Nonselective cation channel as a Ca$^{2+}$ influx pathway in pepsinogen-secreting cells of bullfrog esophagus

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Kimura, Seiichiro, Hiroshi Mieno, Kenji Tamaki, Masaki Inoue, and Kazuaki Chayama. Nonselective cation channel as a Ca$^{2+}$ influx pathway in pepsinogen-secreting cells of bullfrog esophagus. *Am J Physiol Gastrointest Liver Physiol* 281: G333–G341, 2001.—In pepsinogen-secreting cells of bullfrog (*Rana catesbeiana*), recent evidence suggests that Ca$^{2+}$ release from internal stores followed by Ca$^{2+}$ influx across the plasma membrane elicits pepsinogen secretion. Such a Ca$^{2+}$ influx could be carried by a background current, potentiated by bombesin, that was found in these cells using the whole cell patch-clamp technique. The permeability ratio of Cs$^+$-Rb$^+$-K$^+$-Na$^+$-Li$^+$-N-methyl-D-glucamine$^-$-Ca$^{2+}$ was 1.01:1.1:0.86:0.72:0.54:0.34. The current was almost totally blocked by the nonselective cation channel blockers La$^{3+}$ (0.1 mM) and Gd$^{3+}$ (0.1 mM) and was activated by intracellular Ca$^{2+}$. These properties demonstrated that the current, which was activated by bombesin, was a nonselective cation current. At the same time, Gd$^{3+}$ suppressed pepsinogen secretion by 29 ± 5.6% in isolated pepsinogen-secreting glands. These results are in accord with the idea that a nonselective cation channel in pepsinogen-secreting cells plays a role as a Ca$^{2+}$ influx pathway leading to secretion of pepsinogen in bullfrog esophageal mucosa.

patch clamp; bombesin; nifedipine; lanthanum; gadolinium; calcium ion

IT HAS BEEN REPORTED THAT pepsinogen secretion was induced by three known second messengers, such as intracellular Ca$^{2+}$, cAMP, and diacylglycerol, in isolated guinea pig gastric glands (21). Of the three, the role of Ca$^{2+}$ was the predominant focus. For instance, bombesin, one of the peptidergic agonists, was reported to stimulate the pepsinogen secretion of bullfrog (*Rana catesbeiana*) esophageal mucosa by increasing the intracellular free Ca$^{2+}$ (6). That group reported that the early phase of Ca$^{2+}$ elevation was relatively independent of external Ca$^{2+}$ and that the sustained phase of Ca$^{2+}$ was eliminated by adding 0.5–1 mM EGTA. Many reports have demonstrated that this increase in the intracellular free Ca$^{2+}$ concentration ([Ca$^{2+}]_i$) derives from the following two Ca$^{2+}$ sources: a release from intracellular Ca$^{2+}$ stores and an influx from extracellular space (7, 16, 23, 28). Until now, however, the mechanism of Ca$^{2+}$ influx has not been established in pepsinogen-secreting cells.

On the other hand, in pancreas acinar cells, an increase in the [Ca$^{2+}]_i$ by agonist stimulation was reported along with the existence of a nonselective cation channel. In human keratinocytes and guinea pig endocardial endothelial cells, a route for Ca$^{2+}$ influx from the extracellular side was attributed to nonselective cation channels (2, 11).

In the present study, we have identified a pathway of Ca$^{2+}$ influx in frog pepsinogen-secreting cells for the first time, using the whole cell patch-clamp technique (5).

MATERIALS AND METHODS

Cell preparations. Pepsinogen-secreting cells were prepared from bullfrog (*Rana catesbeiana*) esophageal mucosa by the procedure previously described (17). Briefly, the cells were isolated by digestion with 0.01% collagenase (collagenase A; Boehringer Mannheim, Mannheim, Germany) in a Ca$^{2+}$-free solution for 12 min at 37.5°C. The cell suspension was then passed through a 120-μm nylon mesh. The filtrate was centrifuged at 200 g, and the sediments were washed three times. The final cell suspension was stored in a normal amphibian solution (specified below) at room temperature.

