J. Membrane Biol. 164, 197–203 (1998)

The Journal of
Membrane
Biology

Potentiation by Isoproterenol on Carbachol-induced K^+ and Cl^- Currents and Fluid Secretion in Rat Parotid

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Received: 6 October 1997/Revised: 16 April 1998

Abstract. Isoproterenol (IPR) and 8-(4-chlorophenylthio)-cyclic AMP (cpt-cAMP) enhanced carbachol (CCh)-induced fluid secretion from rat parotid glands, but had no effect by themselves. The enhancement by IPR was blocked by propranolol. In dispersed parotid acinar cells, IPR and cpt-cAMP potentiated CCh-induced K^+ and Cl^- currents (I_K and I_{Cl}). IPR at the concentration of 0.1 μM significantly potentiated the CCh-induced increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), but 1 mM cpt-cAMP did not. The incidence of the potentiation by IPR in CCh-induced Mn^{2+} entry was 31% and that by cpt-cAMP was 21%. The potentiation by IPR in the ionic currents and the $[Ca^{2+}]_i$ was suppressed by propranolol. These results suggest that the CCh-induced fluid secretion from rat parotid glands is enhanced by IPR through the potentiation of I_K and I_{Cl} mainly by the increased cyclic AMP level and partially by the potentiated Ca^{2+} influx and $[Ca^{2+}]_i$ increase, and that IPR is more effective than cpt-cAMP in the enhancement of the CCh-induced $[Ca^{2+}]_i$ increase.

Key words: Parotid — Potassium current — Chloride current — Carbachol — Isoproterenol — Calcium

Introduction

The autonomic nervous system regulates salivary secretion [2]. The secretions of salivary juice and amylase are controlled through the increase in the intracellular calcium ions and adenosine 3′,5′-cyclic monophosphate (cAMP) by parasympathetic and sympathetic nerves, respectively [2]. Both nerves act on the salivary secretion in a synergistic manner [1]. Amylase secretion from parotid glands by adrenergic agonists is potentiated by cholinergic agonists [4]. Interaction between intracellular signals such as calcium ions and cAMP has been well investigated on the potentiation of amylase secretion [12, 21]. Fluid secretion by cholinergic agonists is also potentiated by forskolin and VIP which increase intracellular cAMP [10]. However, the mechanism of the potentiation of fluid secretion by cAMP-increasing agents still remains unclear.

The fluid secretion is mediated through the increase in intracellular calcium ions by cholinergic agonists [13]. The increase in intracellular calcium ions by carbachol (CCh) is due to the release from intracellular pools and to the influx of calcium ions from extracellular spaces [16]. The fluid secretion requires the increase in intracellular calcium ions from both pathways [22]. Addition of cAMP increases the release of calcium ions from intracellular pools in rat parotid glands [18]. The potentiation of fluid secretion by cAMP-increasing agents might be induced by a further increase in the intracellular concentration of calcium ions ($[Ca^{2+}]_i$) from intracellular stores and/or extracellular spaces.

The increase in $[Ca^{2+}]_i$ by CCh activates potassium channels and chloride channels which are thought to be in basolateral membranes and luminal membranes, respectively, and the activation of ionic fluxes plays a key role in the fluid secretion [15]. The ion fluxes through ion channels are measured as ionic currents, potassium current (I_K) and chloride current (I_{Cl}), by whole-cell patch-clamp techniques [3, 5, 8, 19]. I_{Cl} and I_K activated by CCh in the salivary glands are dependent on the level of $[Ca^{2+}]_i$ [5, 8], while cAMP-dependent I_{Cl} is reported

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in other exocrine glands [11]. The study on the ionic currents induced by CCh and adrenergic agonists may clarify the mechanism of potentiated fluid secretion.

In this paper, we investigated the effects of isoproterenol (IPR), a cAMP-increasing β-adrenergic agonist, on CCh-induced fluid secretion, calcium response and current responses in rat parotid glands. We demonstrated that IPR enhanced CCh-induced saliva secretion from the parotid glands through the potentiation of K^+ and Cl^- currents in the acinar cells, depending on cAMP and Ca^{2+} influx.

