^-) bathing solutions were used. In these solutions, Cl^- was replaced by aspartate. The 140 choline $^+$ -146 Cl^- bathing solution contained (in mM): 140 choline chloride, 2 MgCl_2 , 1 CaCl_2 , 5 ouabain and 10 HEPES. The 133 K^+ -13 Cl^- pipette solution contained (in mM): 133 potassium aspartate, 7 NaCl, 3 MgCl_2 , 0.062 CaSO_4 , 0.1 EGTA, 2 ATP and 10 HEPES (pCa 7). The 140 choline $^+$ -146 Cl^- pipette solution contained (in mM): 140 choline chloride, 3 MgCl_2 , 0.062 CaSO_4 , 0.1 EGTA, 2 ATP and 10 HEPES (pCa 7). The 140 choline $^+$ -146 Cl^- (Ca^{2+} -free) pipette solution contained (in mM): 140 choline chloride, 3 MgCl_2 , 2 BAPTA, 2 ATP and 10 HEPES. All the bathing and pipette solutions were adjusted to pH 7.3 with KOH or Tris. The membrane potential was recorded in the current-clamp mode. To obtain the current-voltage relationship with the 140 choline $^+$ -146 Cl^- bathing and 140 choline $^+$ -146 Cl^- pipette solutions, command pulses were superposed on the holding potential (0 to ± 100 mV, voltage jump by ± 20 mV, duration of 400 ms). This pulse was delivered using the PULSE+PULSEFIT program (HEKA, installed on a Macintosh computer); resulting currents were stored on the computer. Whole-cell Cl^- currents were recorded continuously with the 133 K^+ -142 Cl^-

bathing and 133 K⁺-13 Cl⁻ pipette solutions at a holding potential of 0 mV, the zero-current potential for K⁺ channels and non-selective cation channels [10,19]. Experiments were performed at 35–37°C.

Noise analysis. Whole-cell Cl⁻ currents were obtained with the 140 choline⁺-146 Cl⁻ bathing and 140 choline⁺-146 Cl⁻ pipette solutions. The single-channel conductance of the Cl⁻ channel was estimated by variance noise analysis during the inhibition of whole-cell Cl⁻ currents (at +20 mV) by 500 μ M NPPB, as described previously [13]. The currents were low-pass filtered (1 kHz), amplified via a low-noise preamplifier (Model 113, Princeton Applied Research, Princeton, NJ, USA) and stored on a tape recorder (SONY A-65). The tape was replayed at the same speed and currents were high-pass filtered (0.3 Hz) via the preamplifier. The roll-off frequencies of the preamplifier were switch selectable and the frequency indicated -3 dB points of a 6 dB/octave roll-off curve. The power spectrum and variance of the fluctuation of the whole-cell Cl⁻ current were calculated (2–200 Hz) using a signal analyzer (SM-2100A, Iwatsu Electric Co., Tokyo, Japan). The unit amplitude of the single Cl⁻ channel current (i) and total number of channels (N) can be estimated from the following equation [20].

$$\sigma^2/I = i - I/N,$$

where I is the mean whole-cell current and σ^2 is the variance of the fluctuation, which was calculated with the signal analyzer.

Measurement of [Ca²⁺]_i. Isolated gastric glands were suspended in the BSA-free standard medium. Fura-2/pentaacetoxymethyl ester (5 μ M) with a detergent, Pluronic F127 (0.025%, w/v), was added to the suspension and incubated for 35 min at 22°C. After loading, the glands were washed and re-suspended in ice-cold standard medium. Then, they were warmed at 35–37°C in the 133 K⁺-142 Cl⁻ bathing solution before measurement. Fura-2-loaded single parietal cells in gastric glands were observed under an inverted microscope (Nikon TMD-EFQ). The total fluorescence intensity from one single parietal cell was monitored at excitation wavelengths of 340 and 380 nm with an emission wavelength of 505 nm (interference filter) using a photon-counting technique (Spex Fluorolog-2 spectrofluorometer, Edison, NJ, USA). After corrections for background fluorescence, the intensity ratio (340/380 nm) and [Ca²⁺]_i were calculated as described previously [21].