Measurement of pepsinogen secretion. Pepsinogen secretion was measured by using a perifusion chamber, as previously described (15). The isolated pepsinogen-secreting glands (multicellular preparation) were also obtained by 0.1% collagenase (collagenase 1; Funakoshi, Tokyo, Japan) digestion. The isolated peptic glands were collected on Teflon mesh (4.75 μm) that was mounted in a perfusion chamber. The normal amphibian solution at 25°C was perfused across the surface of the glands, and the perfusate was collected at 1-min intervals. In addition, an aliquot of the supernatant was assayed for pepsinogen activity. Pepsinogen was measured using acid-denatured Hb at pH 2.0 as the substrate by the modified Anson-Mirsky method. After 18 h of incubation, pepsinogen activity was measured as tyrosine concentration...
The cell pellet was homogenized with a Potter-Elvehjem homogenizer (Polytron) in 2 ml water and was used to estimate the total cellular pepsinogen content (16). Pepsinogen secretion was expressed as a percentage of the total pepsinogen initially present in the cells.

Solution and chemicals. The normal amphibian solution contained (in mM) 110 NaCl, 2 KCl, 1 MgCl₂, 1 CaCl₂, and 10 HEPES-NaOH, and pH was adjusted to 7.3. The Ca²⁺-free solution contained (in mM) 111.5 NaCl, 2 KCl, 1 MgCl₂, and 10 HEPES-NaOH, and pH was 7.3. In several pilot experiments with a pipette solution containing no ATP, the currents were quiescent and the secretagogue could not activate any currents in these cells. Therefore, we decided to use a pipette solution containing 1 mM ATP. The standard pipette solution contained (in mM) 110 KCl, 0.354 CaCl₂, 1 EGTA, 1 Tris-ATP, and 10 HEPES-NaOH; pH was adjusted to 7.3 and pCa (=log Ca²⁺ concentration) was 7, unless otherwise stated. The free Ca²⁺ concentrations of the solutions were estimated using the Chelator program (22).

Tris-ATP, LaCl₃, GdCl₃, N-methyl-D-glucamine (NMDG)-Cl, EGTA, anthracene-9-carboxylic acid (9-AC), and Rb were purchased from Sigma (St. Louis, MO). Bombesin was ob-

![Diagram](http://ajpgi.physiology.org/)

Fig. 1. Current-voltage relationships of the bombesin-induced current. A: after establishment of whole cell configuration, square pulse protocol was applied by pulses from a holding potential of −60 mV to different potentials between −140 and +40 mV in 20-mV steps of 800-ms duration. a: Current traces in normal amphibian solution (in mM: 110 NaCl, 2 KCl, 1 MgCl₂, 1 CaCl₂, and 10 HEPES-NaOH, pH = 7.3, adjusted with NaOH) in the absence (b) and presence (c) of 3.2 × 10⁻⁷ M bombesin. Horizontal lines indicate the 0 current level. B: current-voltage relationships in normal amphibian solution before (●) and after (○) the addition of 3.2 × 10⁻⁷ M bombesin. The pipette was filled with standard pipette solution (in mM: 110 KCl, 0.35 CaCl₂, 1 EGTA, 1 MgCl₂, 1 ATP-Tris, and 10 HEPES-NaOH, pH = 7.3, adjusted with KOH, intracellular Ca²⁺ concentration ([Ca²⁺]ᵢ) = 10⁻⁷ M). These are representative data. C: after establishment of whole cell configuration, a ramp pulse protocol was applied from a holding potential of 0 mV to different potentials between −120 and +30 mV of 150-ms duration. The bath contained normal amphibian solution. The pipette contained standard pipette solution. D: representative data for the current-voltage relationships in normal amphibian solution before (a) and after (b) the addition of 3.2 × 10⁻⁷ M bombesin. The reversal potential of the agonist-induced current was 0 mV.
tained from the Peptide Institute (Osaka, Japan). All other chemicals were of the highest purity available.

