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Research Article

Effects of H^+-K^+ ATPase Inhibitors (Omeprazole and Lansoprazole) on Fertilization-Induced Bioelectrical Potential Changes in the Egg of the Frog, *Rana cameranoi**

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Abstract: Fertilization triggers Ca^{2+} increase and alkalization in the eggs of some species such as the frog. Ooplasmic Ca^{2+} increase triggers fertilization potential (FP), and alkalization can have a permissive effect in Ca^{2+} increase. It can be expected that pH regulation in the egg can affect both ooplasmic Ca^{2+} levels and the events triggered by increasing Ca^{2+}. The evidence of the possibility of H^+-K^+ ATPase in different tissues in recent studies prompted us to wonder about the probability of the existence of this pump in the egg. Therefore, we decided to investigate the presence of H^+-K^+ ATPase by bioelectrical potential recordings in frog eggs. Thus, frog eggs were inseminated while the resting membrane potential (RMP) in the solution containing different concentrations (0.3, 0.6, 0.9 and 1.2 mM) of pump inhibitors (omeprazole and lansoprazole) was recorded. Evidence of H^+-K^+ ATPase was sought from the effects of these pump inhibitors on FP variables.

While omeprazole had a significant effect on RMP and FP variables at a 1.2 mM concentration alone, lansoprazole had significant effects at concentrations higher than 0.6 mM in the frog eggs. It was determined that the effects of the 2 inhibitors were similar.

In conclusion, H^+-K^+ ATPase may be present in frog eggs due to the similar effects of both pump inhibitors on the potential change triggered by fertilization. Thus, this pump might have a function in alkalization triggered by fertilization in the frog egg.

Key Words: Frog egg, H^+-K^+ ATPase, bioelectrical potentials, omeprazole, lansoprazole

Rana cameranoi Türü Kurbağa Yumurtasında Fertilizasyonda Ortaya Çıkan Biyoelektrik Potansiyel Değişikliklere H^+-K^+ ATPaz İnhibitörlerinin (Omeprazol ve Lansoprazol) Etkileri

Özet: Fertilizasyon, yumurtada Ca^{2+} artışını ve kurbağa gibi bazı canlılarda alkalizasyonu tetiklemektedir. Ooplazmik Ca^{2+} artışı Fertilizasyon Potansiyeli'ni (FP) başlatmakta, alkalizasyonun ise Ca^{2+} artışında permisif bir etkisi bulunmaktadır. Dolayısı ile yumurta içi pH regülasyonunun, hem ooplazmik Ca^{2+} düzeyini, hem de Ca^{2+} artışı ile tetiklenen olayları etkilemesi beklenebilir. Son yıllarda yapılan araştırmalarda farklı dokularda H^+-K^+ATPaz varlığının gösterilmesi, yumurtada da bu pompanın olabileceğini düşündürmektedir. Bu nedenle araştırmada, biyoelektrik potansiyellerinin kaydı ile kurbağa yumurtasında H^+-K^+ATPaz varlığının araştırılması amaçlanmıştır. Bunun için değişik yoğunluklarda (0.3, 0.6, 0.9 ve 1.2 mM) pompa inhibitörleri (omeprazol ve lansoprazol) içeren solüsyonlar uygulanan ortamlarda istirahat membran potansiyeli (İMP) kaydedilerek kurbağa yumurtaları döllendi ve FP parametrelerine inhibitörlerin etkileri incelenerek pompa varlığı hakkında bilgi edinilmesine çalışıldı.

Kurbağa yumurtasında İMP ve FP parametrelerini omeprazol yalnız 1.2 mM yoğunluğunda anlamlı olarak etkilerken, lansoprazolün 0.6 mM ve üstündeki yoğunluklarda anlamlı etkileri vardı. Her iki pompa inhibitörü etkisinin benzer yönde olduğu bulundu.

