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Contractile Activity in the Chick Uterus

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Abstract: Uterine contractile activity has never been investigated in chicks. The aim of this study was to investigate spontaneous contraction and response to an agonist of chick uterus. Chicks were scarified by cervical dislocation. The uteri were isolated and contraction measured *in vitro*. Spontaneous contraction can be observed in 4 days chick uterus. External calcium is necessary for the spontaneous activity and it enters the uterus via L-type voltage-gated calcium channels. The chick uterus was responsive to an agonist such as Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). As with spontaneous contraction, the contraction stimulated by $PGF_{2\alpha}$ is dependent on external calcium. However, $PGF_{2\alpha}$ can induce a transient contraction when external calcium is omitted suggesting the ability of the uterus to release calcium from internal sources. Taken together, the data suggest that in the chick uterus spontaneous activity is already present by day 4th and that receptor coupling and excitation-contraction signaling pathways are also functional.

Key words: Uterus, shell gland, chick, calcium, prostaglandin, contraction

INTRODUCTION

Much is known for contractile activity of the uterus in the laying hens (Kupittayanant *et al*., 2009; Shimada and Asai, 1978). Little is known, however about contractile activity of the uterus in chick. It is therefore, unclear when spontaneous contractile activity occurs and which mechanisms lead to contraction. Moreover, the researchers can find no data concerning excitation-contraction coupling in chick uterus. To better understand, the aim of this study was therefore to investigate these processes in chick uterus. Force was recorded in chick uterus.

Spontaneous contraction and response to the agonist Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Toth *et al*., 1979) with and without external calcium present in the bathing solution were observed.

MATERIALS AND METHODS

All procedures were approved by the Institutional Animal Care and Use Committee, Suranaree University of Technology, SUT, Thailand.

Experimental animals: The 4 days old chicks (*Gallus domesticus*) were used in the present study. They were purchased from commercial hatcheries. All of them were Bobans Gold Line.

Uterine preparations: Chicks were killed humanely by cervical dislocation. The entire oviduct was removed and immediately immersed in physiological solution (pH 7.4) containing (mM): NaCl (154), KCl (5.4), $MgSO_4$ (1.2), glucose (8), $CaCl_2$ (2) and 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (10). The oviduct was placed in a shallow dissecting dish containing physiological solution under a microscope for dissection. Excess blood was carefully removed. Under microcopy examination, the uterus was clearly visible and distinguishable from other parts of the oviduct. The whole uterus was then separated.

Measurements of uterine contraction: The uterus was placed vertically in a 25 mL temperature controlled organ bath (Panlab s.l. for AD Instruments Pty Ltd., Spain). Using silk threads, one end of the uterus was attached to a fixed support at the bottom of the chamber and the other end was connected to an isometric force transducer (AD Instruments Pty Ltd., Spain) supporting a stainless steel rod. Passive resting tension of 1 g was applied. The tissue-bathing medium used was physiological solution maintained at temperature of 37°C. The electrical signal from the transducer was amplified and converted to a digital signal and recorded on a computer using Chart software (AD Instruments Pty Ltd., Australia). Force was recorded and analyzed using Microcal Origin Software (Achema Pte Ltd., Singapore).

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Effects of drugs: The uterus was allowed to contract spontaneously and an equilibrium period of 30 min was given before an application any drug. The effects of drugs were made whilst the uterus was continually perfused with physiological solution (control) or physiological solution containing 1 μM $PGF_{2\alpha}$ (Kupittayanant *et al*., 2009)/a blocker of L-type voltage-gated calcium channels (10 μM nifedipine) (Kupittayanant *et al*., 2009; Noble and Wray, 2002). In some experiments, free calcium solutions were used; physiological solution in which $CaCl_2$ had been omitted and 1 mM Ethylene Glycol Tetraacetic Acid (EGTA) added (Kupittayanant *et al*., 2009; Noble and Wray, 2002).

Data presentation: Data are given as mean and SEM and n represents the number of samples each one from a different chick.

RESULTS AND DISCUSSION

Spontaneous uterine activity: In chicks (33%), spontaneous contractions could be observed at day 4th after hatching (Fig. 1a). The frequency of contractions was 0.3±0.04 contractions per min (n = 12).