Statistics. Results are presented as the mean $\pm$ SEM. Statistical significance was evaluated by Student's t -test and analysis of variance (ANOVA), and a

Fig. 1. Effects of K⁺ and Cl⁻ concentrations on the resting membrane potential (E_m) of rat parietal cells. A: Effects of various $[K^+]_o$ on the E_m at a constant $[Cl^-]_o$. The 7 K⁺-142 Cl⁻, 50 K⁺-142 Cl⁻ and 133 K⁺-142 Cl⁻ bathing solutions and the 133 K⁺-13 Cl⁻ pipette solution were used. $n=5-12$. **B:** Effects of various $[Cl^-]_o$ on the E_m at a constant $[K^+]_o$. The 133 K⁺-142 Cl⁻, 133 K⁺-50 Cl⁻ and 133 K⁺-20 Cl⁻ bathing solutions and the 133 K⁺-13 Cl⁻ pipette solution were used. $n=5-12$.

p value below 0.05 was considered to be significant.

RESULTS

Effects of altered extracellular concentrations of either K⁺ or Cl⁻ on membrane potential

In the whole-cell configuration at the current-clamp mode, we measured the membrane potential (E_m) of parietal cells in rat gastric glands. Ouabain (5 mM) was added to the bathing solution to eliminate the possible contribution of Na⁺,K⁺-ATPase to E_m [12]. When E_m was recorded with the 7 K⁺-142 Cl⁻ bathing and 133 K⁺-13 Cl⁻ pipette solutions, i.e., standard ionic conditions, the value was -34.6 ± 2.9 mV ($n=8$). This value is similar to that obtained with rabbit parietal cells (-35.1 mV [12]). Increasing extracellular (basolateral) K⁺ concentration ($[K^+]_o$) at a constant $[Cl^-]_o$ did not significantly affect the E_m (Fig. 1A). Furthermore, E_m recorded in a K⁺- and Na⁺-free bathing solution (140 choline⁺-142 Cl⁻) was -35.2 ± 3.2 mV ($n=5$), the value of which was not significantly different from those in the 133 K⁺-142 Cl⁻, 30 K⁺-142 Cl⁻ and 7 K⁺-142 Cl⁻ bathing solutions (Fig. 1A). In contrast, decreasing $[Cl^-]_o$ at a constant $[K^+]_o$

Fig. 2. Effects of basolateral application of Cl⁻ channel blockers on whole-cell Cl⁻ currents. The Cl⁻ currents were continuously recorded from a gastric parietal cell with the 133 K⁺-13 Cl⁻ pipette and 133 K⁺-142 Cl⁻ bathing solutions at a holding potential of 0 mV. A–D: Representative traces of whole-cell Cl⁻ currents before and after additions of 500 μ M of NPPB (A), SITS (B), DPC (C) and niflumic acid (Nif, D). These inhibitors were added at the arrows. E: Inhibitory effects were assessed 40 s after application of inhibitors. $n=6-9$. ** Significantly different from the value of NPPB ($p<0.01$).

depolarized the cell (Fig. 1B). The slope of Fig. 1B was about 46 mV per decade, which value is smaller than the value calculated from the Nernst equation under the assumption that only Cl⁻ conductance contributed to E_m (64 mV). But this may be due to partial permeation of the aspartate ion in the solutions via the Cl⁻ channel as described previously [12]. Our present data suggest that the resting membrane potential of rat parietal cells was predominantly determined by the basolateral membrane conductance to Cl⁻ but not to K⁺ and Na⁺.

