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Voltage-Dependent Ionic Currents and Their Regulation by GTP and Phorbol Ester in the Unfertilized Eggs of Mouse and Hamster

Ik-Hyun Kim, Yang-Mi Kim, Jae-Hee Haan
Choon-Ok Park and Seong-Geun Hong

Department of Physiology, Gyeongsang National University College of Medicine, Chinju, 660-280

= ABSTRACT =

The present study was performed to investigate the properties of ionic currents elicited by voltage pulses in the unfertilized eggs of mouse and hamster by using the whole-cell voltage-clamp techniques and to find out if there are any differences in properties between eggs of the two rodents. In addition, the modulatory effect of G-proteins and protein kinase C (PKC) on the ionic channels were observed. The inward current in hamster eggs was shown to be due to Ca^{2+} current (i_{Ca}). The current-voltage relations of these currents in hamster egg were analogous to those in mouse eggs. The amplitude of i_{Ca} in the hamster egg was larger than that in the mouse egg (-3.12 ± 1.07 nA vs. -1.71 ± 0.71 nA, mean $\pm$ SD). These results suggest that the Ca^{2+} channels in both kinds of eggs have similar channel properties but their density, and/or conductance per unit area is higher in hamster eggs than in mouse eggs. Outward currents in eggs of both mouse and hamster were carried by K^+. In hamster eggs, they appeared to comprise at least two components; a transient outward component (i_{to}) and a steady-state component (i_∞). The i_{to} was found to be dependent on intracellular Ca^{2+} concentration; whereas on the other hand i_∞ was Ca^{2+}-independent.

Ca^{2+} currents were increased in eggs treated with GTP (or GTPγS) or fluoroaluminate (AlF_4^-). In the hamster egg these increments were antagonized by GDP (or GDPβS) application. In contrast to the enhancement of i_{Ca}, i_K was reduced following GTP (or GTPγS) perfusion in mouse eggs. The transient component (i_{to}) in hamster eggs was increased by adding GTP but decreased by phorbol ester, TPA or dioctanoyl glycerol (DOG). Simultaneous application of GTPγS and DOG suppressed i_{to} more effectively than a single application or DOG or TPA.

From the above results, we have shown that ionic currents elicited by voltage pulses existed in the unfertilized eggs of mouse and hamster. There are at least two types of currents, i_{Ca} and i_K in mouse eggs, while three types, i_{Ca}, Ca^{2+}-dependent i_K and Ca^{2+}-independent i_K exist in hamster eggs. Ionic channels in these eggs may be regulated either directly by GTP and PKC or indirectly by the substances linked with GTP and PKC.

Key Words: Ionic currents, Mouse egg, Hamster egg, GTP, Protein kinase C, Phorbol ester, Whole cell current

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INTRODUCTION

The elucidation of electrical properties of the oocyte membrane would provide a basis for understanding the nature of the excitable membrane from the view point of ontogenesis and differentiation. For this purpose, eggs from a wide range of animals have been studied (Hagiwara & Jaffe, 1979; Hagiwara, 1983). In eggs from mammals such as mouse and hamster, Ca^{2+} and K^+ currents or channels have been implicated either directly from voltage-clamp study or indirectly from their electrical phenomena (Hong et al, 1991; Hong et al, 1992; Okamoto et al, 1977; Peres, 1987; Park et al, 1992; Yoshida et al, 1990). Ca^{2+} current (i_{Ca}) in the mouse egg was confirmed and its properties were relatively well documented on the basis of observation of Ca^{2+} spikes elicted by electrical stimuli, while that in the hamster egg was neither confirmed nor documented. Furthermore the presence of specific K^+ channels in the two mammalian oocytes is unclear (Okamoto et al, 1977; Peres, 1987).

It is now known that many ionic channels are regulated by cell surface receptors and well-known second messengers such as cAMP (Adenosine 3' : 5'-cyclic monophosphate), cGMP (Guanosine 3' : 5'-cyclic monophosphate) and inositol trisphosphate ($InsP_3$). The regulation of many ionic channels by second messengers predicted a role for G-proteins (Brown, 1991). There are several implications that the substances involved in cell-signalling may affect ionic channels in mouse and hamster eggs. Both the β-adrenergic agonist, isoprenaline, and cAMP increases i_{Ca} in the mouse egg (Cheong, 1992). In the hamster egg, the intracellular Ca^{2+} level ($[Ca^{2+}]_i$) is regulated by GTP, $InsP_3$, and protein kinase C (PKC) (Miyazaki, 1988;, 1991; Miyazaki et al, 1990). If Ca^{2+} channels in hamster eggs are regulated by G-proteins or protein kinases, Ca^{2+} influx via Ca^{2+} channels can contribute to the rise of $[Ca^{2+}]_i$. This assumption requires the presence of Ca^{2+} channels in the hamster oocyte membrane. However there are few reports of Ca^{2+} current in the hamster egg. Elucidation of channel properties in mouse and hamster eggs will allow a better understanding of the physiological role of ionic channels in differentiation and provide information about the development of excitability.

As the first trial to elucidate the role(s) of ionic channels in cell differentiation and development of mammals, the present study was performed to find the ionic currents and modulation of ionic channels by GTP (Guanosine 5'-triphosphate) and PKC (Protein kinase C) in mouse and hamster eggs. Unfortunately, there is little information about the physiological ionic composition of eggs surrounded by oviductal fluid. In order to compare electrical properties of the two kinds of egg two assumptions were made to keep the experimental condition constant; first, that the unfertilized egg be an independent excitable cell. Second, that the internal environment of hamster eggs be similar to that of mouse eggs although this would not be the case.