Sonuç olarak, kurbağa yumurtasında fertilizasyon ile tetiklenen potansiyel değişiklikler üzerine her iki pompa inhibitörünün de benzer etkilerde bulunması, kurbağa yumurta membranında H^+-K^+ATPaz olabileceğini gösterebilir. Böylece, bu pompanın kurbağa yumurtasında fertilizasyonla gözlenen alkalizasyonun gelişmesine katkıda bulunabileceği düşünülebilir.

Anahtar Sözcükler: Kurbağa yumurtası, H^+-K^+ ATPaz, bioelektrik potansiyeller, omeprazol, lansoprazol

Introduction

Ionic and metabolic events that lead to embryogenesis in the egg are triggered by fertilization. An increase in ooplasmic calcium has been shown in all species investigated and an alkaline shift of ooplasmic pH (pH_i) shown in some species such as frogs triggered ionic

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events (1-5). By increasing calcium, fertilization potential (FP), which was clearly shown in externally fertilizing organisms, develops and so the egg is protected from secondary sperm entry until the permanent, mechanical block is established (1,2,6). It has also been indicated that ooplasmic calcium increase and alkalization were necessary for the initiation of protein synthesis and DNA replication in Xenopus (3). Since ooplasmic alkalization is important for the activation of the egg, pH_i has to be held at low levels to prevent activation in externally reproducing species, because their eggs are directly exposed to alterations in environmental pH (5,7) and it is suggested that increased pH_i alone is not sufficient for activation, but it may play a permissive role for the activation of calcium-dependent events (7).

It has been reported that membrane potential change (fertilization potential) is a calcium-dependent event triggered by fertilization (1,2,6) and all the factors that affect ooplasmic calcium levels also affect the electrical properties of the membrane (8,9). This is because increases in Ca^{2+}-induced Cl^- conductance cause FP in the frog (6). Furthermore, the peak level and the duration of FP may change according to the ooplasmic Ca^{2+} level (10). It has also been claimed that during alkaline shift in the pH of hamster and Xenopus eggs, there is an increase in the ionic conductance as a result of the increase in ooplasmic Ca^{2+} (4,9). Due to the close relationship between pH and calcium, any factors that can affect pH (fertilization itself or treatment that can affect ooplasmic pH) may also affect the membrane potential of the egg (9,10).

Besides the well known function of H^+-K^+ ATPase in stomach parietal cells, it has been reported that it may be present in vascular smooth muscle cells (11), airways in the guinea pig and human (12) and in rat colon crypt cells (13,14). Furthermore, it has been recently noted that the asymmetric localization of H^+-K^+ ATPase α mRNA occurs during the first 2 cell cycles and this asymmetrically localized proton pump might have a function in the laterality of visceral organs such as the heart and gallbladder in Xenopus eggs (15). Therefore, it was thought that this pump may be present in a *Rana cameranoi* egg due to the importance of alkalization in egg activation. Based on this knowledge, H^+-K^+ ATPase may be present in the egg membrane and functional in ooplasmic pH regulation. There could also be an increase in the activation of this pump by fertilization, thus shifting ooplasmic pH to alkali values may play a permissive role in Ca^{2+} increase, and this is reflected in FP parameters. If a significant change is found in the FP parameters recorded with H^+-K^+ ATPase inhibitors, this finding may provide evidence for the presence of the pump. With this in mind, we aimed to investigate the effects of 2 different H^+-K^+ ATPase inhibitors on the membrane potential properties of frog egg recorded during fertilization.

Materials and Methods

The species *Rana cameranoi*, selected according to Başoğlu and Özeti (16), was used (N = 66) in the study. All procedures were approved by the Committee of Animal Care and Use, Çukurova University.

Obtaining eggs and spermatozoa: Insemination procedure

For induction of ovulation in sexually mature females, the pituitary glands, which were removed from same species of female frogs, were homogenized and injected intraperitoneally, and progesterone (Sigma, P-0130) was injected into the thigh muscle (17). The numbers of injected pituitary glands and amounts of progesterone were adjusted according to the months: in January and February 4, in March 3 pituitary glands and in April 2; in January 2 mg of progesterone, in February and March 1 mg and in April 0.5 mg (18). The injected frogs were kept at 18 ºC for 36 h, or at 25 ºC for 24 h, and then mature eggs were obtained by squeezing the cloaca.

