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SHORT COMMUNICATION

Ni2+ treatment causes cement gland formation in ectoderm explants of Xenopus laevis embryo

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ABSTRACT

We found T-type calcium channel blocker Ni^{2+} can efficiently induce the formation of cement gland in Xenopus laevis animal cap explants. Another T-type specific calcium channel blocker Amiloride can also induce the formation of cement gland, while L-type specific calcium channel blocker Nifedipine has no inductive effect. These results may offer us an new approach to study the differentiation of cement gland through the change of intracelluar calcium concentration.

Key words: *Ni^{2+}, cement gland, gastrulation, T-type calcium channel.*

INTRODUCTION

The cement gland is a mucus-secreting organ located anterior most of the body axis of Xenopus laevis embryo. Although cement gland is itself a provisional and simple organ, research on the mechanism of its formation has important bearing in the understanding of anteroposterior pattern formation. The formation of cement gland can be easily influenced by dorsalizing, ventralizing, neuralizing or posteriorizing factors, either positively or negatively[1-4].

Although current research focused on the effect of secretory factors such as growth factors of TGF- β family, we tried to study this problem from a different way. As it has been reported that the signaling of the posteriorizing factor FGF is coupled with Ca^{2+} influx and without FGF signaling, the neuralizing factor Noggin induces the formation of cement gland rather than the expression of neural markers in animal cap explants[5], we

tried to use calcium channel blocker to study the possible effects of Ca^{2+} in this process. We have found that T-type calcium channel blocker Ni^{2+} can induce the formation of cement glands in animal cap explants. Another T-type specific calcium channel blocker Amiloride can also induce the formation of cement gland while L-type specific calcium channel blocker Nifedipine has no inductive effect. This phenomenon may offer us some insight into the intracellular mechanism involved in the differentiation of cement gland.

MATERIALS AND METHODS

The embryonic manipulation was carried out mainly according to Cold Spring Harbor Course Manual[6]. Briefly, Xenopus laevis female and male were both injected with 400 IU human chorionic gonadotrophin (hCG) and eggs were fertilized either naturally or in vitro. Embryos were dejellied in 2% cysteine (pH 7.8) and then transferred into Holtfreter solution. Embryos were incubated at room temperature (18-22 ℃).

The embryos were staged according to the time table of Nieuwkoop and Farber's[7]. The animal cap explants (animal pole ectoderm) were dissected from stage 9 embryos and cultured in 1 × MBS (modified Barth's solution) with or without Ni^{2+} or other reagents. After different time of Ni^{2+} treatment, the animal cap explants were transferred into 1 × MBS for further incubation.

$NiCl_2$ and Amiloride were purchased from Sigma; Nifedipine was purchased from Shanghai Tian-ping Pharmaceutical Factory; other reagents were all of analytic grade.

RESULTS AND DISCUSSION

Ni^{2+} can induce the formation of cement gland in animal cap explant

Animal cap explants from stage 9 embryos were routinely used for Ni^{2+} treatment in our experiment. From the data summarized in Fig 1, animal cap explants from stage 8-10 embryos could all be effciently induced to form cement gland by Ni^{2+} treatment, stage 9 embryos were preferred simply because they were easy to handle.

The optimal Ni^{2+} concentration was determined to be 100 μM. From Tab 1, it can be

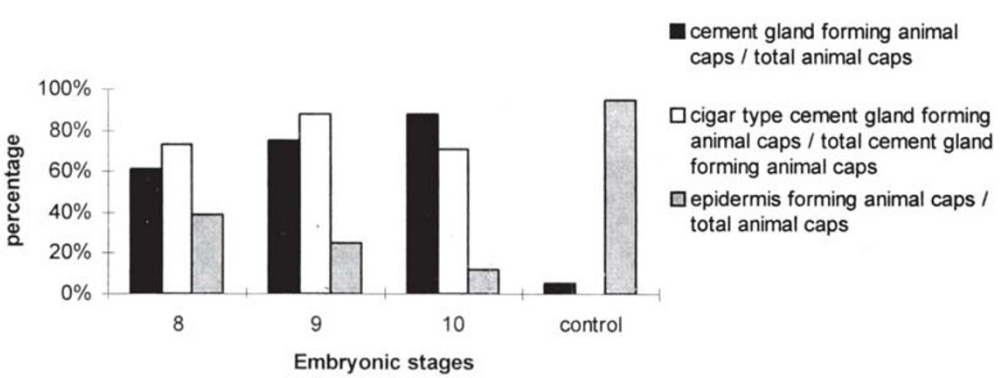
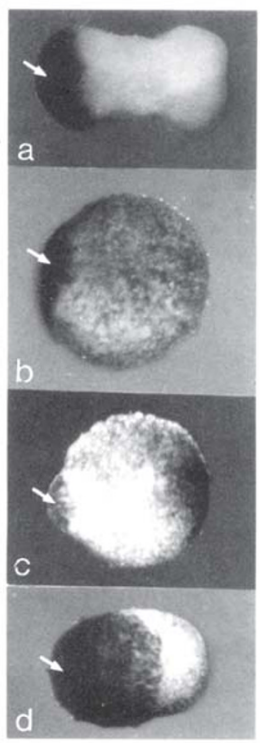


Fig 1. The effect of Ni^{2+} treatment (MBS containing 100 μ M Ni^{2+}) on the formation of cement gland in animal cap explants dissected from different stages of embryos. (Control using stage 9 embryo and MBS instead of Ni^{2+} containing MBS).

seen that Ni^{2+} concentration from 20μM to 300μM can all induce the formation of cement gland, and the size of induced cement gland was proportional to the concentration of Ni^{2+}. The most strongly induced type was dubbed as "cigar" type, shown in Fig 2a. and Fig 3. At Ni^{2+}concentration higher than 100μM, the outer layer had a tendency to separate form the inner layer. Treatment with extremely high concentration of Ni^{2+} could cause the dissociation of the animal cap.