METHODS

Preparation of eggs

Mice (mixed breed ICR strain supplied from Yuhan Research Center, older than 6 weeks) were used as donors of unfertilized eggs. Zona-free eggs were prepared by the same procedure that used by Park et al (1991).

Sexually mature female hamsters, older than 8 weeks, were also used as egg donors. The animals were induced to ovulate with intraperitoneal injection of 20 units of pregnant mare serum gonadotropin and 20 units of human chorionic gonadotropin (HCG). The eggs were collected from the oviducts 12~17 hours after HCG injection. Cumulus cells surrounding the eggs were removed by treatment with 100 units hyaluronidase (Sigma Type 1-S) for 2~3 min

at room temperature and the zona pellucida was subsequently freed with 1 unit of protease (Sigma Type VII) for 10~15 sec at room temprature. After digestion of the zona pellucida, the eggs were washed several times with a medium containing (in mM): NaCl, 140; Na-pyruvate, 0.1; Lactate, 10; HEPES (N-2-hydroxy ethylpiperazine-N'-2-ethanesulfonic acid), 20; $MgCl_2$, 1.2; $CaCl_2$, 2; KCl, 6; and 2 mg/ml polyvinylpyrrolidone. Zona-free eggs were transferred to a 35 mm plastic Petri dish. All eggs were used within 8 hours of collection from the oviduct. They were kept in a refrigerator before use (−4℃).

Solutions

The control external solution contained (in mM): NaCl 120; KCl, 6; Lactate, 10; Na-pyruvate, 0.1; $MgCl_2$, 1.2; $CaCl_2$, 10; HEPES, 20. In some experiments $CaCl_2$ was substituted by equimolar amounts of $SrCl_2$ or $BaCl_2$. When adding 20 mM tetraethylammonium chloride (TEA-Cl), equimolar NaCl was lowered to balance the tonicity of external solution.

The pipette solution contained (in mM): KCl, 140; $MgCl_2$, 2; phosphocreatine-ditris, 5; ATP (adenosine triphosphate), 1; HEPES, 10. In some experiments KCl in the pipette solution was replaced by CsCl (120 mM) and TEA-Cl (20 mM), EGTA (10 mM) was dissolved and diluted with the normal pipette solution. When the extracellular K^+ or Ca^{2+} concentration was changed the tonicity of solution was balanced by altering the extracellular Na^+ concentration.

GTP, GTPγS [Guanosine-5'-0-(3-thiotriphosphate)], or GDPβS [Guanosine 5'-0-(2-thiotriphosphate)] obtained from Sigma were added to the pipette solution without modifying ionic composition. Concentrations of GTP and GTP γS used in this study were 1~10 mM and 100 ~500 μM respectively. Instead of GTP or GTP γS, fluoroaluminate (AlF_4^-) was sometimes used by using NaF (5 mM) in the presence of $AlCl_3$ (5 μM). 1, 2-dioctanoyl glycerol (DOG, Boerhinger Mannheim Biochemicals) and phorbol ester, 12-0-tetradecanoyl glycerolphorbol-13-acetate (TPA, Boerhinger Mannheim Biochemicals) were dissolved in dimethyl sulfoxide (DMSO) and stored at −20℃. On the day of experiment, stock solutions were diluted in the external solution with final DMSO concentration not exceeding 0.1% (v/v). All solutions were buffered at pH 7.4.

Experimental procedure and electrophysiology

Current traces were recorded by using the whole-cell voltage-clamp technique. The bathing solution was perfused at a rate of 1 ml/min. Eggs were left in the bathing solution at least 5 min to equilibrate before beginning the experiment. The patch pipette resistance was 2~3 M Ω. After establishment of a giga-seal, the patch membrane was usually disrupted by suction. At this point the conditions of the egg were examined visually; eggs in which the cytoplasm was squeezed into the pipette were discarded.

Signals were digitized by an analog-to-digital converter (TL-1-125, Axon) and collected on a personal computer. Stimulation, data acqusition and analysis were performed with pClamp software (Version 5.51, Axon). The currents were amplified by a patch clamp amplifier (EPC-7, List).

A series of depolarizations from a holding potential (V_h) of −80 mV and going from −50 mV to +50 mV in 10 mV steps was given. Each voltage step was 300 ms long for the mouse egg and 500 ms to 1000 ms long for the hamster egg. When recording the current changes before and after adding drugs, a voltage pulse which elicited the maximum current from V_h = −80 mV was given at regular intervals of 30 sec~2 min. In some experiments, a 1000 ms-long ramp pulse from −60 mV to +50 mV (V_h = −80 mV) was used.

All experiments were carried out room temperature. Data represent mean ± standard deviation (SD) with number of observations. Current traces are leak subtracted.