Materials and Methods

Measurement of Stimulated Saliva

Male Wistar rats (290–350 g) were anesthetized with pentobarbital sodium (Nembutal, 70 mg/kg, i.p.). Either the right or left duct of rat parotid glands was cannulated. Saliva was collected every minute and weighted. The saliva secretory rate was calculated from the weight of the saliva collected for every 1 min divided by the body weight of the rat. The drugs were injected in a volume of 0.29–0.35 ml in the femoral vein [10]. Each injection took 15 sec and was followed by flushing the cannula with saline for 15 sec. All experiments were performed at room temperature (25–27°C).

Measurement of Ionic Currents

Parotid glands were removed from male Wistar rats (260–350 g), which were anesthetized by pentobarbital sodium (Nembutal, 70 mg/kg), and digested for 7–10 min at 37°C by 0.05% trypsin (Nakarai, Kyoto, Japan) dissolved in phosphate-buffered saline containing 0.016% EDTA and then for 30 min at 37°C by 0.16% collagenase type I (Sigma, St. Louis, MO). Dispersed cells were filtered with 50 μm nylon mesh and placed on cover glasses with a diameter of 5 mm. After the cells were attached, one of the cover glasses was placed in a 0.2 ml chamber which was constantly perfused at a rate of 1 ml/min with modified Krebs-Henseleit Ringer (KHR) solution containing (in mM) 103 NaCl, 4.7 KCl, 2.56 $CaCl_2$, 1.13 $MgCl_2$, 2.8 glucose, 4.9 sodium pyruvate, 2.7 fumaric acid disodium salt, 4.9 L-glutamic acid monosodium salt, 12.5 HEPES-NaOH (pH 7.4), 25 $NaHCO_3$, and 1.15 NaH_2PO_4. Patch-clamp pipettes were pulled from borosilicate glass capillaries (Sutter Instrument, Novato, CA). Pipettes were filled with the standard KCl-rich pipette solution containing (in mM): 140 KCl, 1 $MgCl_2$, 10 HEPES, 0.5 ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), 10 glucose [6], and 1 ATP [5] and was adjusted to pH 7.4 by KOH. A patch/whole-cell clamp amplifier CEZ-2400 (Nihon Kohden, Tokyo, Japan) was used to measure whole-cell current. We used only parotid acinar cells, distinguishable from duct cells and other cell types, with the shape of the cells and intracellular localization of secretory granules. Chloride and potassium currents of the cells were separated by a method basically similar to those that many workers used [3, 5, 8], changing the membrane potential from the holding potential (−80 mV) to 0 mV briefly (100 msec) at 5-sec intervals. The currents were recorded on the chart of a pen recorder. The series resistance (14.2 ± 5.9 MΩ, mean ± SD, $n = 87$) was not compensated, but it did not affect the relative comparison of the amplitude of the I_K and I_{Cl} in different cells. All measurements were performed at room temperature (24–27°C). For the calculation of the mean amplitude of CCh-induced currents, current amplitude was averaged for 1 min at the period of maximum response in each cell. To evaluate IPR-evoked potentiation of CCh-induced currents, we calculated the amplitude ratio of CCh-induced currents averaged for 1 min after the addition of IPR / CCh-induced currents averaged for 1 min just before the addition of IPR. The ratio of the averaged currents was smaller than 1.2 in the presence of 1 μM propranolol, a potent antagonist of β-adrenergic receptor. Therefore, we set the threshold ratio of the potentiation at 1.2 for the calculation of the incidence of the potentiation.

Measurement of $[Ca^{2+}]_i$ and Mn^{2+} Entry

Cells prepared for $[Ca^{2+}]_i$ measurement were obtained by almost the same methods as previously described, except for the incubation with enzymes. Parotid glands were digested with 0.1% collagenase (type S-1, Nitta Gelatin, Osaka, Japan) at 37°C for 30 min. Trypsin was not used in this case. The dispersed cells were then incubated with 2 μM fura-2/AM (Dojindo, Kumamoto, Japan) at 37°C for 20 min. $[Ca^{2+}]_i$ was calculated from the fluorescence ratio (F340/F360) measured with the ARGUS-50/CA system (Hamamatsu Photonics, Hamamatsu, Japan). Calibration of calcium concentration was made using artificial internal solutions which contain (in mM): 150 KCl, 0.8 $MgCl_2$, 10 MOPS-KOH (pH 7.2), 10 EGTA, and 0-10 $CaCl_2$. The average and the standard deviation of $[Ca^{2+}]_i$ were calculated from the measurements in 7 cells. Other details were the same as those described by Sugita et al. [20]. To get the time course of Mn^{2+} entry, the quenching of 360 nm fluorescence of fura-2 was measured in the presence of 200 μM of Mn^{2+} in the external solution and normalized (initial value = 100%). All measurements were performed at 24–26°C.