The induction of the formation of cement gland need at least 4 h of Ni^{2+} treatment after



▷ **Fig 2.** Cement gland (arrow indicated) of different sizes, induced by different concentration of Ni^{2+}.
a. the whole outer layer turned into cement gland, this type is dubbed as "cigar" type;
b. a patch of cement gland formed near the edge of the outerlayer, fre quently seen and so referred as nor mal sized cement gland;
c.a small sized spot of cement gland formed;
d. a "cigar" type in its formation.

Fig 3. Section of a "cigar" type: showing well differentiated cement gland cell (arrow indicated) in the outerlayer; inner layer has a tendency to separate eg.

the animal cap was dissected from stage 9 embryo and directly treated with Ni^{2+} containing

medium. It can be seen from Fig 4 that the effciency of induction did not change significantly from 4 h to overnight of Ni^{2+} treatment. But animal caps that were treated for only 3 h in Ni^{2+}containing medium showed a drastically low effciency of cement gland induction.

Tab 1. The effect of different concentrations of Ni^{2+} on animal cap explants from stage 9 embryos

$[Ni^{2+}]\mu M$	Total Number	Cement gland	Size of cement gland	epidermis	Other changes
20	34	11	Very small	23	
50	18	17	Small	1	
100	44	33	29 cigar	11	
300	18	8	5 cigar	10	Inner outer layers separated
1000	20	0		0	all dissociated

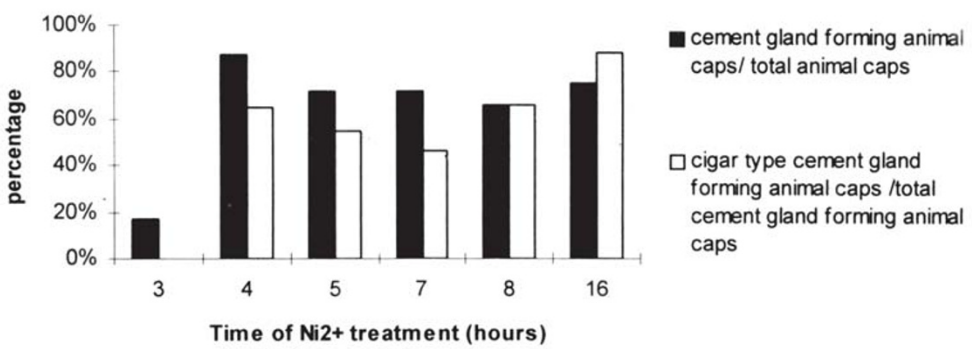


Fig 4. The effect of the treatment duration on the formation of cement glandin animal caps dissected from stage 9 embryos, using MBS containing 100 μM $Ni2^{+}$.

However, when animal cap explants were first cultured in neutral saline such as MBS for 3 h and then transferred into Ni^{2+} ontaining medium for further incubation, the effciency of cement gland induction was no lower than those were directly cultured in Ni^{2+} containing medium after dissection. But animal cap explants being first cultured in MBS for 4 h instead of 3 h before they were transferred into Ni^{2+} containing medium, the result was entirely different. A drastic lowered effciency of induction happened. So it seems that the animal caps may have a time window for Ni^{2+} treatment between 3 and 4 h after being dissected from stage 9 embryos. We repeated this experiment for three times and results were in parallel.

The effect of calcium channel blockers on cement gland formation

Being a factor that has not often been used in biological research, especially in development biology, Ni^{2+} has little established biological effects. One of those known

effects is that Ni^{2+} can specifically block the T-type calcium channel[8-13]. The T-type calcium channel is unique for being a low-voltage gated calcium channel and its activation can cause prolonged rise of cytosolic calcium concentration. But whether this is causally related to the cement gland inducing ability of Ni^{2+} and whether there actually exists T-type calcium channel so early in the embryo are not known.

Since Nifedipine is a specific L-type calcium channel blocker[12], and Amiloride blocks T-type calcium channel specifically, we used these two chemicals to further identify which kind of calcium channel is responsible in this phenomenon. We found even with concentration substantially higher than the Kd of Nifedipine (10 μM), there was no formation of cement gland at all. But Amiloride, another specific blocker of T-type calcium channel [12, 14] could induce the formation of cement gland, although less effciently in comparison with Ni^{2+} treatment. About 50% of the animal cap explants treated with Amiloride formed cement glands of sizes that tended to be smaller than those found in Ni^{2+} treatment.

Possible mechanism of Ni^{2+} treatment

Animal cap explants dissected from blastula embryos will form atypical epidermis if cultured in neutral medium such as MBS[6, 15]. The ability of Ni^{2+} to induce the animal cap explants to form cement gland may be resulted from the disturbance on certain induction or differentiation events of normal development. Ni^{2+} treatment may directly influence certain steps in the differentiation by changing the cytosol calcium concentration. Ni^{2+} wa reported to be a specific T-type calcium channel blocker and its Kd range from 30μM to 780μM in different systems[8]. We showed above that specific T-type calcium channel blocker Ni^{2+} and Amiloride can both induce the formation of cement gland while specific L-type calcium channel blocker Nifedipine has no effect on the development of cement gland. As the activation of T-type calcium channel was considered to result in sustained increase of cytosol calcium[13, 16], the blockage of T-type calcium channel may disturb the calcium concentration fluctuation during normal development.

Therefore, a possible mechanism of Ni^{2+} treatment is that the blockage of the T-type calcium channel resulted in the hindrance of calcium influx which is vital for FGF signaling. The attenuated FGF signaling, in turn, cause the induction of cement gland. We are now trying to verify this possibility.

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