Recording methods. The whole cell currents were recorded using a patch-clamp amplifier (Axopatch 200A; Axon Instruments, Foster City, CA). Records were filtered through a 4-pole Bessel low-pass filter with a cutoff frequency at 2 kHz and were digitized at a rate of 1–2 kHz. Data were analyzed with the “pClamp” program (Axon Instruments). The patch pipettes had resistances of 4–5 MΩ when filled with the standard pipette solution. All experiments were carried out at room temperature (22–24°C).

Ramp pulse and square pulse experiments. Current-voltage (I-V) relationships were obtained either by applying ramp pulses or square pulse sequences. Ramp pulses ranging between −120 and +30 mV were given from a holding potential of 0 mV at a ramp speed of 1 V/s. Square pulses were given in 20-mV step increments between −140 and +40 mV from a holding potential of −60 mV. The slope conductance was determined by calculating a line over an appropriate short segment, about −60 mV, of the I-V curve. The reversal potentials of the currents were measured as the potentials where the I-V curves of the control (without stimulation) crossed over those either stimulated by bombesin or blocked by La3+ or Gd3+. Cell capacitance, measured by the test-pulse mode of the patch-clamp amplifier under the whole cell configuration, was 6.4 ± 0.8 pF (n = 90).

Data are expressed as means ± SE. Statistical significance between groups was determined by Mann-Whitney U-test, with P < 0.05 considered statistically significant.

RESULTS

Bombesin-induced currents. Bombesin, which is a peptidergic agonist, is known to stimulate pepsinogen secretion by increasing [Ca2+]i in bullfrog esophageal mucosa. The ability of bombesin to increase [Ca2+]i is most prominent compared with other agonists in these cells. The effect of bombesin is shown in Fig. 1. An inactivated sustained current in the normal amphibian solution without bombesin was elicited by applying square pulses in 20-mV steps in the range between −140 and +40 mV from a holding potential of −60 mV (Fig. 1A). Bombesin (3.2 × 10−7 M) induced a significant increase in the conductance of the current (Fig. 1C). The resultant I-V curves are displayed in Fig. 1B. The slope conductance increased significantly (P < 0.05) from 69.3 ± 3.9 to 194.4 ± 15.1 pS/pF (n = 3). The reversal potential for the bombesin-sensitive current was 6 ± 0.8 mV. The I-V curves obtained by the currents elicited by the ramp pulse protocol (Fig. 1C) exhibited a result similar to that elicited by square pulses.

Fig. 2. Time course of the slope conductance after bombesin stimulation. A: after establishment of whole cell configuration, the ramp pulse protocol shown in Fig. 1C was applied after 3.2 × 10−7 M bombesin stimulation in normal amphibian solution. The time course of the slope conductance after bombesin stimulation is displayed. The pipette contained standard pipette solution. *Values were significantly different (P < 0.05) from respective values of 30, 40, and 50 s. †Values were significantly different (P < 0.05) from respective values of 10 and 20 s (Mann-Whitney U-test). B: current-voltage relationships after bombesin stimulation. Left: relation at 10–40 s; right, 50–70 s.
pulse sequences (Fig. 1D). The slope conductance of the current was increased significantly \((P < 0.05)\) by bombesin from 47.2 ± 13.7 to 213.1 ± 9.6 pS/pF \((n = 3)\). Bethanechol \((3.2 \times 10^{-5} \text{M})\), a cholinergic agonist that is known to increase \([\text{Ca}^{2+}]_{i}\) in pepsinogen-secreting cells, also exhibited an enhancing effect on this current. The slope conductance increased significantly from 35.9 ± 10.5 to 124.3 ± 29.4 pS/pF \((n = 4; \text{data not shown})\).

With the use of the ramp pulse protocol, the time-dependent development of the bombesin \((3.2 \times 10^{-7} \text{M})\) effect was observed. As shown in Fig. 2, the slope conductance started to increase within 10 s after bombesin application \((\text{time } 0)\) and reached a maximum between 30 and 50 s. Thereafter, the current gradually decreased, and the slope conductance at 60 and 70 s was significantly lower than that from 30 to 50 s.