Chemicals

Carbamylcholine chloride (carbachol), ATP, and 8-(4-chlorophenylthio)-adenosine 3′,5′-cyclic monophosphate (cpt-cAMP) were purchased from Sigma (St. Louis, MO). DL-isoproterenol hydrochloride and DL-propranolol hydrochloride were obtained from Nakarai (Kyoto, Japan).

Results

Potentiation of CCh-induced Secretion by IPR and cAMP

Intravenous application of CCh (20 nmol/kg) induced a transient secretion of saliva from the parotid (Fig. 1*A*, a). IPR (20 nmol/kg), applied 3 min after CCh administration, potentiated the CCh-induced salivary secretion from the gland (Fig. 1*A*, b), although IPR had no effect by itself (Fig. 1*A*, c). This enhancement by IPR was inhibited by preapplication of propranolol (200 nmol/kg) (Fig. 1*B*). Intravenous application of cpt-cAMP (100 μmol/kg) did not induce salivary secretion by itself but enhanced the CCh-induced secretion of saliva (Fig. 2). Typical data from more than three sets of measurements are shown in each experiment.

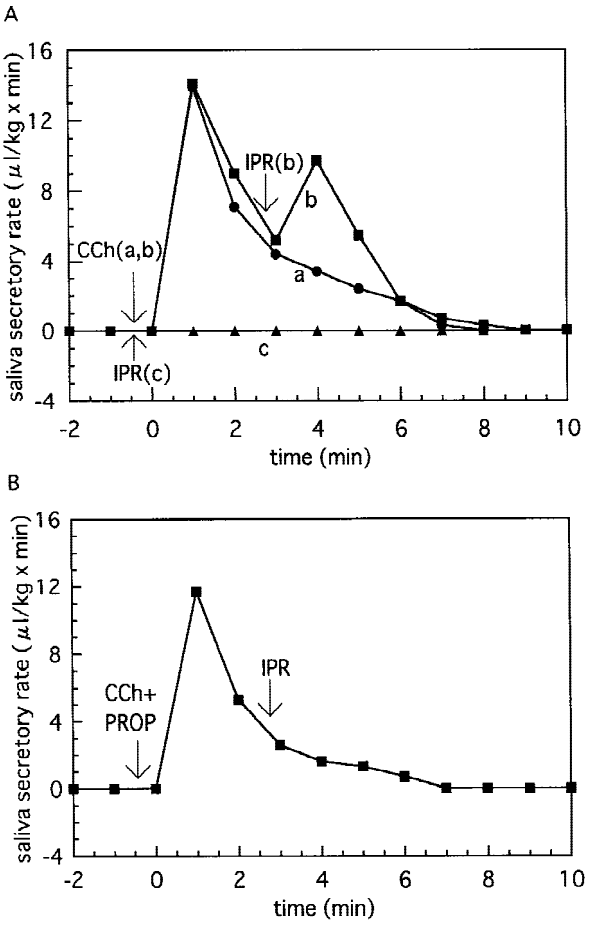

Fig. 1. (*A*) Enhancement of CCh-induced salivary secretion by IPR in rat parotid gland. CCh-induced salivary secretion (a), enhancement of the secretion by IPR (b) and no salivary secretion by IPR alone (c). Amount of injected drugs was 20 nmol/kg for both CCh and IPR. Each injection started at the points indicated by arrows. IPR was injected 3 min 15 sec after the injection of CCh in b. (*B*) Inhibition of IPR-evoked potentiation by propranolol (200 nmol/kg) in CCh-induced salivary secretion.