RESULTS

Ionic currents in the mouse and the hamster egg

Depolarization from V_h of −80 mV to poten-

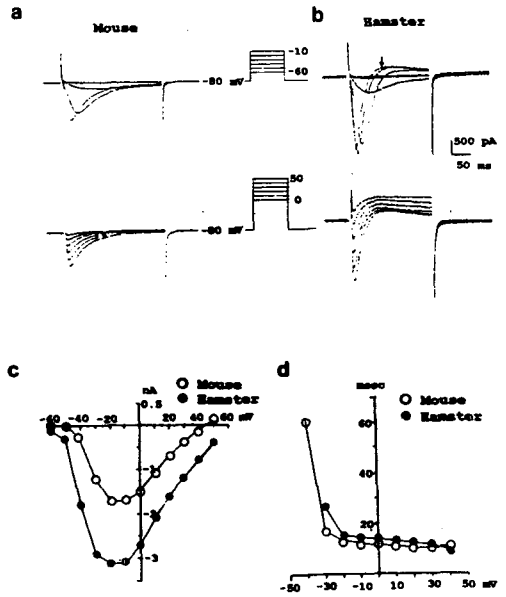

Fig. 1. Typical current traces recorded in the mouse egg and the hamster egg. a, currents evoked by the voltage pulses in the mouse egg. b, traces of current elicited by the various depolarizations in the hamster egg. c, Current-voltage (I-V) relations of inward currents in mouse and hamster eggs. d, inactivation time constants (τ) versus the inward currents in the mouse egg and the hamster egg. Data in panels c and d represent the averages measured from 29 eggs of the mouse and 25 eggs of the hamster, respectively.

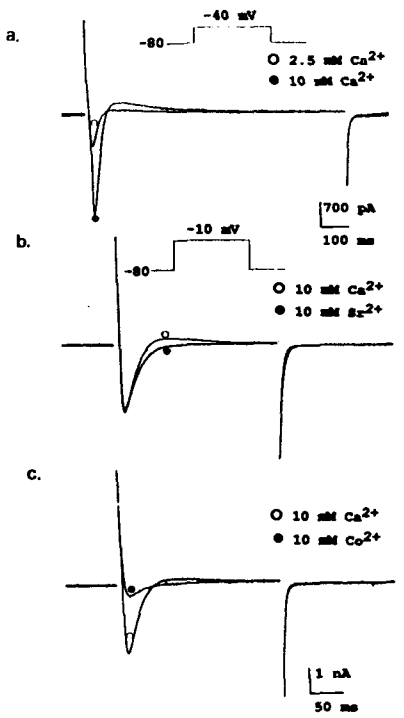

Fig. 2. Effects of divalent cations on the inward currents in the hamster egg. a, dependence of current amplitude on $[Ca^{2+}]_o$. b and c, effects of Sr^{2+} and Co^{2+} on the inward currents. Sr^{2+} and Co^{2+} replaced the equimolar Ca^{2+} outside the egg. Currents were elicited at -40 mV in panel a and -10 mV in panel b and c. Traces of currents in each panel were obtained from the three different eggs. Note the decrease of i_{to} when Ca^{2+} was replaced by Sr^{2+} or Co^{2+}. Scale bar in the bottom is common to panel b and c.

tials more positive than -50 mV elicited transient i_{Ca} in the mouse egg as shown in Fig. 1a. In the hamster egg the large and transient inward currents and outward currents following them were evoked by step depolarizations (Fig. 1b). Inward currents in the hamster egg could be observed in the range of potentials from -60 mV to potential beyond -50 mV, while i_{Ca} in the mouse egg reversed at about -45 mV. Their peak was reached at -20 mV in the hamster egg and -10 mV in the mouse egg (Fig. 1c). The peak amplitude was larger in the hamster egg(-3.12 ± 1.07 nA, n=25) than in the mouse egg (-1.71 ± 0.71 nA, n=29). The inward currents decayed rapidly in hamster eggs as well as mouse eggs. As shown in Fig. 1d, there was not a significant difference in time constants of inactivation (τ) between the two kinds of eggs: 14.4 ± 3.69 ms (n=8) and 12.4 ± 2.06 ms (n=5), respectively in hamster eggs and mouse eggs (at -10 mV).

In a previous experiment (not shown), inward current was not altered in the absence of external NaCl, which was replaced by choline chloride to maintain tonicity of solution. This led to the assumption that inward current in the hamster egg was not due to Na^+ current. It

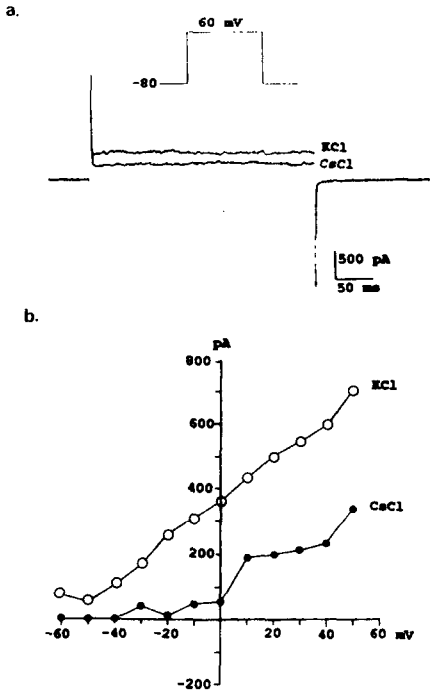

Fig. 3. The outward current in the mouse eggs. a, current traces elicited at 60 mV from the holding potential (V_h) of −80 mV. b, I-V relations of the outward currents. This current was decreased when CsCl was substituted for KCl in the pipette solution. Data in panels a and b were obtained in different eggs.