**Selectivity for monovalent and divalent cations.** To determine the ion selectivity of the channel, reversal potentials \(E_{\text{rev}}\) for various conditions of external \(\text{Na}^{+}\) and \(\text{K}^{+}\) concentrations were obtained while the internal \(\text{K}^{+}\) concentration was maintained at 100 mM. NMDG\(^+\) was used as a replacement for the external monovalent cations. Measurements of the \(E_{\text{rev}}\) of bombesin-induced currents were carried out to find potentials where \(I-V\) curves in the presence of bombesin \((3.2 \times 10^{-7} \text{M})\) crossed those in the absence of bombesin, as shown in Fig. 3. The resultant \(E_{\text{rev}}\) values should be accurate, assuming that bombesin only activated this channel and leakage and other currents were not affected by the application of bombesin. Data are summarized in Table 1.

**Table 1. Slope conductance of bombesin-induced current**

<table>
<thead>
<tr>
<th>Cation Species</th>
<th>Concentration, mM</th>
<th>(E_{\text{rev}}), mV</th>
<th>Change in Conductance by Bombesin, pS/pF</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{K}^+)</td>
<td>100</td>
<td>-0.6 ± 0.1</td>
<td>98 ± 16 → 238 ± 19*</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>-11 ± 1.2</td>
<td>69 ± 8 → 149 ± 20*</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>-16 ± 2</td>
<td>35 ± 3 → 86 ± 10*</td>
</tr>
<tr>
<td>(\text{Na}^+)</td>
<td>100</td>
<td>-5 ± 0.5</td>
<td>115 ± 6 → 213 ± 33*</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>-14 ± 4</td>
<td>64 ± 3 → 134 ± 9*</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>-17 ± 1.3</td>
<td>48 ± 7 → 95 ± 10*</td>
</tr>
<tr>
<td>(\text{Ca}^{2+})</td>
<td>20</td>
<td>-10 ± 2.1</td>
<td>30 ± 2 → 71 ± 8*</td>
</tr>
</tbody>
</table>

Values are means ± SE of 3 experiments in each group. \(E_{\text{rev}}\) reversal potential. *Significant difference \([P < 0.05]; \text{(Mann-Whitney } U\text{-test)]\) compared with the value before stimulation.
Changes in $E_{\text{rev}}$ corresponding to changes in the extracellular $K^+$ concentration or the extracellular $Na^+$ concentration did not obey the Nernst equation, indicating that they were not selective permeant ions. These results suggest that permeant ions other than $K^+$ or $Na^+$ were present. Because anions do not permeate, the only possible permeant ion under this condition is NMDG$^+$, which will be described as follows. A permeability ($P$) ratio of $P_{\text{NMDG}}/P_{K^+} = 0.58$ was calculated from the measured $E_{\text{rev}}$ values according to the following equation

$$E_{\text{rev}} = \frac{RT}{F} \ln \left( \frac{P_X[X^+]}{P_K[K^+]} \right)$$

where $R$ is the gas constant, $T$ is the absolute temperature, and $F$ is Faraday's constant. $X$ is any monovalent cation that was employed in the experiments. $P_X$ is a permeability ratio of $X$ ion.

The permeability ratios of other ion species like Cs$^+$, Rb$^+$, and Li$^+$ against $K^+$ were measured as in Fig. 3, and these experiments are shown in Fig. 4. The averaged $E_{\text{rev}}$ values were $0.28 \pm 0.81$ (Cs$^+$), $0.16 \pm 1.08$ (Rb$^+$), $0 \pm 0$ (K$^+$), $-3.63 \pm 0.56$ (Na$^+$), $-8.16 \pm 0.67$ (Li$^+$), and $-15.6 \pm 0.4$ (NMDG$^+$) mV. These data yielded permeability ratios of $P_{\text{Cs}}/P_{K^+}=0.28$ (Cs$^+$), $P_{\text{Rb}}/P_{K^+}=0.68$ (Rb$^+$), $P_{\text{Na}}/P_{K^+}=0.72$ (Na$^+$), and $P_{\text{Li}}/P_{K^+}=0.54$ (Li$^+$). The permeability ratio of $P_{\text{NMDG}}/P_{K^+}(0.58)$ separately obtained in Fig. 3 is well in accord with that (0.54) obtained here. This nonselective nature toward monovalent cations fits the definition of nonselective cation channels.