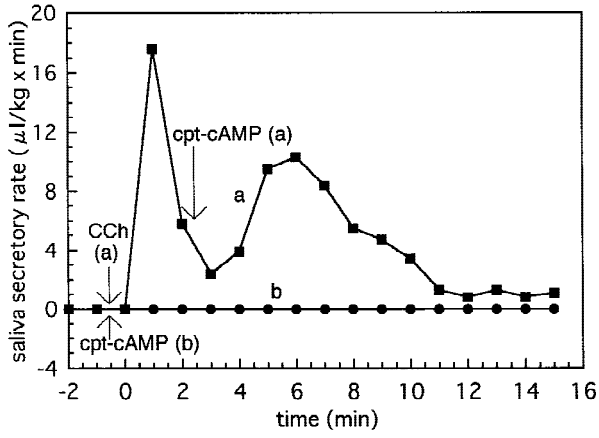

Fig. 2. Enhancement of CCh-induced salivary secretion by cpt-cAMP (a) and no salivary secretion by cpt-cAMP alone (b). Amount of injected drugs was 20 nmol/kg for CCh and 100 μmol/kg for cpt-cAMP. Each injection started at the points indicated by arrows.

Potentiation of CCh-induced Membrane Currents by IPR

Current responses induced by CCh were investigated by whole-cell patch-clamp techniques on the isolated acinar cells. CCh at 0.25 μM evoked the oscillatory inward current and the sustained outward current (Fig. 3). The inward current and outward current were I_{Cl} and I_K, respectively, as described in Materials and Methods.

The incidence and mean amplitudes of I_K and I_{Cl} induced by 0.25 μM CCh are summarized in Table 1. In almost all acinar cells we tested, CCh evoked I_k and I_{Cl}. The mean amplitudes of I_K and I_{Cl} were 0.55 and 0.28 nA, respectively.

Addition of 0.1 μM IPR did not induce any ionic current in 6 of 7 cells tested. In only one of the 7 cells, IPR induced slowly activated outward and inward currents with amplitudes of 0.1 nA (outward) and 0.02 nA (inward), respectively. The incidence and the amplitude of the IPR-induced currents were negligible compared to those of the CCh-induced currents. When 0.1 μM IPR was added in the presence of 0.25 μM CCh, the current responses were potentiated. The incidence and the % increase in the current amplitude in the potentiation of the CCh-induced I_K and I_{Cl} are summarized in Tables 2 and 3. The incidence of the potentiation in the CCh-induced I_K and I_{Cl} by IPR was 100% and 55%, respectively, in the cells tested ($n = 20$). The mean amplitudes of potentiated I_K and I_{Cl} by IPR increased by almost 80% and 100%, respectively, at 2.5 ± 1.0 min (mean ± SD, $n = 20$ in I_K) and 2.8 ± 1.1 min ($n = 11$ in I_{Cl}) after the addition of IPR. Figure 4 shows a typical case of the potentiation of CCh-induced current responses by IPR.

Effects of Propranolol on the Potentiation and the Effect of cAMP on the Currents

The potentiation by IPR of the CCh-induced membrane currents was significantly blocked by a β-adrenergic antagonist propranolol (1 μM) (Table 2). To determine whether cAMP was involved in the IPR-induced potentiation, cpt-cAMP, a potent analogue of cAMP, was added to the external solution during the application of CCh. The CCh-induced ionic currents were potentiated by 1 mM cpt-cAMP (Tables 2 and 3). However, the incidence of the potentiation by cpt-cAMP in the CCh-induced I_K was significantly lower than that by IPR. In the CCh-induced I_{Cl}, the incidence of the potentiation by cpt-cAMP was similar to that by IPR.

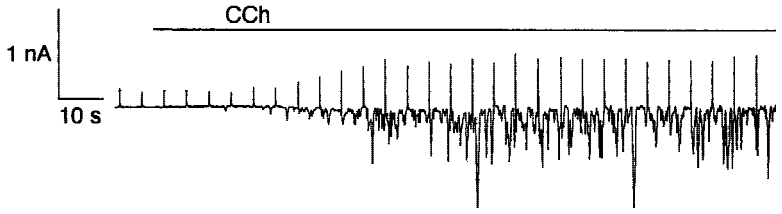

Fig. 3. CCh (0.25 μM)-induced I_K and I_{Cl} in a parotid acinar cell. I_K is the peak current of each pulse measured at 0 mV, and I_{Cl} is the inward oscillatory current measured at −80 mV.