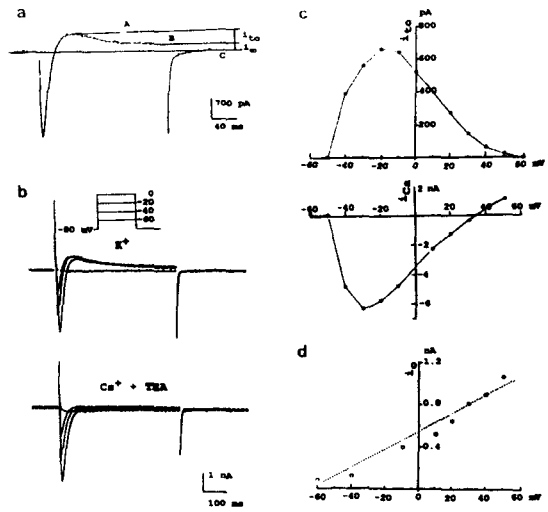

Fig. 4. Ca^{2+}-dependency of the transient outward current in the hamster egg. a, two components of the outward current of the hamster egg. b, blockade of the outward currents by K^+ channel blockers, Cs^+ and TEA. c, symmetrical I-V relations between the inward current and i_{to}. d, I-V relation of the voltage-dependent i_{ss}. The outward current was arbitrarily divided into the two components: transient outward component (i_{to}), and steady-state component (i_{ss}) between line B and line C. The amplitude of i_{to} was determined as the height of the shaded area in panel a, the difference between line A and line. B. Note the different scale in upper and lower current axis in panel c.

is conceivable that this current could be carried by Ca^{2+}, the main cation outside the egg. As shown in Fig. 2, inward current in the hamster egg was affected by the external Ca^{2+} concentration ($[Ca^{2+}]_o$), blocked when Co^{2+} replaced external Ca^{2+} and presented when Sr^{2+} substituted for Ca^{2+}. These results suggest that this is a current carried via specific Ca^{2+} channels, i_{Ca}.

Outward currents responding to voltage pulses were observed and, unlike the inward currents, there were differences in them between the two kinds of eggs. In the mouse egg, i_{Ca} was followed by a outward current which maintained a steady state level (Fig. 3A). The I-V relation of the outward currents showed a marked outward rectification (see Fig. 8B). This outward current was reduced when internal K^+ was replaced by Cs^+ (Fig. 3B). This suggests that the outward current is carried by K^+. A considerable outward component remained even in the absence of KCl, however the property of the remaining portion was not examined in this study.

In contrast with the outward current in mouse eggs, a distinctive outward current could be observed in the hamster egg (Fig. 4A). Typically this outward current increased transiently and then decreased to reach a steady state level

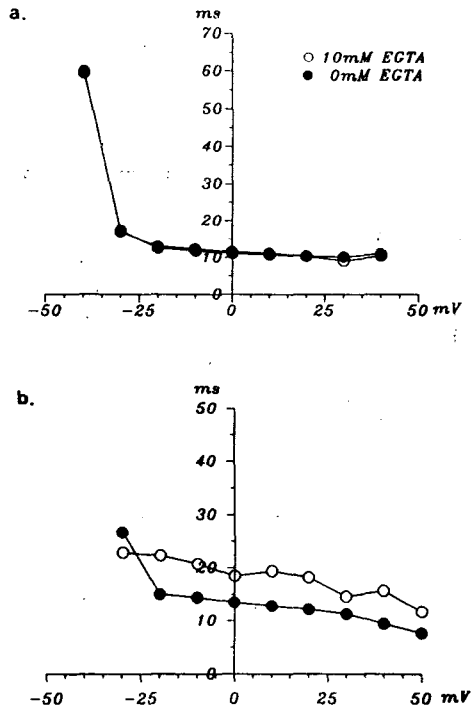

Fig. 5. Effect of EGTA on the inactivation time constant in mouse and hamster eggs. a, inactivation time constants (τ) of the inward current in mouse eggs before and after adding EGTA to the pipette solution. b, changes of τ of the inward current in hamster eggs. When adding 10 mM EGTA inside eggs, the concentration of KCl in pipette solution was subtracted to maintain the tonicity. Data in panels a and b are the averages in 8 eggs of the mouse and 7 eggs of the hamster. In the hamster egg, τ was decreased in the presence of EGTA.

by end of the step depolarization. This typical outward current was arbitrarily divided into two components: a transient outward component (i_{to}) and a steady-state outward component (i_∞). The transient outward component (i_{to}) appeared earlier following the i_{Ca} (shaded area in Fig. 4A), while the steady-state outward current (i_∞) was defined as the current remaining after the decay of i_{to}. The two outward components were completely blocked in the presence of Cs^+ and TEA (Fig. 4B). The I-V relation of i_{to} was almost the mirror image of that of i_{Ca}, while i_∞ increased linearly with depolarization (Fig. 4C & D).