Similarly, the permeabilities for divalent cations could be obtained by measuring $E_{\text{rev}}$ using Eqs. 2 and 3 in the presence of external divalent cations

$$E_{\text{rev}} = \frac{RT}{F} \ln \left( \frac{P^Y[Y^{2+}]}{P_K[K^+]} \right) + P_{\text{NMDG}}[\text{NMDG}^+]/[P_K[K^+] + P_{\text{Na}}[\text{Na}^-]]$$

$$P_{\gamma} = P_{\gamma} \times [1 + \exp(\frac{E_{\text{rev}} \times F/RT}{RT})]^{-1}$$

where $Y$ denotes any divalent cation, brackets denote concentration, subscript $i$ denotes intracellular, and subscript $o$ denotes extracellular.

As shown in Fig. 5A, in the presence of 20 mM Ca$^{2+}$ and 80 mM NMDG$^+$, bombesin $(3.2 \times 10^{-7}$ M) stimulated the current, leading to an increase in the slope conductance (data are shown in Table 1). The resultant $E_{\text{rev}}$ gives a permeability ratio of $P_{\text{Ca}}/P_K = 0.34$. Data are summarized in Table 2.

Other evidence for Ca$^{2+}$ permeation through the channel is provided by the concentration dependence of the relevant ions on the conductance. As shown in Fig. 5B, $[Ca^{2+}]_o$ was increased from 1 to 3 mM and to 10 mM while other cations were substituted with manni-

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**Fig. 4.** Inward current carried by Cs$^+$ (A), Rb$^+$ (B), K$^+$ (C), Na$^+$ (D), Li$^+$ (E), and NMDG$^+$ (F) After establishment of the whole cell configuration, ramp pulses, such as shown in Fig. 1C, were applied. Current-voltage relationships in a 110 mM chloride solution of each cation in the absence (a) and presence (b) of $3.2 \times 10^{-7}$ M bombesin. The pipette was filled with standard pipette solution. These are representative data.
tol in the presence of bombesin (3.2 × 10⁻⁷ M). I-V curves were obtained at the maximum effect of bombesin. The slope conductances for the current were increased significantly (P < 0.05), from 41.7 ± 0.9 pS/pF (1 mM Ca²⁺; n = 3) to 53.9 ± 4.6 pS/pF (3 mM Ca²⁺; n = 4) and to 181.2 ± 21.4 pS/pF (10 mM Ca²⁺; n = 3). These findings produced the important result that this nonselective cation channel is permeable to divalent cations and monovalent cations.

Ca²⁺ sensitivity of the nonselective cation channel. Another property specific to nonselective cation channels is their Ca²⁺ sensitivity. There are many reports regarding this property (4, 12). An increase in [Ca²⁺]ᵢ activated the background current of the cells bathed in an external solution containing 110 mM Na⁺ without bombesin. As shown in Fig. 6, the slope conductances obtained in three conditions of [Ca²⁺]ᵢ were 32.7 ± 4.0 pS/pF (n = 4) for 0.01 μM, 133.7 ± 7.6 pS/pF (n = 4) for 0.1 μM, and 167.4 ± 17.9 pS/pF (n = 4) for 1 μM. The elevation of intracellular Ca²⁺ activated these currents in a dose-dependent manner. The values for 0.1 μM [Ca²⁺]ᵢ and 1 μM [Ca²⁺]ᵢ were significantly larger than those for 0.01 μM [Ca²⁺]ᵢ. These results indicate that at least 0.1 μM [Ca²⁺]ᵢ is necessary for the channel to be in an activated state.