Sperm suspensions were obtained by macerating frog testes in 10% Ringer solution, 3-5 h after a male was injected with 300 IU of human chorionic gonadotropin (hCG, Sigma C-8554) intraperitoneally. Sperm samples were examined for motility and morphology under a microscope (Nikon) at 400x magnification.

Between 5 and 10 mature frog eggs were put into plastic petri dishes and stabilized due to the natural stickiness of the gel layer (19). After addition of the recording solution, a microelectrode was inserted into the egg and after the stabilization of the recorded membrane potential, insemination was carried out by putting 1 or 2 drops of sperm solution at a concentration of 5×10^6 sperm/ml. Approximately 5 min after insemination, fertilization occurred. Normal fertilization was scored by a shift in membrane potential towards positive values,

rotation and normal first cleavage, and neural fold stage (6). Eggs that showed membrane depolarization and rotation but did not cleave normally or did not develop until the neural fold stage were considered activated. Experiments were performed at 21-23 °C.

Control recordings and treatment of H^+-K^+ ATPase inhibitor

Control records were carried out in standard 10% Ringer solution containing (in mM): NaCl, 11.1; KCl, 0.19; $CaCl_2$, 0.11; $MgSO_4$, 0.08; NaOH, 0.4 and HEPES, 0.25 (pH 7.80) (6). Two different inhibitors were used, omeprazole (Sigma O-104) and lansoprazole (Sigma L-8533). The effects of the inhibitors were investigated using quantities of omeprazole/lansoprazole added to the control solution (the pH of all inhibitor solutions was also adjusted to 7.80). A dose-response curve was plotted for both inhibitors (0.3, 0.6, 0.9 and 1.2 mM) and for the 3 determined doses (0.6, 0.9 and 1.2 mM) and the number of recordings were increased. In each experimental group, the number of frogs used (N) and recorded eggs (n) were as follows: 0.3 mM, N = 3, n = 5; 0.6 mM, N = 3, n = 8; 0.9 mM, N = 6, n = 12 and 1.2 mM, N = 6, n = 13 for omeprazole; 0.3 mM, N = 4, n = 5; 0.6 mM, N = 5, n = 9; 0.9 mM, N = 3, n = 8 and 1.2 mM, N = 3, n = 8 for lansoprazole. The effects of each dose were examined with the control recordings obtained before the treatment of these doses. All these control recordings served together as the control group (N = 33, n = 67).

Electrophysiological recordings

Intracellular records were obtained by conventional microelectrode techniques. The electrodes were inserted into the egg by transiently increasing the negative capacitance of the amplifier (Nihon Kohden MEZ-8201) to produce an oscillating current. Membrane potentials were monitored on a storage oscilloscope (Nihon Kohden VC-10), and recorded on a chart recorder (Palmer Bioscience). The following parameters were investigated from the electrical recordings: resting membrane potential (RMP), peak fertilization potential (FP_p), fertilization potential amplitude (FP_a), fertilization potential duration (FP_d) and membrane potential of the fertilized egg (MP_f) (Figure 1).

Statistical analysis

Before the treatment of each inhibitor, control recordings were obtained and the effects of the inhibitors were compared. Student's t test was used to compare each dose and its control recording. One-way ANOVA was also used to compare the control group with mentioned control values, the same doses of different inhibitors and different doses of the same inhibitor (20). The Student-Newman-Keuls test was used to determine statistically significant differences for the one-way ANOVA test. The criterion significance was 0.05 and all averages were expressed as mean ± SEM.