Table 1. Incidence and amplitude of current responses (I_K and I_{Cl}) induced by CCh

	Incidence (%)	*n*	Amplitude (nA)	*n*
I_K	92	52	0.55 ± 0.41	48
I_{Cl}	79	52	0.28 ± 0.24	41

CCh (0.25 μM) was added to the external solution (modified KHR solution). The incidence is indicated as the percentage of the responding cells in total cells used in the experiment. Current amplitude was averaged for 1 min at the period of maximum response in each cell, and the value is shown by mean ± SD in only responding cells.

Potentiation of CCh-induced $[Ca^{2+}]_i$ Increase by IPR and Effect of cAMP

The effect of IPR on the CCh-induced increase in $[Ca^{2+}]_i$ was investigated to clarify the involvement of $[Ca^{2+}]_i$ in the potentiation of current responses, because the CCh-induced I_K and I_{Cl} were well known to be activated by the increase in $[Ca^{2+}]_i$ [8]. Addition of CCh (0.25 μM) induced a rapid increase in $[Ca^{2+}]_i$, and then the sustained high level of $[Ca^{2+}]_i$ as shown in Fig. 5, where the time course of $[Ca^{2+}]_i$ in 7 cells of the same experiment is shown in mean ± SD.

The increase in $[Ca^{2+}]_i$ induced by 0.25 μM CCh was significantly enhanced by 0.1 μM IPR (Fig. 5), while IPR itself did not change $[Ca^{2+}]_i$ in all cells tested (n = 54). The IPR-enhanced increase in $[Ca^{2+}]_i$ was sustained after the removal of IPR. Propranolol (1 μM) inhibited the potentiation by IPR of the CCh-induced increase in $[Ca^{2+}]_i$ (Fig. 6*A*). Addition of cpt-cAMP (1 mM) tended to potentiate slightly, but did not significantly, the CCh-induced increase in $[Ca^{2+}]_i$ (Fig. 6*B*).

Potentiation of CCh-induced Mn^{2+} Entry by IPR and cAMP

In the presence of 200 μM external Mn^{2+}, CCh (0.25 μM) increased the fura-2 quenching rate (line b in Fig. 7). IPR (0.1 μM) did not increase the fura-2 quenching rate by itself (*data not shown*), but the addition of 0.1 μM IPR potentiated the CCh-induced increase in the quenching rate of fura-2 (line a in Fig. 7), indicating that IPR potentiated the CCh-induced Mn^{2+} entry. The incidence of the IPR-evoked potentiation in the CCh-induced Mn^{2+} entry was 31% (n = 93), and the % increase in the potentiated Mn^{2+} entry was 142 ± 29% (mean ± SE, n = 29). Propranolol (1 μM) decreased the IPR-induced potentiation of the Mn^{2+} entry. The incidence of the potentiation with propranolol was 5% (n = 20). The addition of 1 mM cpt-cAMP also potentiated the CCh-induced Mn^{2+} entry (Fig. 8). The incidence of the potentiation by cpt-cAMP was 21% (n = 52) and the % increase in the potentiated Mn^{2+} entry was 102 ± 14% (mean ± SE, n = 11).

Discussion

Intravenous application of IPR (20 nmol/kg) potentiated the CCh-induced volume secretion of saliva from the parotid gland, as is the case for vasoactive intestinal polypeptide and calcitonin gene-related peptide which also increase the fluid secretion evoked by cholinergic agonists [10]. Propranolol suppressed the potentiation of the CCh-induced fluid secretion by IPR, and cAMP potentiated the CCh-induced fluid secretion, indicating that the CCh-induced fluid secretion is potentiated by IPR through β-action. On the other hand, amylase secretion from the parotid, induced by β-adrenergic agonists or other cAMP-increasing agents, is enhanced by cholinergic agonists or other Ca^{2+}-mobilizing agents [4, 12]. Taken together, the signaling of Ca^{2+} is accelerated by the signaling of cAMP, and vice-versa. CCh-induced fluid secretion and IPR-induced amylase secretion from the rat parotid glands are regulated through the synergistic action between intracellular signals such as cAMP and Ca^{2+}. However, the CCh-induced fluid secretion is enhanced by IPR at low concentrations, while the synergistic release of amylase is evoked by the combination of low concentration of CCh and high concentration of IPR.