Cl⁻ conductance through the nonselective cation channel. The data presented so far do not exclude Cl⁻ permeation through the channel. We observed the currents with total replacement of Cl⁻ by aspartate⁻. Irrespective of the presence or the absence of Cl⁻, the cation permeability was not altered (data not shown). Eᵣₑᵥ were 3.7 ± 1.5 mV (potassium aspartate) and −1 ± 0 mV (KCl). These results indicate that this channel is not permeable to Cl⁻. Another source of evidence is provided by the experiments with the Cl⁻ channel blocker 9-AC. Application of 0.5 mM 9-AC did not suppress the conductance of the current of cells bathed in an external solution containing 110 mM NaCl (data not shown), nor was Eᵣₑᵥ changed by the application of 9-AC.

Innsensitivity of bombesin-sensitive current to the dihydropyridine blocker nifedipine. One possible route for Ca²⁺ influx through the plasma membrane is the L-type Ca²⁺ channel found in a variety of excitable cells. We therefore examined the effects of nifedipine on bombesin-induced currents. The enhanced currents induced by bombesin (3.2 × 10⁻⁷ M) were unaltered by nifedipine (5 × 10⁻⁶ M; data not shown). The slope conductances of the application of bombesin alone (3.2 × 10⁻⁷ M) and both bombesin (3.2 × 10⁻⁷ M) and

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Table 2. Eᵣₑᵥ and permeability ratio for monovalent and divalent cations

<table>
<thead>
<tr>
<th>Cation Species</th>
<th>Eᵣₑᵥ, mV</th>
<th>Permeability Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca⁺</td>
<td>0.28 ± 0.81</td>
<td>1.01</td>
</tr>
<tr>
<td>Rb⁺</td>
<td>0.16 ± 1.08</td>
<td>1</td>
</tr>
<tr>
<td>K⁺</td>
<td>0 ± 0</td>
<td>1</td>
</tr>
<tr>
<td>Na⁺</td>
<td>−3.63 ± 0.56</td>
<td>0.86</td>
</tr>
<tr>
<td>Li⁺</td>
<td>−8.16 ± 0.67</td>
<td>0.72</td>
</tr>
<tr>
<td>NMDG⁺</td>
<td>−15.6 ± 0.40</td>
<td>0.54</td>
</tr>
<tr>
<td>P_Ca/P_K</td>
<td>0.34</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE of 3 experiments in each group. NMDG, N-methyl-D-glucamine; P_Ca, Ca²⁺ permeability; P_K, K⁺ permeability.
nifedipine ($5 \times 10^{-6}$ M) were 181.6 ± 11.9 pS/pF ($n = 3$) and 185.0 ± 7.2 pS/pF ($n = 4$). These data suggest that the bombesin-induced current was not through voltage-gated L-type Ca$^{2+}$ channels.

**Blockers for the nonselective cation current.** It is known that La$^{3+}$ and Gd$^{3+}$ are effective blockers of nonselective cation channels (9, 11, 25). We therefore tested the effects of 100 μM Gd$^{3+}$ ($n = 3$) and 100 μM La$^{3+}$ ($n = 4$) on bombesin-induced currents (Fig. 7). Bombesin-induced currents were suppressed both by La$^{3+}$ and Gd$^{3+}$ to a level lower than that without bombesin, indicating that channel activation by bombesin is completely suppressed with La$^{3+}$ and Gd$^{3+}$. Gd$^{3+}$ (100 μM) reduced the membrane conductance by $93.5 \pm 0.5\%$ and La$^{3+}$ (100 μM) by $95.0 \pm 0.3\%$ with respect to current maximally activated by bombesin ($3.2 \times 10^{-7}$ M). It should also be noted that all of the I-V curves in the three conditions crossed at one point (Fig. 7, points a, b, and c). This confirmed that the $E_{rev}$ values had been measured accurately because the assumption that only the nonselective cation channel was influenced by bombesin was supported. Based on the $E_{rev}$ obtained here, $-5.20 \pm 1.37$ mV ($n = 3$) with Gd$^{3+}$ and $-3.18 \pm 1.06$ mV ($n = 4$) with La$^{3+}$, $P_{Na}/P_{K}$ ratios were calculated to be 0.81 and 0.88, respectively. These are consistent with data obtained earlier in this study.