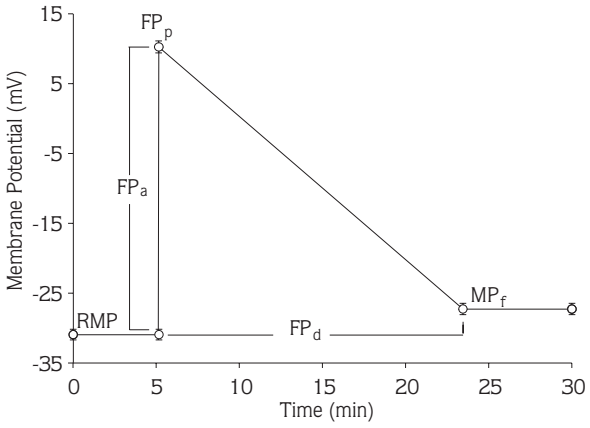

Figure 1. Graphical illustration of evaluated parameters drawn by using mean values determined in the control group.

Results

Representative recordings of frog egg bioelectrical potentials from the control solution and the solutions of 0.6 M and higher concentrations of omeprazole are shown in Figure 2 and those of lansoprazole in Figure 3.

Resting membrane potential

The RMP of *R. cameranoi* eggs was determined to be -30.96 ± 0.75 mV (n = 67). There was no significant difference between measurements of control RMP obtained before all concentrations of the pump inhibitors (Tables 1 and 2).

Omeprazole and lansoprazole influenced the RMP in a dose-dependent manner in the direction of depolarization. While this effect was significant only in 1.2 mM concentrations of omeprazole, 0.6 mM and higher concentrations of lansoprazole showed significant differences ($P < 0.05$) (Tables 1 and 2). There was no significant difference between the same concentrations of the 2 pump inhibitors on RMP.

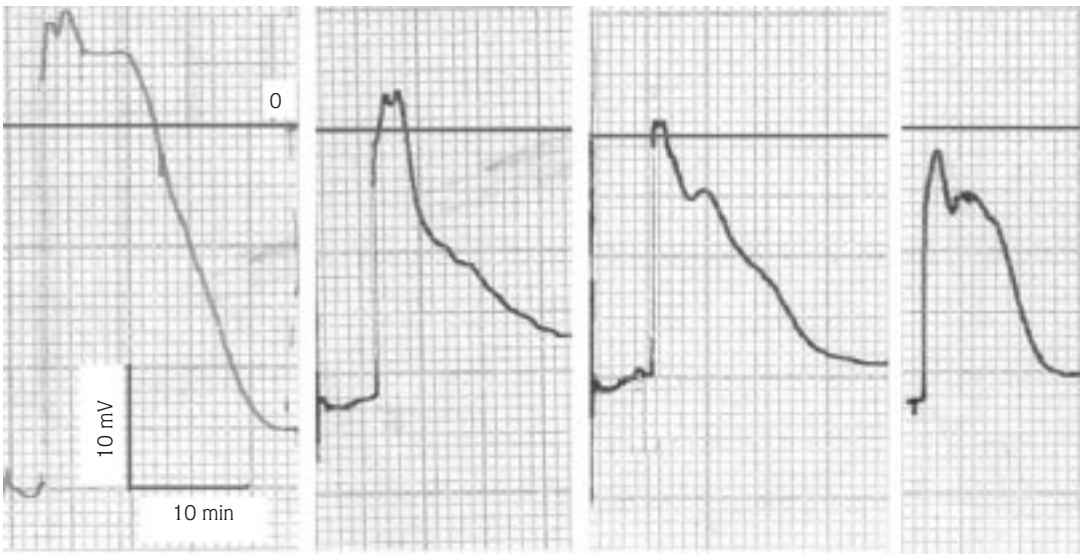

Figure 2. Illustration of records taken from control (A), and 0.6 (B), 0.9 (C) and 1.2 mM of omeprazole (D).