Fig. 3. Variance noise analysis of whole-cell Cl⁻ currents. A: Inhibition of the whole-cell Cl⁻ current by 500 μ M NPPB. The Cl⁻ current was recorded with the 140 choline⁺-146 Cl⁻ pipette solution and the 140 choline⁺-146 Cl⁻ bathing solution. Holding potential was +20 mV. B: σ^2/I is plotted against I . The mean value of σ^2 was calculated at four different periods (each 9-s duration), the centers of which are indicated by asterisks in A. The four periods did not overlap. The system noise was corrected prior to the calculation of the variance; that is, the power spectrum at zero-current potential (0 mV) was subtracted from each power spectrum. The line was drawn with the least-squares fit. The value of the correlation coefficient is -0.996 . Representative data from four similar experiments are shown.

Effects of Cl⁻ channel blockers on the whole-cell Cl⁻ current

As shown in Fig. 2A, inhibition of the whole-cell Cl⁻ current was observed immediately after the addition of anion channel blockers, NPPB, SITS, DPC and niflumic acid to the basolateral bathing solution, suggesting that the blockers directly acted on the Cl⁻ channel in the basolateral membrane. At 500 μ M, the inhibitory effect of NPPB was significantly greater than those of SITS, DPC and niflumic acid (Fig. 2B). This pharmacological profile is similar to that observed with rabbit parietal cells [10,13].

Noise analysis

Single Cl⁻ channel events could not be observed in cell-attached and excised patches of the basolateral

membrane of parietal cells. Therefore, the unit conductance of the Cl⁻ channel was calculated from variances of the current fluctuation during the period of the NPPB (500 μ M)-induced inhibition process (Fig. 3), as previously performed for the basolateral Cl⁻ channel of rabbit parietal cells [13]. As shown in Table 1, the Cl⁻ channel has a sub-pS unit conductance (0.29 pS) similar to the Cl⁻ channel of rabbit parietal cells (0.35 pS [13]). Furthermore, open probability of the Cl⁻ channel ($P_o=0.52$; Table 1) and the total number of Cl⁻ channels in the basolateral membrane ($N=3.42\times 10^4$; Table 1) are similar to those obtained with rabbit parietal cells ($P_o=0.45$ and $N=$

2.92×10^4 [13]).

Activation of the housekeeping Cl⁻ channel by PGE₂ and ONO-NT-012

We have recently found that PGE₂ activates the membrane potential-dominating (housekeeping) Cl⁻ channel via the EP3 receptor-mediated Ca²⁺/NO/cGMP pathway in rabbit parietal cells [11]. Here, we examined if PGE₂ similarly increases the Cl⁻ current in rat parietal cells. PGE₂ (10 μ M) increased the whole-cell Cl⁻ current as shown in Fig. 4. The maximal effect was obtained around 6 min after the application of PGE₂. This effect was significant; outward and inward Cl⁻ currents at ± 100 mV increased during the 6 min period, from 336 ± 65 to 836 ± 72 pA and from -322 ± 54 to -782 ± 24 pA, respectively ($p<0.01$, $n=3$). PGE₂ did not change the linearity of the current-voltage relationship (Fig. 4), indicating that the characteristics of the Cl⁻ channel observed before and after stimulation by PGE₂ were the same. Both the basal and PGE₂-elicited currents were inhibited by 500 μ M NPPB. When the Ca²⁺-free pipette solution was used, PGE₂ (10 μ M) did not significantly increase the whole-cell Cl⁻ currents (3 observations, data not shown). ONO-NT-012 (10 μ M), a novel bifunctional EP3 agonist/EP1 antagonist [22], also increased the Cl⁻ current (Fig. 5); outward and inward Cl⁻ currents

Table 1. Characteristics of single Cl⁻ channel obtained by variance noise analysis.

i (fA)	5.86 ± 0.69
γ (pS)	0.29 ± 0.03
I (pA)	94 ± 11
N	$(3.42\pm 0.74)\times 10^4$
P_o	0.52 ± 0.06

i , unit amplitude of the single Cl⁻ channel current; γ , single Cl⁻ channel conductance; I , mean total Cl⁻ current before the addition of NPPB; N , total number of Cl⁻ channels in the basolateral membrane; P_o , open probability of the Cl⁻ channel calculated from $I=NP_o i$. The data are mean $\pm$ SEM from four observations.