**Effect of nonselective cation channel blockers on pepsinogen secretion.** If Ca$^{2+}$, via the nonselective cation channel, plays a role in pepsinogen secretion, Gd$^{3+}$ should suppress that secretion. That was found to be the case, and the evidence is shown in Fig. 8. Pepsinogen secretion was expressed as a percentage of total pepsinogen per hour and was monitored every few minutes. Bombesin (Fig. 8) stimulated pepsinogen secretion by $3.4 \pm 0.07$-fold without Gd$^{3+}$ ($n = 5$). Gd$^{3+}$ (100 μM) significantly suppressed bombesin-induced pepsinogen secretion by $25.1 \pm 0.3\%$ ($n = 5$) with respect to data without Gd$^{3+}$. These data indicate that nonselective cation channels are indeed involved in pepsinogen secretion in bullfrog esophageal mucosa.

**DISCUSSION**

Using the whole cell patch-clamp technique, we revealed the presence of a nonselective cation channel in pepsinogen-secreting cells of bullfrog during agonist stimulation. The channel was activated by bombesin or...
bethanechol, a secretagogue that elevates intracellular Ca$^{2+}$ and stimulates pepsinogen secretion.

The permeabilities to monovalent cations in the agonist-sensitive channel of frog pepsinogen-secreting cells found in this study were in the order $P_{Na}/P_K = P_{K_i}/P_{Na_i}/P_{Li_i} = 1.01:1.0086:0.72$. With regard to monovalent cation permeability, it was reported that the nonselective cation channels were equally permeable to Na$^+$ and K$^+$ but impermeable to Cl$^-$. Other studies have reported permeability ratios ($P_{Na}/P_K$) of nonselective cation channels of 0.89 in a cultured secretory epithelial cell line (4), 1.0 in pig coronary artery endothelial cells (1), 0.67 in guinea pig endocardial endothelial cells (11), and 1.0 in mouse neuroblastoma cells (30). Among these cell types, the following properties are common in nonselective cation channels: poor discrimination between monovalent cations, activation by intracellular Ca$^{2+}$ (1, 3, 4, 10, 30), and considerable permeability to Ca$^{2+}$ (1, 4, 10, 11, 14, 19). All of these characteristics were found in our newly discovered nonselective cation channels.

Previous studies reported that Ca$^{2+}$ was one of the major intracellular messengers of pepsinogen secretion. The concentration of intracellular Ca$^{2+}$ was elevated by the stimulation by ACh and/or bombesin (24, 26). It was also reported that a transient increase in [Ca$^{2+}$]$_i$ depends on the intracellular Ca$^{2+}$ store, and a sustained plateau of [Ca$^{2+}$]$_i$ depends on extracellular Ca$^{2+}$ (23, 27). Furthermore, as we have previously reported, when the extracellular Ca$^{2+}$ was chelated by EGTA, the sustained plateau of intracellular Ca$^{2+}$ was abolished, and, as a result, pepsinogen secretion was also abolished (6). There are many other reports concerning the role of intracellular Ca$^{2+}$ in pepsinogen secretion (16, 23, 28). All of these studies predicted the existence of a Ca$^{2+}$ entry pathway from the extracellular space, although the nature of such a pathway had not been elucidated. In these experiments, we demonstrated the existence of nonselective cation channels in these cells. It has been shown that nonselective cation channels have a relative permeability to Ca$^{2+}$ in many cells. In exocrine cells, there are some reports that these channels can provide a route for Ca$^{2+}$ entry (4, 13, 20). In this study, we demonstrated that the nonselective cation channel was permeable to Ca$^{2+}$ in a dose-dependent manner on the extracellular Ca$^{2+}$ concentration.

