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Effects of Lithium on Formation of the Larval Body Pattern of Xenopus Laevis

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Abstract: Xenopus laevis embryos ranging in development from the 2-to 4-cell stage to the early blastula stage were exposed to Lithium chloride concentrations of from 0.01 M to 0.1 M. (4 hours). Fertilized eggs of Xenopus laevis were obtained by natural mating induced by injecting a male and a female frog with 400 I.U. and 600 I.U. of human chorionic gonadotropin, respectively, and keeping them together overnight in an aquarium at 24 ± 2°C. Fertilized eggs were selected under dissection microscope.

Xenopus embryos failed to neurulate and developed without discernible anteroposterior axis. Treatment with Lithium at progressively later stages reduced developmental defects, and neural differentiation was returned to normal levels. It was observed that from the 2-cell to the early blastula stage, lithium resulted in the reduction of posterior structures (anteriozation).

Key Words: Lithium, Xenopus laevis, neural development, neurulation

Lityumun Xenopus Laevis Larval Vücut Paternine Etkisi

Özet: Xenopus embriyoları Lityum klorüre 0.01M'den 0.1 M'e kadar 6 değişik konsantrasyonda, 2 - 4 hücreli evreden sonra erken blastula safhasına kadar (Evre 7) dört saat maruz bırakıldı.

Xenopus'tan fertil yumurta eldesi, doğal yolla erkeğe 400 I.U, dişiye 600 I.U. human koriyonik Gonadotropin zerkedilerek elde edildi. Xenopus'lar gece boyunca aynı akvaryumda 24 ± 2°C'de beraber tutuldu. Elde edilen yumurtaların fertil olup olmadığı disseksiyon mikroskobunda incelendi.

Xenopus embriyoları nörülasyonda başarısızlığa uğradılar ve anterio-posterior aksisde görülür bir değişim olmadı. Lityuma geç fazda muamele gelişim defektlerini azalttı ve nöral farklılaşma tekrar normal düzeye geldi. Sonuç olarak, lityum'un larval vücut üzerine 2 hücreli evreden erken blastulaya kadar posterior yapının redüksiyonu yönünde (anteriozasyon) etki ettiği gözlendi.

Anahtar Sözcükler : Lityum, Xenopus laevis, nöral gelişim, nörülasyon

Introduction

Lithium has long been known (1-7) to affect the larval body pattern in various species of amphibians. Many biochemical studies have shown that Li blocks the inositol triphosphate-diacylglycerol second messenger pathway by inhibiting the conversion of inositol

phosphate to inositol (Sherman et al. 1981; Berridge, 1987). This pathway transduces e tracellular signals to intercellular calcium fluxes, thus leading to changes in cell physiolo The observation that Li alters pattern formation suggests that cleavage-stage intercellul communications mediated by the inositol triphosphate pathway normally play a role in lat morphogenesis (7). Furthermore, studies have shown that exposure of embryos to lithiu at an early stage (cleavage stage) usually results in the reduction of posterior structure such as the tail fin and segmented somites in the larval stage. On the other hand, exposu to lithium at a later stage (gastrula stage) results in the reduction of anterior structure such as the head, optic vesicles and the cement gland. These findings imply that the effec of lithium on the formation of the larval body pattern change from the blastula to gastru stage (1).

Furthermore, and abrupt slowing down and synchronization of the cleavage cycle, pre ently known as midblastula transition (MBT), occurs during the blastula stage, i.e., just a ter the 11th (Ambylostoma) or 12th (Xenopus and Cynops) cleavage. The synthesis of var ous RNAs and proteins required for later morphogenesis is known to start at the time MBT (1).

Backstrom et al. (1954) obtained similar results, and further suggested that neural di ferentiation was reduced. Consequently, they proposed that lithium "ventralized" the em bryo. Both sea urchin and amphibian embryos are sensitive to lithium treatment during th early cleavage stages (sea urchin: Lallier, 1964; amphibian: Pastells, 1945; Hall, 1942 Backstrom, 1954) (2). During the gastrulation stage, exposure of the embryos to lithiur treatment has little effect. Attempts to clarify the consequences of lithium treatment by ex posure of isolated ectoderm stripped from the amphibian embryo to lithium have dem onstrated a confusingly wide range of effects. These include the induction of derivations c three characteristic germ layers (Englander, Johnen, 1967), the formation of mesoderma structures (Ogi, 1961; Masui 1961; Grunz 1968; Johnen 1970) and the induction of neu ronal differentiation (Barth & Barth, 1962, 1974) (2).

In this study, we examined the consequences of treatment of early amphibian embryo with lithium chloride in order to test whether previous suggestions that lithium ions migh interfere with neural induction are correct.

Materials and Methods

Xenopus adults were obtained (Xenopus I Ann Arbor, Michigan) and kept in a glas aquarium in dechlorinated tap water. During experiments the temperature was 24 ± 2°C Adult Xenopus were fed twice a week with chicken liver. To induced mating, the femal and male received 600 and 400 I.U, respectively, of human chorionic gonadotropin (Or ganon) via injection into the dorsal lymph sac.