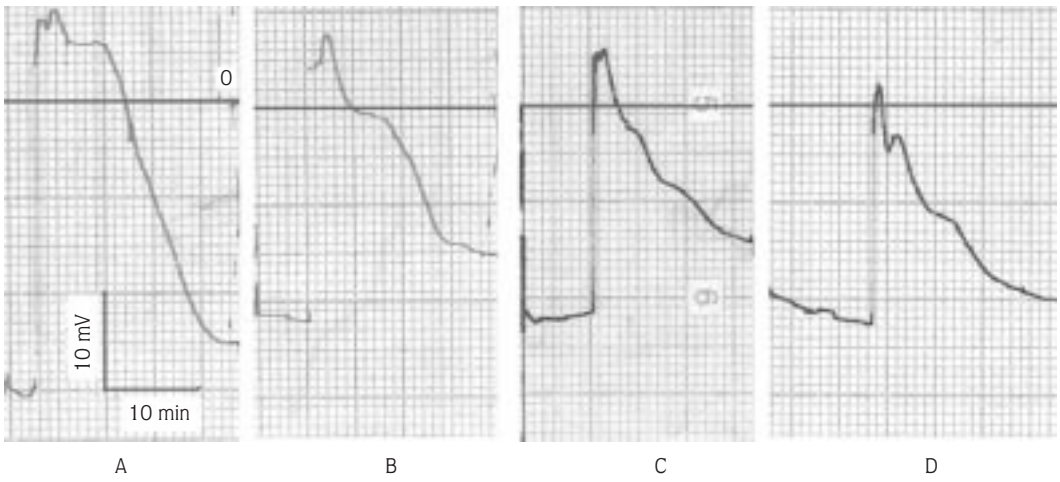

Figure 3. Illustration of records taken from control (A), and 0.6 (B), 0.9 (C) and 1.2 mM of lansoprazole (D).

Peak fertilization potential

FP_p values were 10.22 ± 0.85 mV (n = 67) in the control recordings. No significant difference was found between control FP_p values (Tables 1 and 2). Both pump inhibitors affected the FP_p value significantly at 1.2 mM, and FP_p could obtain less positive values (Tables 1 and 2, Figures 2–5).

Fertilization potential amplitude

FP_a was 41.18 ± 1.05 mV (n = 67) in the control group. Similarly, no significant difference was found between the control recordings of all the concentrations and the control group (Tables 1 and 2). A significant difference was found only those receiving 1.2 mM of omeprazole (Table 1, Figure 4); however, in the lansoprazole group significant differences were determined at 0.6 mM and in higher concentrations (Table 2, Figure 5). The same concentrations of inhibitors showed similar effects on the FP_a and no significant difference was determined (Tables 1 and 2).

Table 1. RMP and FP parameters in the control and 0.3, 0.6, 0.9 and 1.2 mM omeprazole groups (mean ± SEM).

Group	RMP (mV)	FP_p (mV)	FP_a (mV)	FP_d (min)	MP_f (mV)
Control (N = 33, n = 67)	-30.96 ± 0.75	10.22 ± 0.85	41.18 ± 1.05	18.10 ± 0.63	-27.30 ± 0.80
0.3 mM (N = 3, n =5)	-36.40 ± 3.47	11.00 ± 3.18	47.40 ± 4.00	19.29 ± 0.55	-32.60 ± 1.94
Control (N = 3, n = 5)	-29.60 ± 2.71	10.40 ± 1.50	40.00 ± 3.89	18.11 ± 1.17	-28.40 ± 1.47
0.6 mM (N = 3, n = 8)	-28.88 ± 1.04	8.50 ± 1.57	37.38 ± 2.47	16.21 ± 1.06	-21.88 ± 2.57
Control (N = 3, n = 8)	-30.13 ± 1.67	8.38 ± 1.75	38.51 ± 2.81	18.27 ± 1.10	-25.00 ± 3.04
0.9 mM (N = 6, n = 12)	-25.42 ± 1.67	6.42 ± 2.31	31.84 ± 2.11	15.31 ± 1.37	-20.00 ± 1.63
Control (N = 6, n = 12)	-29.25 ± 1.37	8.58 ± 1.82	37.83 ± 1.73	17.48 ± 1.45	-26.92 ± 3.43
1.2 mM (N = 6, n = 13)	-22.62 ± 1.14*	1.54 ± 1.71*	24.16 ± 1.97*	11.03 ± 1.11*	-22.67 ± 2.47
Control (N = 6, n = 13)	-28.62 ± 1.29	11.46 ± 1.72	40.08 ± 2.60	14.20 ± 0.59	-24.58 ± 1.78

*: Significant when compared to its control ($P < 0.05$).
RMP, resting membrane potential; FP_p, peak fertilization potential; FP_a, fertilization potential amplitude; FP_d, fertilization potential duration; MP_f, membrane potential of the fertilized egg

Table 2. RMP and FP parameters in the control and 0.3, 0.6, 0.9 and 1.2 mM lansoprazole groups (mean ± SEM).