CCh induced the membrane currents in rat parotid acinar cells. With the current-recording method described in Materials and Methods, the outward and inward currents were expected to be I_K and I_{Cl}, respectively, which were supported by our preliminary data on the blockage of I_K by charybdotoxin and I_{Cl} by diphenylamine-2-carboxylate (Hirono et al., *unpublished data*). Addition of IPR at a low concentration (0.1 μM) potentiated the CCh-induced ionic currents (I_K and I_{Cl}), while IPR itself did not evoke any marked current response. IPR does not induce ionic current even at the

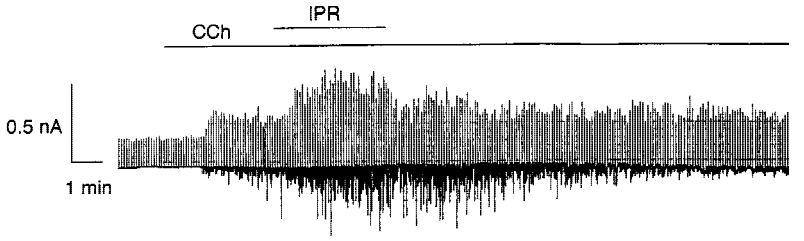

Fig. 4. Potentiation of 0.25 μM CCh-induced I_K and I_{Cl} by 0.1 μM IPR.

Table 2. Incidence in the potentiation of CCh-induced I_K and I_{Cl} by IPR and cpt-cAMP

Ext. soln	Incidence of potentiation		
	I_K (%)	I_{Cl} (%)	*n*
IPR			
Control	100	55	20
Propranolol	7	13	15
cpt-cAMP	73	64	11

CCh (0.25 μM) was added to each external solution 3–5 min before the addition of 0.1 μM IPR or 1 mM cpt-cAMP. Propranolol (1 μM) was applied simultaneously with CCh. Propranolol significantly inhibited the potentiation by IPR in each current.

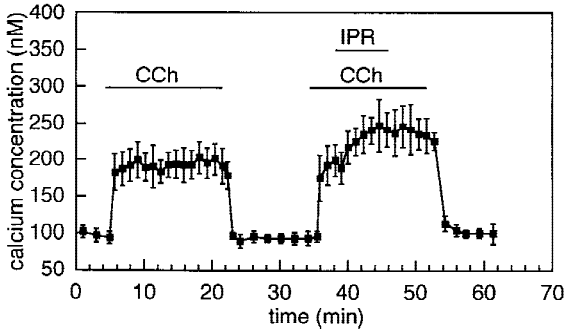

Fig. 5. Increase in $[Ca^{2+}]_i$ induced by 0.25 μM CCh (the first application of CCh) and 0.1 μM IPR-evoked potentiation of 0.25 μM CCh-induced increase in $[Ca^{2+}]_i$ (the second application of CCh).

Table 3. % increase in the potentiation of CCh-induced currents by IPR and cpt-cAMP

Ext. soln	% increase in the response			
	I_K (%)	*n*	I_{Cl} (%)	*n*
IPR				
Control	80 ± 10	20	97 ± 25	11
Propranolol	46	1	47 ± 3	2
cpt-cAMP	69 ± 13	8	123 ± 24	7

% increase in amplitude (mean ± SE) of 0.25 μM CCh-induced currents potentiated by 0.1 μM IPR or 1 mM cpt-cAMP was calculated against CCh-induced currents just before the addition of IPR or cpt-cAMP. For this calculation, the CCh-induced currents were averaged for 1 min only in the cells with potentiated currents.

concentration of 1 μM [5]. The CCh-induced I_K and I_{Cl} were also potentiated by cpt-cAMP, suggesting that the effluxes of potassium ions and chloride ions were enhanced by IPR via the mediation of cAMP as was the case of fluid secretion. In addition, propranolol suppressed the IPR-evoked potentiation of both CCh-induced fluid secretion and CCh-induced ionic currents. Since the fluid secretion requires the efflux of potassium ions and chloride ions [13], the potentiation of I_K and I_{Cl} with cpt-cAMP and IPR may be responsible for the potentiation of fluid secretion. Modulation of Ca^{2+}-dependent responses by cAMP is also observed in the rat submandibular gland, where a cAMP-increasing agent, forskolin, enhances the transient initial phase of fluid secretion induced by A-23187 and TEA-insensitive, Ca^{2+}-activated K^+ conductance, and reduces the smaller sustained phase of fluid secretion induced by A-23187 [7].