Also i_{to} was dependent on the external Ca^{2+} concentration and the amplitude of i_{Ca}, disappeared in the absence of Ca^{2+} when replaced by Sr^{2+}, and blocked by the divalent Ca^{2+} channel antagonist, Co^{2+} (Fig. 2). On the contrary, i_∞ was neither dependent on the amplitude of the inward currents (Fig. 4D) nor altered by the presence of Sr^{2+} and Co^{2+} (Fig. 2). These results suggest that i_{to} is a Ca^{2+}-dependent K^+ current different from i_∞. Also the inactivation time constants (τ) of i_{Ca} were not altered by the presence of intracellular EGTA (Fig. 5A), while in hamster eggs those were significantly increased by about 7 ms in the presence of EGTA: 22.4 ± 2.64 ms ($n=7$) and 15.1 ± 3.77 ms ($n=8$), respectively, with and without EGTA at -20 mV (Fig. 5B). This implices that i_{to} in the hamster egg is dependent upon the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) increased by Ca^{2+} influx via Ca^{2+} channels and it is present during i_{Ca} thus facilitating the more rapid decay of i_{Ca}.

Effects of GTP and GDP on the ionic currents of the mouse and the hamster egg

It was confirmed that there are at least two ionic currents (i_{Ca} and i_K) in mouse eggs and three types of currents (i_{Ca} i_{to} and i_∞) in hamster eggs. Several reports suggested that G proteins concerned with cAMP and protein kinases were involved in fertilization in mouse and hamster eggs (Downs et al, 1992; Jones & Schultz, 1990; Miyazaki et al, 1990). In this experiment, we observed the response of the ionic currents to the application of GTP to examine the possibility of the regulatory effect of G proteins on the ionic channels in mouse and hamster eggs.

GTP (or GTPγS, AlF_4^-) increased i_{Ca}. The increase in mean i_{Ca} (ratio of increase to control) was 169 pA ($119 \pm 9.78\%$, $n=15$) in the mouse eggs and 217 pA ($113 \pm 9.18\%$, $n=11$) in the hamster eggs (Fig. 6). These data were selected from 45 mouse eggs and 25 hamster eggs examined in this experiment. Data were excluded

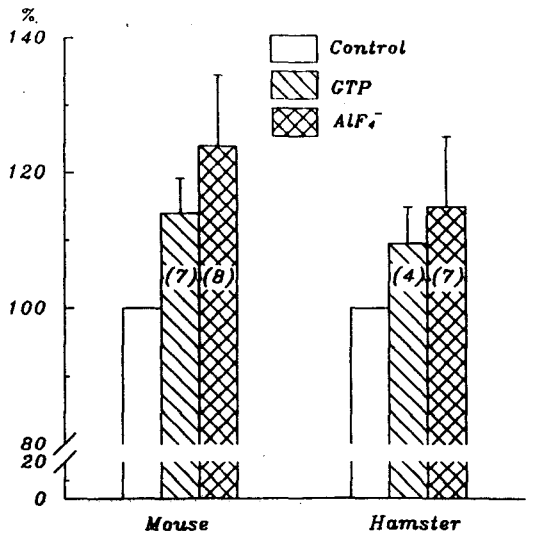

Fig. 6. Increase of i_{Ca} by either GTP or AlF_4^- in mouse and hamster eggs. The peaks of i_{Ca} were increased up to 114±5.21% by GTP or GTPγS, 124 ±10.5% by AlF_4^- in mouse eggs and 110±5.43% by GTP or GTPγS, 115±10.2% by AlF_4^- in hamster eggs, compared with those obtained before adding GTP (or GTPγS) and AlF_4^-. The left bar on each panel represents the peaks of i_{Ca} obtained before adding GTP or AlF_4^- and was regarded as control (100%). The middle and right bars represents the mean and the standard deviation of those increased after adding GTP or GTPγS, or after adding AlF_4^-, respectively. Figures in parentheses express the number of eggs which revealed the data to be analyzed.

from the analysis when it was difficult to discriminate the effect of GTP, since i_{Ca} did not recover after reperfusing the control solution due to run-down phenomena.

Fluoroaluminate (AlF_4^-) increased i_{Ca} more effectively than GTP or GTPγS did, however there was not a significant difference between their effect on these currents (Fig. 6). i_{Ca} was fully enhanced 30 sec~5 min after beginning perfusion of GTP (or GTPγS) or AlF_4^- and then decreased slightly (Fig. 7A). The effect of GTP on i_{Ca} was antagonized by GDPβS. As shown in Fig. 7B, i_{Ca} increased by the presence of 500 μM GTPγS was suppressed after exchanging the pipette solution for solution containing the equimolar concentration of GDPβS in the hamster egg. These results implies that Ca^{2+} channels in the egg may be regulated either directly by the G protein or indirectly by the substance(s) involved in the pathway triggered by the G protein.

Unlike the effect of GTP on i_{Ca}, outward currents in mouse eggs decreased after perfusing the pipette solution with 10 mM GTP (Fig. 8). They were reduced markedly at potentials more positive than −30 mV (Fig. 8A). In Fig. 8B, the I-V relations of outward current were plotted to compare the changes in outward currents obtained before and after adding GTP to the pipette solution. The slope of the I-V relation