Fig. 4. PGE₂-induced increase in whole-cell Cl⁻ currents. The Cl⁻ currents were recorded from a parietal cell equilibrated with the 140 choline⁺-146 Cl⁻ pipette solution and 140 choline⁺-146 Cl⁻ bathing solution. Membrane potential (V_m) was changed by a ± 20 mV step from the holding potential (at 0 mV). **A:** Representative traces. **Left**, control; **middle**, 6 min after the application of 10 μ M PGE₂; **right**, 40 s after the subsequent application of 500 μ M NPPB. Typical relations from 3 similar experiments are shown. **B:** Relationship between the whole-cell Cl⁻ current (I) and V_m recorded from the same single cell. The currents were measured 350–400 ms after the application of voltage steps. The I - V_m relationships of the control (○), 6 min after the application of 10 μ M PGE₂ (●) and 40 s after the subsequent application of 500 μ M NPPB (▲) are shown.

Fig. 5. ONO-NT-012-induced increase in whole-cell Cl^- currents. Experimental procedures were the same as those in Fig. 4. **A:** Representative traces. **Left,** control; **middle,** 6 min after the application of $10 \mu\text{M}$ ONO-NT-012; **right,** 40 s after the subsequent application of $500 \mu\text{M}$ NPPB. Typical relations from 3 similar experiments are shown. **B:** Corresponding I - V_m relationships of the control ($\circ$), after the application of ONO-NT-012 ($\bullet$) and after the additional application of NPPB ($\blacktriangle$) are shown.

Fig. 6. PGE_2 - and ONO-NT-012-induced increases in $[\text{Ca}^{2+}]_i$ in single parietal cells. Representative traces of the change in $[\text{Ca}^{2+}]_i$ of single parietal cells in gastric glands are shown. PGE_2 (**A**) or ONO-NT-012 (**B**) ($10 \mu\text{M}$) was perfused from the time indicated by the arrows. The data represent 5 similar experiments.

at $\pm 100 \text{ mV}$ increased during the 6 min period from 618 ± 50 to $1,026 \pm 29 \text{ pA}$ and -485 ± 86 to $-1,064 \pm 36 \text{ pA}$, respectively ($p < 0.01$, $n = 3$). As observed with PGE_2 (Fig. 4), ONO-NT-012 did not change the linearity of the current-voltage relationship, and the ONO-NT-012-elicited current was inhibited by 500

μM NPPB (Fig. 5), indicating that the PGE_2 -induced effect was mediated by the EP₃ receptor.

Elevation of $[\text{Ca}^{2+}]_i$ by PGE_2 and ONO-NT-012 in parietal cells

We measured $[\text{Ca}^{2+}]_i$ in fura-2-loaded single parietal cells. As shown in Fig. 6, PGE_2 and ONO-NT-012 transiently increased $[\text{Ca}^{2+}]_i$. PGE_2 ($10 \mu\text{M}$) and ONO-NT-012 ($10 \mu\text{M}$) increased $[\text{Ca}^{2+}]_i$ by 49 ± 8 ($n = 5$) and $20 \pm 2 \text{ nM}$ ($n = 5$), respectively.

DISCUSSION

It was reported that exposure of the rat stomach to ethanol induced tissue damage and reduction of the transmucosal potential difference (PD), and that 16,16-dimethyl PGE_2 significantly recovered both the tissue damage and PD reduction [23]. In isolated rabbit gastric parietal cells, we recently found that the cell membrane potential may be related to ethanol-induced cytotoxicity and PGE_2 -elicited cytoprotection [14]. Ostrowski *et al.* [24] reported that modulation of the membrane potential caused functional and structural changes of isolated rat parietal cells. These findings suggest that the membrane potential, which is modulated by PGE_2 , may be essential for maintaining

the housekeeping function of gastric parietal cells.