Effects of inhibiting external calcium entry: To investigate whether spontaneous contractions were dependent upon external calcium entry, the solution was changed to one with 0-$CaCl_2$ and 1 mM EGTA (Fig. 1b). Spontaneous force was rapidly abolished but restored upon return of calcium to the bathing solution (Fig. 1b). Nifedipine (10 μM), an inhibitor of L-type voltage-gated calcium channels also rapidly abolished spontaneous force transients in the chick uterus (Fig. 1c). Taken together, these data indicate that spontaneous uterine activity in these 4 days chick uteri is dependent upon external calcium entry through voltage gated L-type calcium channels.

Agonist-induced contraction: The agonist, 1 μM $PGF_{2\alpha}$ was applied to the chick uterus following a control spontaneous contraction. Figure 2a shows the effect of $PGF_{2\alpha}$ in the chick uterus. The response to 1 μM $PGF_{2\alpha}$ in the chick was significantly prolonged. It is known that $PGF_{2\alpha}$ can induce contraction by increasing calcium entry via L-type voltage-gated calcium channels and releasing calcium from internal stores (Kupittayanant *et al*., 2009; Luckas *et al*., 1999). To investigate whether $PGF_{2\alpha}$-induced contractions were dependent upon external calcium entry, nifedipine was applied in the continued presence of 1 μM $PGF_{2\alpha}$. As can be shown in Fig. 2b, contraction induced by 1 μM, $PGF_{2\alpha}$ was rapidly abolished.

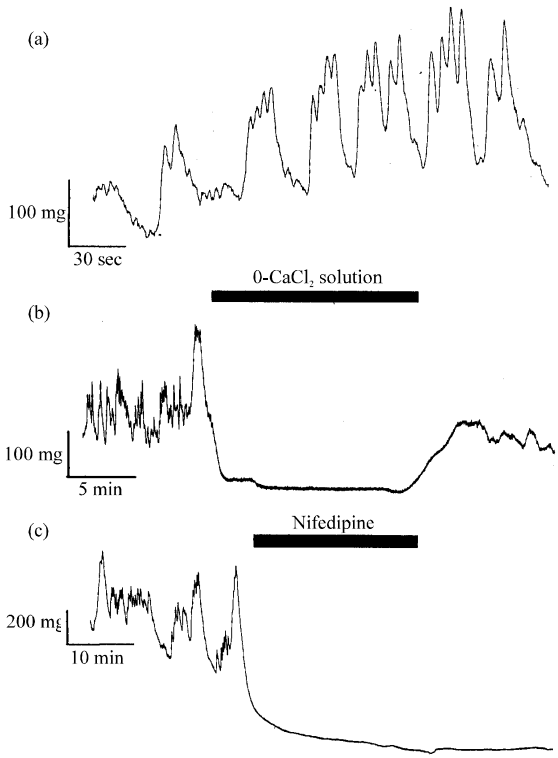

Fig. 1: Effects of 0-$CaCl_2$ and nifedipine on spontaneous force in uterus from 4 days old chicks; a) spontaneous activity (n = 12); b) effect of 0-$CaCl_2$ solution containing 1 mM EGTA on spontaneous force (n = 5) and c) effect of 10 μM nifedipine on spontaneous force (n = 5)

To investigate whether $PGF_{2\alpha}$ could release calcium from the sarcoplasmic reticulum, 1 μM $PGF_{2\alpha}$ was added in the absence of external calcium entry. This protocol was used to ensure that the only source of calcium is from the sarcoplasmic reticulum (Kupittayanant *et al*., 2009; Luckas *et al*., 1999).

As can be shown in Fig. 2c spontaneous contractions stopped upon changing to 0-$CaCl_2$ and 1 mM EGTA solution. In the presence of 1 μM $PGF_{2\alpha}$ but with out external calcium some force was produced and always larger when compared to spontaneous force (Fig. 2c).

Taken together these data indicate that $PGF_{2\alpha}$ stimulates uterine activity in these 4 days chick uteri is dependent upon external calcium entry through voltage gated L-type calcium channels as well as internal calcium release form the sarcoplasmic reticulum.