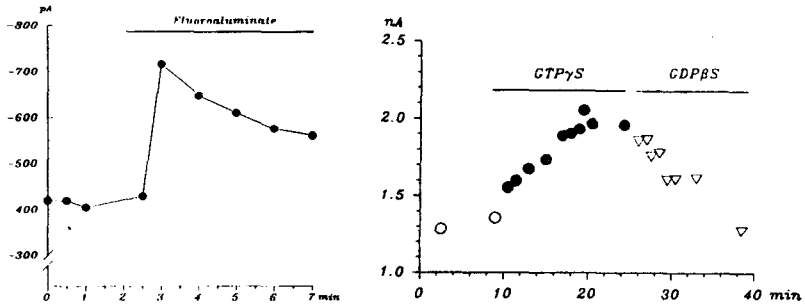

Fig. 7. Effects of GTP analogues and AlF_4^- on the inward currents in the mouse egg and the hamster egg. a, effects of AlF_4^- on the inward current of the mouse eggs. b, antagonistic action between GTPγS and GDPβS on i_{Ca} of hamster egg. Data in panels a and b were obtained from the current peaks elicited at −20 mV from V_h=−80 mV. The amplitude of the depolarization was selected as the test potential giving the maximal current from the I-V relation of the egg examined before recording.

was reduced with continuous perfusion of GTP, but the reversal potential of −20 mV was not changed. In this case a bathing medium containing 10 mM Co^{2+} instead of Ca^{2+} was used to block i_{Ca}.

In only one case out of several trials could an antagonistic effect between GTPγS and GDPβS on i_{to} be observed in a hamster egg perfused with GDPβS and succeeding GTPγS. Fig. 9A by shows that i_{to} in the hamster egg was de-

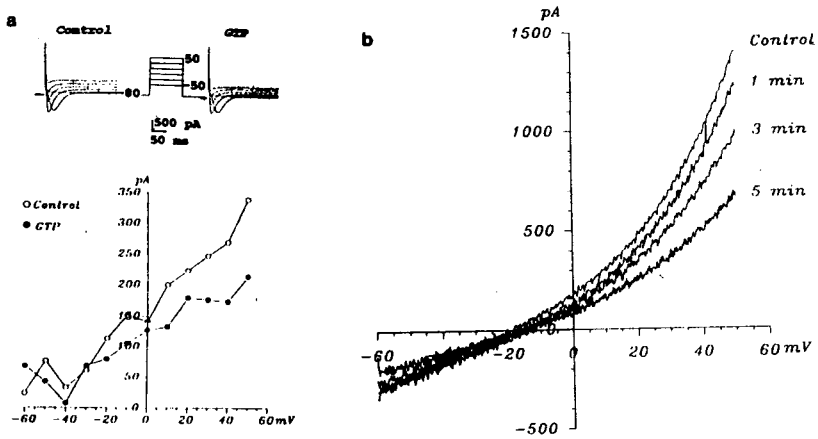

Fig. 8. Effect of GTP on the outward current in the mouse egg. a, decrease in the current traces and their I-V relations before and after adding GTP to the pipette solution. b, gradual decrease of the slope of the I-V curves rectified outwardly with a time lapse after GTP perfusion of mouse eggs. I-V relations in panel b were obtained from the traces evoked by the 1 sec-long ramp pulse going from −60 mV to +50 mV. In this experiment, Ca^{2+} was replaced with Co^{2+} in order to eliminate the inward component.

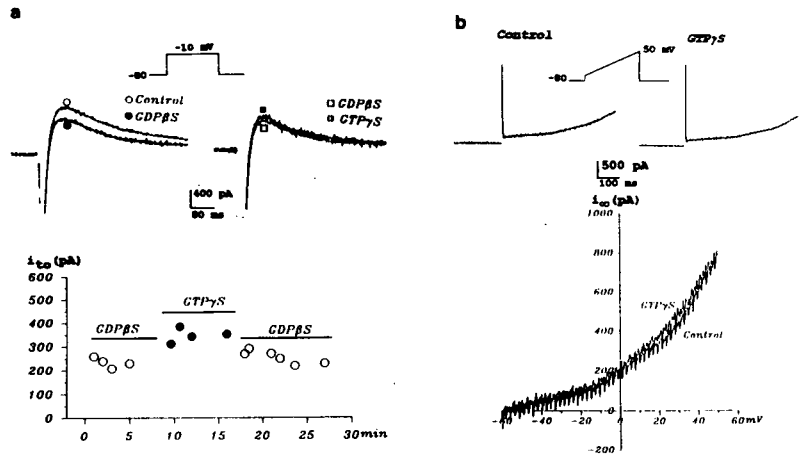

Fig. 9. Effects of GTP on i_{to} and i_{∞} in the hamster egg. a, antagonistic acitons of GTPγS and GDPβS on i_{to} in the hamster egg. b, ineffectiveness of GTPγS on the outward current in the hamster egg. I-V relations in panel b were obtained by the same method as in Fig. 8b.

creased about 250 pA by 500 μM GDPβS and recovered by about 100 pA by 500 μM GTPγS. Partial recovery of i_{to} by GTPγS (100 pA) against the fraction reduced by GDPβS may be due to the remaining GDPβS inside the egg. In this experiment we could not further examine whether their effects on i_{to} were secondary to the changes of Ca^{2+} influx. On the contrary, the I-V relation of the steady-state component (i_{∞}) was insensitive to GTPγS (Fig. 9B).

Effect of the activators of PKC on i_{to} in the hamster egg

In relation to the $[Ca^{2+}]_i$ rise during the fertilization of hamster eggs, it has been reported that Ca^{2+} transients activate Ca^{2+}-dependent K^+ (K_{Ca}) channels to evoke membrane hyperpolarization. Either GTP or $InsP_3$ facilitates hyperpolarization, while PKC inhibits it (Miyazaki et al, 1990; Swann et al, 1989). However, it is still unclear whether PKC exerts an inhibitory effect directly on K_{Ca} channels or a negative feedback effect indirectly via the hydrolysis of phosphoinositide bisphosphate and subsequent Ca^{2+} release (Berridge, 1987; Cobbold et al, 1990).