Our present study shows that the membrane potential of rat parietal cells is predominantly determined by Cl⁻ conductance, and that K⁺ and Na⁺ conductances are not major determinants of the membrane potential (Fig. 1). Similar results were obtained in cultured rat parietal cells using conventional microelectrodes; about a 5-fold change in [Na⁺]_o (143→30 mM) or [K⁺]_o (30→147 mM) did not significantly affect the resting membrane potential, while only a 2.5-fold change in [Cl⁻]_o (160→66 mM) significantly shifted the potential by 8.7 mV [25]. We found in the present study that Cl⁻ conductance was originated from the sub-pS Cl⁻ channel that has a housekeeping role in rat gastric parietal cells.

The reason why the gastric parietal cell keeps a lower intracellular Cl⁻ concentration may be explained by the partial contribution of basolateral Na⁺,K⁺-ATPase to the membrane potential. In fact, ouabain significantly changed the membrane potential (from -40.6 to -35.1 mV) of rabbit parietal cells [12].

There are at least four subtypes of prostaglandin E receptor, the EP1, EP2, EP3 and EP4 receptors [7,26]. When they are expressed in mammalian cells, EP1 and EP3 elicit the elevation of [Ca²⁺]_i, EP2 and EP4 stimulate adenylate cyclase and EP3 inhibits the enzyme [7,27]. In the present study, ONO-NT-012, a bifunctional EP3 agonist/EP1 antagonist, induced increases in both Cl⁻ current (Fig. 5) and [Ca²⁺]_i (Fig. 6), suggesting that the PGE₂-elicited responses in rat gastric parietal cells are mediated via the EP3 receptor. Interestingly, PGE₂ also inhibits gastric acid secretion mediated via the EP3 receptor in the parietal cells [28], however this effect is associated with a decrease in [cAMP]_i [28,29]. In the rat, so far two isoforms of the EP3 subtype (rEP_{3A} and rEP_{3B}) have been cloned and characterized [30]. The activation of rEP_{3A} and rEP_{3B} is associated with a decrease in [cAMP]_i and an increase in [Ca²⁺]_i, respectively. Ding *et al.* [15] recently found that the EP3 receptor mRNA was abundant in rat gastric parietal cells.

We suggest that the molecule of the gastric housekeeping Cl⁻ channel of the rat is similar to that of the rabbit [11,13], because 1) both channels have sub-pS single-channel conductance and a linear current-voltage relationship, 2) both channels determine the cell membrane potential, and 3) both channels are activated by PGE₂ via the EP3 receptor and inhibited by NPPB, a Cl⁻ channel blocker. PGE₂-induced transient increase in [Ca²⁺]_i may link to the NO/cGMP pathway and slowly activate the Cl⁻ channel, as reported in rabbit gastric parietal cells [11].

Kajita *et al.* [31] reported that a mini (sub-pS) Cl⁻

channel (0.46–0.6 pS) in the basolateral membrane of guinea pig parietal cells was inhibited by NPPB as found here, but was not activated by PGE₂, which is different from the cases of rabbits and rats. Such different sensitivities of the gastric Cl⁻ channels to PGE₂ may be due to different animal species. Another possibility is due to different experimental conditions; they performed the experiment at 23–25°C, while our experiments with rat and rabbit parietal cells were performed at 35–37°C. We observed that PGE₂ (10 μM) transiently increased [Ca²⁺]_i in guinea pig parietal cells incubated at 35–37°C (A. Ikari, K. Inoue, H. Sakai, and N. Takeguchi, unpublished data), giving the possibility that the mini (sub-pS) Cl⁻ channel of guinea pig parietal cells may be activated at 37°C.

Opening of the sub-pS Cl⁻ channel may be essential for the parietal cells to survive against severe environmental circumstances, because this channel has a housekeeping function through dominating the cell membrane potential [12,13] and a cytoprotective function against ethanol-induced injury [14]. PGE₂ has two important activities in the gastric parietal cells, antisecretory activity via the cAMP pathway and cytoprotective activity via the Ca²⁺/NO/cGMP pathway.

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