There have been a few reports concerning a possible Ca$^{2+}$ influx pathway in pepsinogen-secreting cells. In guinea pig gastric chief cells, Konada et al. (8) reported that ethanol stimulated pepsinogen secretion by enhancing Ca$^{2+}$ influx through Ca$^{2+}$ channels. Because La$^{3+}$ blocked the increase in [Ca$^{2+}$]$_i$ by ethanol, whereas neither nifedipine nor verapamil could inhibit it, they concluded that the extracellular Ca$^{2+}$ passed through La$^{3+}$-sensitive Ca$^{2+}$ channels but not through L-type Ca$^{2+}$ channels. However, it has been reported that La$^{3+}$ blocks L-type Ca$^{2+}$ channels and nonselective cation channels (18). Therefore, the blockage by La$^{3+}$ is not conclusive evidence that the influx pathway by ethanol is via Ca$^{2+}$ channels. Similarly, in frog esophageal pepsinogen-secreting cells, we have shown that the nonselective channel were blocked by La$^{3+}$ but were not blocked by nifedipine. In these cells, La$^{3+}$ blocked both bombesin-stimulated Ca$^{2+}$ influx and pepsinogen secretion (29). These data suggest that guinea pig gastric cells probably also have nonselective cation channels.

Most nonselective cation channels are sensitive to intracellular Ca$^{2+}$. In pepsinogen-secreting cells, the channels were activated by intracellular Ca$^{2+}$ in the range of 10$^{-7}$ to 10$^{-6}$ M. We demonstrated that the channel was activated by secretagogues that elevate [Ca$^{2+}$]$_i$. However, it has not been clarified whether such activation was attributable to a direct receptor-coupled mechanism or secondary to intracellular Ca$^{2+}$ elevation. In this study, we successfully demonstrated that a prerequisite for the activation of nonselective cation channels was an increase in [Ca$^{2+}$]$_i$, rather than the receptor stimulation by bombesin or other secretagogues. Nevertheless, the mechanisms that activate the channel by Ca$^{2+}$ and the signal pathways leading to pepsinogen secretion after an increase in [Ca$^{2+}$]$_i$ remain to be clarified. Because the channels could be seen only in the circumstances of a few millimolar intracellular ATP if the Ca$^{2+}$ concentration of the bathing solution was in the physiological range, the channel activation would seem to depend on the phosphorylation pathway.

The evidence for the involvement of nonselective cation channels in secretion is thought to be compatible with many other reports on peptic cells. First, the nonselective cation channel was activated by secretagogues, which stimulated pepsinogen secretion by intracellular Ca$^{2+}$ elevation. Second, this channel was activated by intracellular Ca$^{2+}$ elevation in a dose-dependent manner. Third, La$^{3+}$ and Gd$^{3+}$ blocked both pepsinogen secretion and channel activity. Fourth, La$^{3+}$ blocked the intracellular Ca$^{2+}$ elevation by ago-
nist stimulation. These data also suggested that the nonselective cation channels might participate as a physiological function of peptic cells. It is speculated that, at least in part, the nonselective cation channel is the physiological pathway of Ca\textsuperscript{2+} influx in peptic cells. In pepsinogen-secreting cells of the bullfrog, Uemura et al. (29) previously described that pepsinogen secretion was suppressed by La\textsuperscript{3+}. As demonstrated in this study, in these cell types, La\textsuperscript{3+} blocked the nonselective cation channel. It was also shown that Gd\textsuperscript{3+} blocked both channel activities and pepsinogen secretion. These data suggest that the activation of nonselective cation channels is certainly an important step in pepsinogen secretion. However, there remains the discrepancy that, although La\textsuperscript{3+} or Gd\textsuperscript{3+} reduced pepsinogen secretion by only 20–50%, these blockers suppressed the nonselective cation channels almost totally. This discrepancy can be explained in part by differences in the final dose of these blockers because of differences in the preparations used; the former was multicellular, whereas the latter was a single cell preparation.

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