Group	RMP (mV)	FP_p (mV)	FP_a (mV)	FP_d (min)	MP_f (mV)
Control (N = 33, n = 67)	-30.96 ± 0.75	10.22 ± 0.85	41.18 ± 1.05	18.10 ± 0.63	-27.30 ± 0.80
0.3 mM (N = 4, n = 5)	-28.00 ± 4.08	7.50 ± 3.12	35.50 ± 4.27	18.01 ± 2.35	-25.75 ± 3.45
Control (N = 4, n = 4)	-32.00 ± 3.37	7.25 ± 4.05	39.25 ± 3.20	19.13 ± 2.23	-26.00 ± 2.48
0.6 mM (N = 5, n = 9)	-26.11 ± 1.78*	8.33 ± 1.89	34.44 ± 1.71*	16.08 ± 2.58	-21.17 ± 2.57
Control (N = 5, n = 9)	-31.33 ± 1.66	14.44 ± 3.24	45.77 ± 2.89	19.19 ± 1.47	-26.67 ± 3.58
0.9 mM (N = 3, n = 8)	-25.88 ± 2.00*	6.00 ± 1.65	31.88 ± 1.65*	14.01 ± 1.46*	-20.00 ± 0.69*
Control (N = 3, n = 8)	-35.38 ± 2.10	8.88 ± 2.96	44.25 ± 2.75	20.20 ± 1.58	-31.00 ± 4.45
1.2 mM (N = 3, n = 8)	-21.25 ± 1.52*	5.50 ± 1.96*	26.75 ± 2.38*	12.55 ± 1.55*	-19.80 ± 2.22*
Control (N = 3, n = 8)	-31.38 ± 1.96	12.38 ± 2.36	43.75 ± 2.74	18.22 ± 2.00	-29.80 ± 3.45

*: Significant when compared to its control ($P < 0.05$).
RMP, resting membrane potential; FP_p, peak fertilization potential; FP_a, fertilization potential amplitude; FP_d, fertilization potential duration; MP_f, membrane potential of the fertilized egg

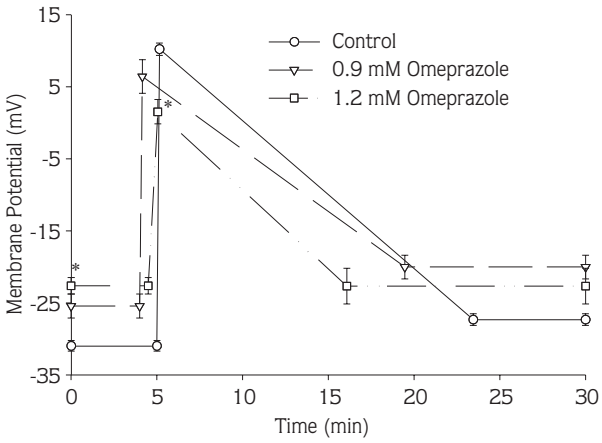

Figure 4. Comparison of values (mean ± SEM) determined in the control, 0.9 and 1.2 mM omeprazole groups (* significant when compared to control, $P < 0.05$).