On the basis that i_{to} might reflect the amount of Ca^{2+} influx in the hamster egg as shown in Fig. 2 and 4, the effect of PKC on i_{to} was examined by using the PKC activators. TPA and DOG. Transient outward current (i_{to}) was decreased in the presence of 10 μM TPA and recovered by removing TPA. After washing for more than 20 min, i_{to} was increased by perfusing 500 μM GTPγS.

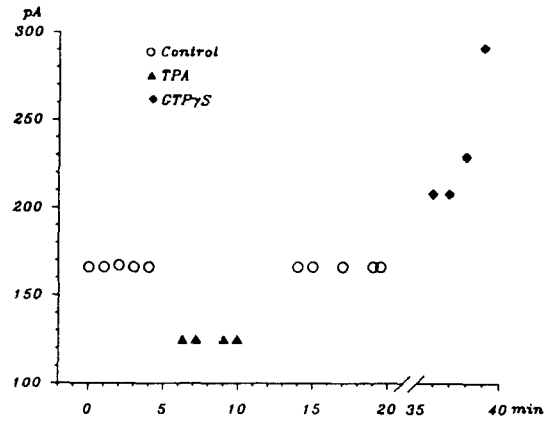

Fig. 10. Inhibitory effect of TPA on i_{to} of the hamster egg. Data represented are the peak of i_{to} measured in the current traces elicited at −10 mV from V_h = −80 mV. Break in ordinate represents the recovery in a control bathing solution for 15 min.

DISCUSSION

In this whole-cell voltage-clamp study of the mouse and hamster egg, several ionic currents were found to be present and the effects of GTP on them were examined. We compared the characteristics of ionic currents in the hamster egg with those in the mouse egg since those in the hamster egg are still relatively unknown.

Ionic currents in the mouse egg and the hamster egg

In this study, two kinds of currents in mouse egg and three kinds of currents in hamster egg were recorded. Inward and outward currents in the mouse egg were carried by Ca^{2+} and K^+ respectively as already reported (Okamoto et al, 1977; Peres, 1987; Park et al, 1991). An inward and two outward currents carried by Ca^{2+} and K^+ respectively were recorded in the hamster egg. These currents in the hamster egg are reported for the first time in the present study.

Apart from its amplitude, the parameters of the inward current in hamster eggs were similar to i_{Ca} in mouse eggs (Figs. 1 and 2). These observations imply that kinetics of the channel permeable to Ca^{2+} in the hamster egg might be similar to those in the mouse egg, although it requires further study to support this implication. In spite of the equal size (approximately 50 μm) of both kinds of eggs, the maximal amplitude of i_{Ca} was larger in the hamster egg than in the mouse egg, indicating that the channel density and/or its conductance to Ca^{2+} was

higher in the hamster egg than in the mouse egg.

There are distinct differences between the outward currents in the mouse egg and the hamster egg. Several results (Figs. 2 and 4) indicate that the outward current was composed of at least of two types of K^+ current in the hamster egg, arbitrarily named the transient outward current (i_{to}) and the steady-state outward current (i_∞), while there was only one in the mouse egg. The transient outward current, i_{to}, appears to be a Ca^{2+} dependent K^+ current, i_{KCa} and it is likely to be the current responsible for the hyperpolarization response (HR) observed in fertilization of hamster eggs (Igusa & Miyazaki, 1983; Igusa et al., 1983). On the contrary, i_∞ is not dependent on Ca^{2+} (Figs. 2 & 4D). In mouse eggs, the outward currents were flat in shape and their amplitudes were entirely dependent upon the amplitude of depolarization. Their I-V relation showed a marked outward rectification (Fig. 8B). These currents were decreased by substituting Cs^+ for K^+ (Figs. 1 & 3). This result suggests that there are channels specific to K^+ in the mouse egg. The possible presence of specific K^+ channels in this study is in conflict with the suggestion by Peres (1987); the specific K^+ channels were not observed and K^+ passed via Ca^{2+} channels in mouse eggs. If Ca^{2+} channels in the mouse egg were permeable to K^+, then the inward i_{Ca} elicited by step depolarization might be reduced by summing up with the outward i_K. However i_{Ca} was neither increased in the presence of CsCl or TEA (unpublished data). Neither reversed nor decreased outward i_K was observed on blocking the channels by replacing Ca^{2+} with Co^{2+} (Fig. 2C). These data imply that the activation of Ca^{2+} channels is a prerequisite to conduction of i_K. Interestingly, a considerable amount of outward current in mouse eggs remained even in the presence of CsCl (Fig. 3) and they reversed around -20 mV (Fig. 8B), which is far from the reversal potential of -78 mV estimated in our experimental condition. Therefore it is likely that K^+ may be carried via other non-specific channels.