Researchers found that the chick uterus was capable of contracting spontaneously and responsive to the agonist. These suggest that at day 4th, all aspects necessary for excitation-contraction coupling and signal

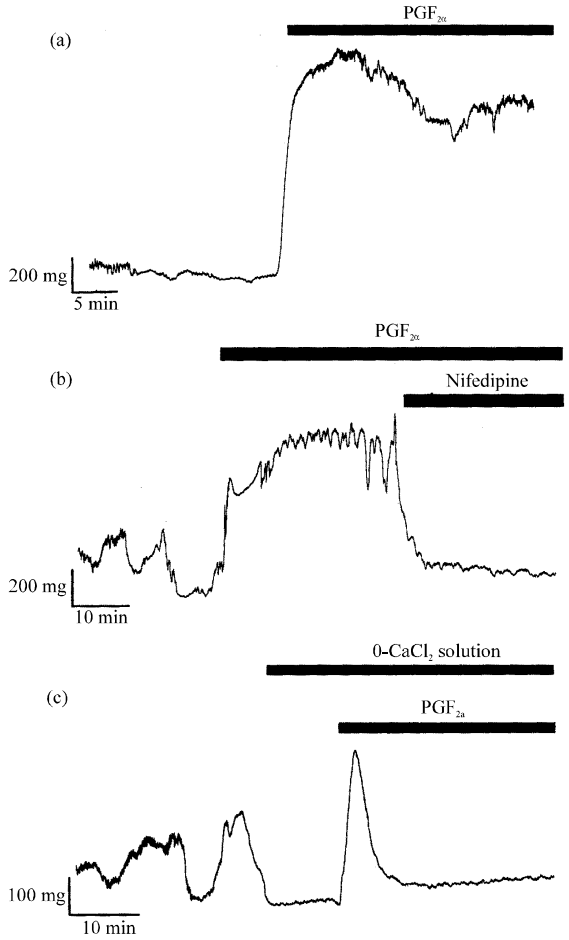

Fig 2: Agonist-induced changes in force in uterus from 4 days old chicks; a) effect of 1 μM $PGF_{2\alpha}$ on spontaneous contracting uterus (n = 5); b) effect of 10 μM nifedipine on 1 μM $PGF_{2\alpha}$-induced contraction and c) $PGF_{2\alpha}$-induced sarcoplasmic reticulum calcium release and force responsiveness. $PGF_{2\alpha}$ (1 μM) was applied for 15 min after exposure to 0-$CaCl_2$ solutions containing 1 mM EGTA (n = 5)

transduction are apparent and functional. However, developed force was not as large or as regular as those present in the laying hens (Kupittayanant *et al*., 2009; Shimada and Asai, 1978). The irregular nature of the spontaneous activity was not well understood. However, this was also found in the uterus of other species (Noble and Wray, 2002). To the best of the knowledge, there is little information on the posthatching development of the uterus. In a study on the oviduct development (Grau *et al*., 1985), it was reported that after hatching there was the beginning of the smooth muscle development but that smooth muscle development was not complete until 20 weeks of age or before the onset of egg laying (Fujii, 1963). This is consistent with the preliminary data showing that the spontaneous activity remained irregularly until 20 weeks of age.

Thus, it might be that force can be developed by the smooth muscle but communication and coordination appear to be functionally and anatomically incomplete at day 4th.

The responses of force to $PGF_{2\alpha}$ suggest that the receptors of $PGF_{2\alpha}$ are present as early as at day 4th in the chick uterus. The clear response to $PGF_{2\alpha}$ and demonstration of a releasable calcium store show that the effects of $PGF_{2\alpha}$ were due to the intracellular signaling pathways in the chick uterus. It is interesting to note that response to $PGF_{2\alpha}$ in the chick uterus was different in shape to that induced in the laying hens. Thus, the response to $PGF_{2\alpha}$ in the laying hens was phasic (Kupittayanant *et al*., 2009). The difference was also observed in the uterus of other species where force and calcium were simultaneously measured (Noble and Wray, 2002). The explanation for the difference is that calcium sensitization mechanisms play a more prominent role in the neonate uterus (Noble and Wray, 2002).

CONCLUSION

The demonstration that the chick uterus is spontaneously active and capable of responding to $PGF_{2\alpha}$, suggests that this phasic activity is an inherent activity of uterine smooth muscle. In addition, researchers have observed differences pertaining to excitation-contraction mechanisms when compared to the laying hens (Kupittayanant *et al*., 2009). It is of interest to highlight mechanisms of these differences in the future.

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