IPR potentiated the rise of $[Ca^{2+}]_i$ elicited by CCh, while having no stimulatory effect of its own. A similar discovery was also made by other workers [12]. The addition of cAMP was reported to stimulate the CCh-induced release of intracellular Ca^{2+} from the intracellular pool in parotid glands [18]. However, the results obtained in this paper suggest that IPR, but not cAMP, markedly increases $[Ca^{2+}]_i$ under the CCh-induced high level of $[Ca^{2+}]_i$, partially through the potentiation of the Ca^{2+} influx. A simple explanation of the discrepancy between the effects of IPR and cpt-cAMP is that the dose of cpt-cAMP was not high enough to evoke the full effect on CCh-induced $[Ca^{2+}]_i$ increase, though cpt-cAMP is a potent and membrane-permeable analogue of cAMP and generally used at the concentration of about 0.1 mM which is one tenth of the concentration we used. An alternative explanation is that cpt-cAMP, not cAMP, might fail to induce full activation of cAMP-dependent protein kinase (PKA), even in high dose, with the specificity for different binding sites of PKA in the parotid gland [17]. In addition, the possibility is not negligible that IPR may exert other actions on Ca^{2+} mobilization not through the increase in cAMP. IPR, but not cAMP, might markedly activate another Ca^{2+} entry mechanism,

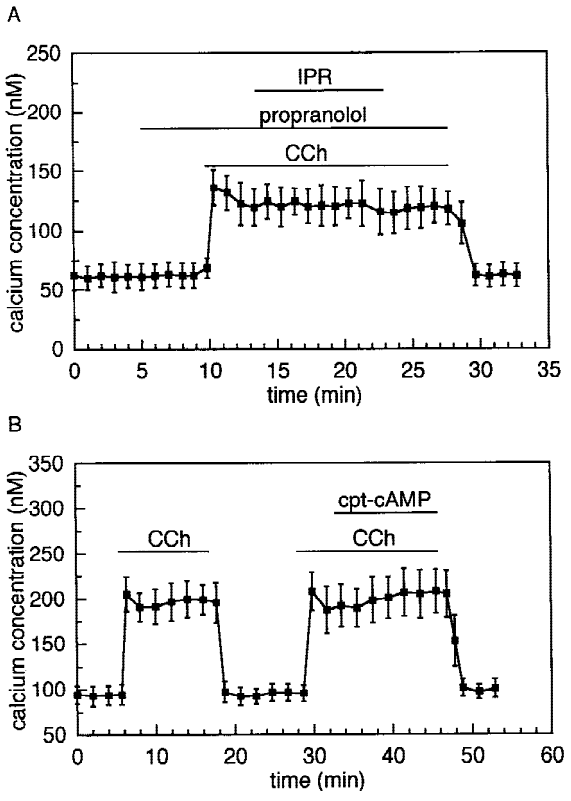

Fig. 6. Inhibition by 1 μM propranolol of the potentiation by 0.1 μM IPR in 0.25 μM CCh-induced increase in $[Ca^{2+}]_i$ (*A*), and the effect of 1 mM cpt-cAMP on 0.25 μM CCh-induced increase in $[Ca^{2+}]_i$ (*B*).