Effect of GTP on the ionic currents in the mouse egg and the hamster egg

One of the main goals of this study was to obtain evidence for the regulatory effects of GTP on ionic channels in the two kinds of egg. It has been well demonstrated that many types of channels are under the control of substances involved in the signal transduction pathways (Dolphin & Scott, 1991; Hille, 1992) and several types of G-protein such as G_s, G_q or G_i are present in mouse eggs (Allworth et al, 1990; Downs et al, 1992; Jones & Schultz, 1990; Williams et al, 1992). It was suggested that the G-protein (G_p) related with the activation of phopholipase C is present in the hamster egg (Miyazaki, 1988). The occurance of $[Ca^{2+}]_i$ transients could be observed frequently in fertilization of the eggs (Curthbertson et al, 1983; Miyazaki et al, 1986). The Ca^{2+} influx from $[Ca^{2+}]_o$ via Ca^{2+} channels or Ca^{2+} release from intracellular stores (or both) could be possible sources for this transient. Only Ca^{2+} release from intracellular stores induced by $InsP_3$ (IICR) has been shown until now (Miyazaki et al, 1992).

Ca^{2+} currents were enhanced by the activation of G-proteins in both kinds of egg. This increase was facilitated by the presence of GTP, and was antagonized by the presence of GDP (Fig. 7). The increase in i_{Ca} by GTP in the present study may provide another route by which intracellular Ca^{2+} rises in the eggs. This is supported by the evidence that i_{Ca} was increased by cAMP, and decreased by a protein kinase inhibitor showing an antagonistic effect on cAMP-dependent protein kinase (PKA) in the mouse egg (Cheong, 1992). Therefore Ca^{2+} influx via Ca^{2+} channels may play a role in the generation of Ca^{2+} transients in the egg. However it is unclear whether the i_{Ca} increase resulted from an alteration in Ca^{2+} conductance by PKC activated by G_p. or from regulation of the channel activity by PKA activated by G_s, as well known in cardiac myocytes and Aplysia neurons (Dolphin & Scott, 1990; Kameyama et

al, 1986; Trauwein & Kameyama, 1987).

Fluoroaluminate (AlF_4^-) was sometimes used instead of GTP (or GTPγS). The effect of AlF_4^- on i_{Ca} was stronger than that of GTP or GTPγS but not significantly so (Fig. 6). Great care is needed in the analysis of this result. In relation to the egg maturation, it has been reported that AlF_4^- stimulates the germinal vesicle-breakdown (GVBD) in the mouse, while a signal transduction pathway involving a Gs-cAMP-PKA cascade inhibited GVBD (Downs et al, 1992). An unknown mechanism for increasing i_{Ca} can not therefore be excluded.

The action of GTP on the three types of K^+ currents differed in the two kinds of egg. In the mouse egg, GTP decreased i_K and the slope of the I-V curve (Fig. 8B), indicating that GTP might reduce the K^+ conductance or the numbers of active channels permeable to K^+. In the hamster egg, i_{to} was increased in the presence of GTP, while i_∞ was unaffected by GTP (Fig. 9). These results led to at least two assumptions. First, the properties of channels permeable to K^+ in the two kinds of eggs are not the same since they showed a different response to GTP. Second, K^+ channels in mouse and hamster eggs are immature and similar in their properties, but they are controlled by a different signal cascade involving GTP. Unfortunately, at present there is little information to discriminate between these assumptions.

Effect of PKC agonists on i_{to} in the hamster egg

In relation to the $[Ca^{2+}]_i$ rises, a signal transduction pathway involving $InsP_3$ and PKC was firmly established in the hamster egg (Miyazaki, 1988; Miyazaki, 1991; Miyazaki et al, 1992). It has been known that PKC suppressed Ca^{2+} transients by a negative feedback inhibition of PLC activation (Miyazaki, 1991; Swann et al, 1989). Similar effect was observed in this study. A PKC activator, TPA suppressed i_{to} which is likely to be i_{KCa} (Fig. 10). Although not presented, this result was confirmed again by using DOG, a synthetic PKC activator. We gave great care to the analysis of this result, because synthetic PKC activators such as TPA or DOG were reported to have various effects on a variety of cells. That is, i_{Ca} stimulation of PKC enhanced in Aplysia neurons (Deriemer et al, 1985; Strong et al, 1987), or DOG reduced i_{Ca} independently of PKC activation (Hockberger et al, 1989). Furthermore, TPA, phorbol ester directly regulated K^+ channels in hippocampal pyramidal neurons (Malenka et al, 1986). Therefore, it is possible that the reduction in either Ca^{2+} influx or i_K by TPA and DOG might result in the i_{to} decrease. In the present experiment, the amplitude of i_{Ca} was not altered during the perfusion of TPA or DOG although this is not shown. From these results, the effect of TPA or DOG on i_{to} by decreasing Ca^{2+} influx can be neglected. Hence the activation of PKC leading to a reduction of i_{to} may result from the negative feedback inhibition of the $[Ca^{2+}]_i$ rise or directly from the inhibition of K_{Ca} channels carrying i_{to} in hamster eggs.

In terms of the physiological functions of the ionic currents recorded in the present study, i_{Ca} may contribute to the $[Ca^{2+}]_i$ rise in the eggs. It was recently reported (Iino & Endo, 1992) that the level of $[Ca^{2+}]_i$ is essential to enhance IICR (IP_3-induced Ca^{2+} release), which is known as a mechanism to elevate $[Ca^{2+}]_i$ in the mouse egg and the hamster egg until now (Miyazaki et al, 1992; Peres, 1990). Therefore providing the condition to elicit i_{Ca}, this current may act either as a mediator to adjust the level of $[Ca^{2+}]_i$ or as an amplifier to elevate $[Ca^{2+}]i$ effectively inside the egg.

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