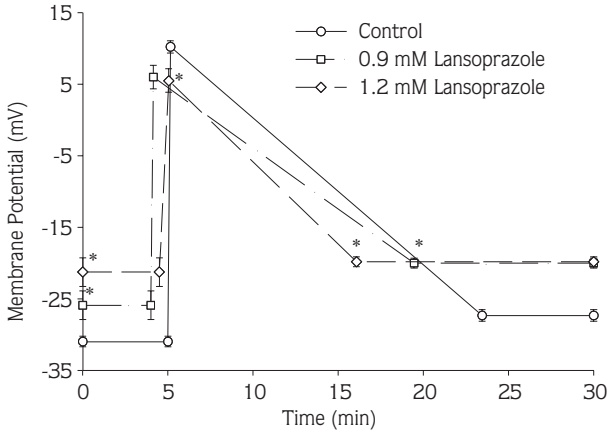

Figure 5. Comparison of values (mean ± SEM) determined in the control, 0.9 and 1.2 mM lansoprazole groups (* significant when compared to control, $P < 0.05$).

Fertilization potential duration

FP_d was 18.10 ± 1.03 min in the control group and showed no significant difference from the other control values. As a result of pump inhibitor treatment, while 1.2 mM omeprazole caused significant shortening (Table 1, Figure 4), for lansoprazole this effect occurred at 0.9 and 1.2 mM (Table 2, Figure 5).

Membrane potential of the fertilized egg

No significant difference was found between the control group value of –27.30 ± 0.80 mV and the other control values (Tables 1 and 2). While omeprazole treatments had no significant effect on MP_f, 0.9 and 1.2 mM lansoprazole treatments showed significant depolarization effects (Table 2).

The 2 pump inhibitors' effects on frog egg bioelectrical potentials showed similarities at 0.3 mM and in higher concentrations.

Discussion

As a main finding of the study, it was found that H^+-K^+ ATPase inhibitors (omeprazole and lansoprazole) showed similar dose-dependent effects on RMP and FP parameters of frog egg triggered by fertilization.

Investigation of the presence of H^+-K^+ ATPase using bioelectrical potential recording

The first change in the egg when it is activated by sperm is the increase in ooplasmic Ca^{2+}, and this has been demonstrated in all species investigated (1,2,4). On the other hand, alkaline shift of ooplasmic pH (pH_i) was also demonstrated at the time of fertilization in some species (3,5). Increased ooplasmic Ca^{2+} leads to a rise in the ionic conductance of the egg resulting in changes in membrane potential (FP) (1,2,6). It has been reported that alkalization is necessary for protein synthesis and DNA replication in Xenopus (3). It has deduced that the increase in ooplasmic calcium is the main trigger for activation in the egg of all species and alkalization may have a permissive effect on this increase (7).

The ooplasmic Ca^{2+} increase triggered by fertilization affects membrane potential (1,2,6,21,22) and the electrical properties of the membrane are affected by other factors like pH (4,8-10). Furthermore, it has been demonstrated that pHi increment may lead to increased ooplasmic Ca^{2+} by decreasing Ca^{2+} pump activity in the oolemma (8,9). It has been reported that the alkaline shift of the pH_i of the hamster and Xenopus eggs may cause the increment of ooplasmic Ca^{2+} and ionic conductance (4,9). Therefore, when the alkaline shift of pH_i occurs due to an increase in the activation of H^+-K^+ ATPase by fertilization, it is expected to play a permissive role in Ca^{2+} increase, and this is reflected in FP parameters. Therefore, when a significant difference can be found from recorded FP parameters by using H^+-K^+ ATPase inhibitors, this data may provide information on the presence of the pump.

The presence of the H^+-K^+ ATPase in gastric parietal cells, vascular smooth muscle cells (11), human and guinea pig airways (12) and rat colon crypt cells (13,14) has been reported. Furthermore, it has been recently determined that H^+-K^+ ATPase α mRNA is present in a radially symmetrical, circular region at the animal pole in the unfertilized Xenopus egg (15). However in the same study, the authors reported that the asymmetric localization of the pump occurs during the first 2 cell cycles, resulting in asymmetrically localized ion flux; thus, an asymmetrically localized proton pump might have a function in the laterality of visceral organs such as heart and gallbladder (15). Equally, it was shown that H^+-K^+ ATPase may contribute to the membrane polarization and inhibition of the pump by using omeprazole and lansoprazole to instigate depolarization (15). The findings of Levin et al. (15), who evaluated membrane potential by using the fluorescent dye technique, are similar to our findings recorded by electrophysiological technique. Therefore, their results and our findings support the presence of H^+-K^+ ATPase in frog eggs. However, it has been demonstrated that the activation of H^+-K^+ ATPase recovers rat colonic crypt cells from an acid load and this may be present as an important control mechanism to regulate pH_i in colonocytes (14). Although this conclusion has been reported in different species and tissues, H^+-K^+ ATPase can be functional in the regulation of pH_i and may be critically important for ooplasmic alkalization occurring at the time of fertilization.