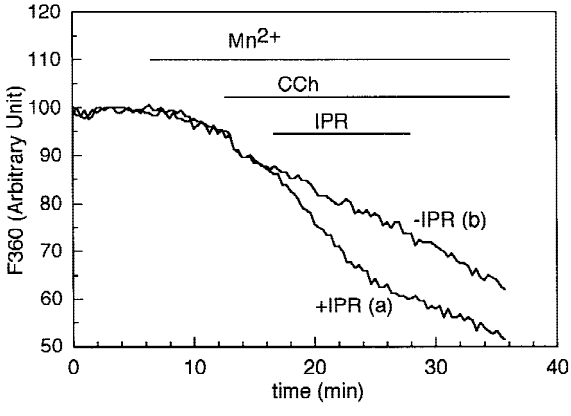

Fig. 7. Potentiation of CCh (0.25 μM)-induced Mn^{2+} entry by 0.1 μM IPR (a) and control CCh-induced Mn^{2+} entry (b). Intensity of 360 nm fluorescence of fura-2 was measured and normalized.

permeable to Ca^{2+} but impermeable to Mn^{2+}, as reported in HSG cells [9].

The effects of IPR and cpt-cAMP on CCh-induced ionic currents and Mn^{2+} entry were not always the same in all cells studied. The sensitivity of the cell to IPR and cpt-cAMP may vary in many cells for unknown reasons

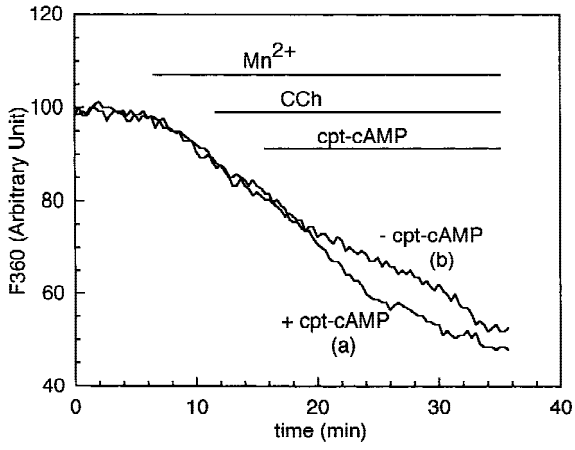

Fig. 8. Potentiation of CCh (0.25 μM)-induced Mn^{2+} entry by 1 mM cpt-cAMP (a) and control CCh-induced Mn^{2+} entry (b).

or possibly by the difference in the secretory cycle of the cell. It is unlikely that the low incidence of the potentiation in I_K, I_{Cl} and Mn^{2+} entry is due to cell damage during preparation or contamination of the duct cell or other cell type because I_K and I_{Cl} were induced by CCh in almost all acinar cells and the CCh-induced I_K was potentiated by IPR in all 20 cells tested.

Both CCh-induced I_K and I_{Cl} were potentiated by IPR and cpt-cAMP. The potentiation of I_K and I_{Cl} by IPR may be basically due to the potentiation of Ca^{2+}-dependent K^+ and Cl^- channel activity by the increase in the cAMP level evoked by IPR during the CCh-induced $[Ca^{2+}]_i$ increase. In addition, the potentiation of I_K and I_{Cl} by IPR may have also partially resulted from the further increase in Ca^{2+} influx and $[Ca^{2+}]_i$. Because both IPR and cpt-cAMP potentiate the CCh-induced saliva secretion, the I_K and I_{Cl} potentiated by cAMP under a high level of $[Ca^{2+}]_i$ may be responsible for the IPR-potentiated fluid secretion.

The activation of cotransporter and exchangers is essential for the maintenance of high intracellular Cl^- [13]. IPR stimulates the Na^+-K^+-$2Cl^-$ cotransporter in the rat parotid glands [14]. However, the electroneutral transport of ions is not detectable in electrophysiological measurements. Although the potentiation of CCh-induced I_K and I_{Cl} by IPR may contribute to the potentiation in CCh-induced fluid secretion by IPR, up-regulation of the Na^+-K^+-$2Cl^-$ cotransporter by IPR also enhances the fluid secretion with increasing intracellular K^+ and Cl^-.

In this paper, IPR and cpt-cAMP are shown to potentiate the CCh-induced fluid secretion and the CCh-induced ionic currents in rat parotid acinar cells. The potentiation of I_K and I_{Cl} through the interaction between cAMP and CCh-induced increase in $[Ca^{2+}]_i$ may contribute to the enhancement of the CCh-induced fluid secretion.

This work was partially supported by a Grant-in-Aid for Scientific Research (06671856) from the Ministry of Education, Science, Sports and Culture of Japan.

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