Bioelectrical potentials and the effects of pump inhibitors

Resting membrane potential: Omeprazole significantly depolarized the membrane at 1.2 mM; however, the same effect occurred at 0.6 mM and higher concentrations for lansoprazole. By inhibiting the proton

pump, an acidic shift of pH_i may develop in the intracellular milieu due to the influence of the pump on intracellular pH regulation. It is known that intracellular acidity inhibits Na^+-K^+ ATPase (23). Because Na^+-K^+ ATPase is an electrogenic pump, it affects membrane potential and contributes to the negativity of the membrane (24). Thus, in a situation where the pump is inhibited, a less polarized membrane may be determined due to decreased contributions of Na^+-K^+ ATPase on the negative charging of the membrane (Figures 4 and 5). The 2 inhibitors used in this study have shown similar effects at the same doses and in a dose-dependent manner in 0.6 mM and above concentrations (Tables 1 and 2). In addition, lower negative potential recordings in the membrane of the fertilized egg may be the result of the chain of events mentioned above. However, the direct effects of the inhibitors and their indirect effects on any transport and exchange mechanism in the membrane could not be eliminated.

Fertilization potential parameters: Peak FP (FP_p), which was affected significantly at 1.2 mM of both inhibitors, could return less positive values. By the time of fertilization, the increase in ooplasmic Ca^{2+} triggers Cl^- conductance in the frog egg. The calculated equilibrium potential for Cl^- of the frog egg in 10% Ringer solution is +18 mV (6). Thus, it may be expected that FP_p can reach +18 mV by increasing Cl^- conductance. However, it has been reported that Cl^- cannot reach the equilibrium potential because of the repolarizing efforts of the increased conductance of K^+ (6,19). The equilibrium potential of K^+ is -150 mV (6), and it is responsible for the repolarization phase of the FP (6,19). In our study, control FP_p values were similar to this value (Tables 1 and 2). On the other hand, if treatment of the pump inhibitors limits the alkaline shift of the pH_i, FP_p could reach less positive values as a result of the decreasing permissive effect of the alkalization on Ca^{2+}. Moreover, although the effects of 0.6 and 0.9 mM concentrations of both inhibitors were not significant, they affected the FP_p in a similar manner, and the amplitude of FP remained at values lower than those on the mentioned doses of the inhibitors. These findings also support this opinion.

FP duration (FP_d) became significantly shorter at 0.9 and 1.2 mM of lansoprazole and 1.2 mM of omeprazole. FP_d is the time that passes from activation triggered by fertilization to the repolarization of the membrane. Therefore, it may be expected that all the factors that affect depolarization and repolarization phases also affect this parameter (19). When it is thought that there is no factor to limit the K^+ conductance responsible for repolarization, as discussed above, a shorter period will be sufficient for the repolarization of the membrane as a result of decreases in the FP_p, and this may cause shorter FP_d recording.

In conclusion, it might be thought that H^+-K^+ ATPase exists on the frog egg membrane due to 2 different pump inhibitors showing a similar effect on RMP and the FP parameters triggered by fertilization in the frog egg and that contribute to alkalization by the time of fertilization. Nevertheless, the presence and function of H^+-K^+ ATPase in ooplasmic alkalization during fertilization can be demonstrated clearly by directly measuring ooplasmic H^+ concentrations while treating H^+-K^+ ATPase inhibitors.

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