Amplexus normally ensued within 2-6 hours, the deposition of eggs taking place from 9 to 14 hours after injection. Following breeding, the adults and their fecal material were re moved from the aquarium, and the embryos were collected in Petri dishes.

The study, was repeated 5 times, and 6 different concentrations were used, ranging from 0.01M to 0.1M. Thirty embryos were soaked in Petri dishes containing a total of 50ml of different concentrations of lithium chloride. Later, the embryos were evaluated under a dissecting microscope to find out whether the eggs were fertile according to Normal Table of Xenopus laevis (8), and then eggs at the 2- to 4-cell stage were selected.

The embryos were exposed to different concentrations of lithium chloride for 4 hours from the 2- to 4-cell stage to the early blastula stage. FETAX solution was used as a control solution (9). After 4 hours embryos were washed twice with distilled water twice to remove excess lithium chloride and then placed in Fetax solution. At 24, 48, 72 and 96 hours, dead embryos were removed and the number of dead embryos recorded. The number of live, malformed embryos and their stage of development were determined according to Nieuwkoop and Faber (8). Deaths at 24 hours (stage 27) and 48 hours (stage 35) were determined by skin pigmentation, structural integrity and irritability of the embryos, while at 72 hours (stage 42) and 96 hours (stage 45), the absence of a heartbeat (visible) was also used as an indicator of death. At 96 hours, dead and malformed embryos were removed and recorded for each group.

The surviving embryos were fixed in formalin (5 to 10%) and the body length (cm) of each embryo was individually measured. The degrees of malformation were designated anteriozation (A1, A2, A3, A4) and posteriozation (P1, P2, P3, P4), according to Yamaguchi and Shinagawa's method (1). Each grade of deficiency was as follows: A1, slight reduction of tail; A2, extreme reduction of posterior structures such as the tail fin and segmented somites; A3, absence of posterior structures but presence of essentially normal eyes and cement gland; A4, absence of any axial structures, but presence of radially located cement gland. Abnormalities of posteriozation were not seen because of exposing time. For statistical analysis, the chi-square test was used.

Results

In this study, the effects of lithium appeared first at the 2-cell stage as a slight reduction of posterior structures (anteriozation) (Figure 1, 2, 3). The results of exposure lithium chloride exposure of from 0.01M to 0.1M on Xenopus embryo survival and the percentage of abnormalities from all five studies are presented in Table 1.

In the study, a total of 150 embryos were used as controls. Seven of these died (4.99%) and 6 of the survivors were malformed (4%). Malformations were limited to a slight reduction of the tail, extreme reduction of the tail fin and segmented somites, the absence of any axial structures, but the presence of radially located cement gland (Table 2). The percentages of surviving embryos were determined for normal and malformed survivals. The malformation percentages for the surviving embryos at concentrations of 0.01M, 0.02M, 0.04M, 0.06M, 0.08M, and 0.1M LiCl were 5.3%, 8.6%, 17.3%, 41.3%, 22.6%, and 0% (because embryos at this concentration all died), respectively (Table 1). As seen in Table 1, at a concentration of 0.06M LiCl were 93.3%, 90%, 87.3%, 78.6%,

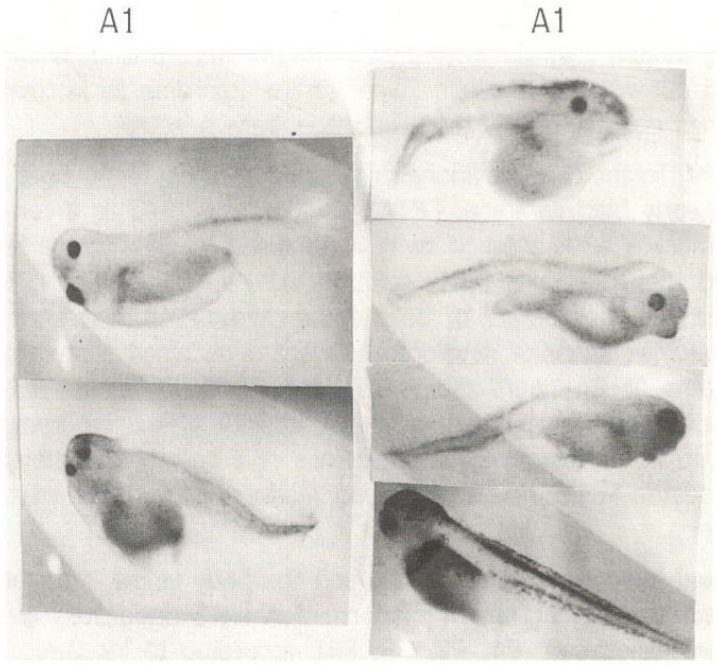

Figure 1. Abnormalities (anteriozation; A1) of Xenopus laevis embryos after 4 hours of exposure to LiCl concentrations 0.01M and 0.02M.

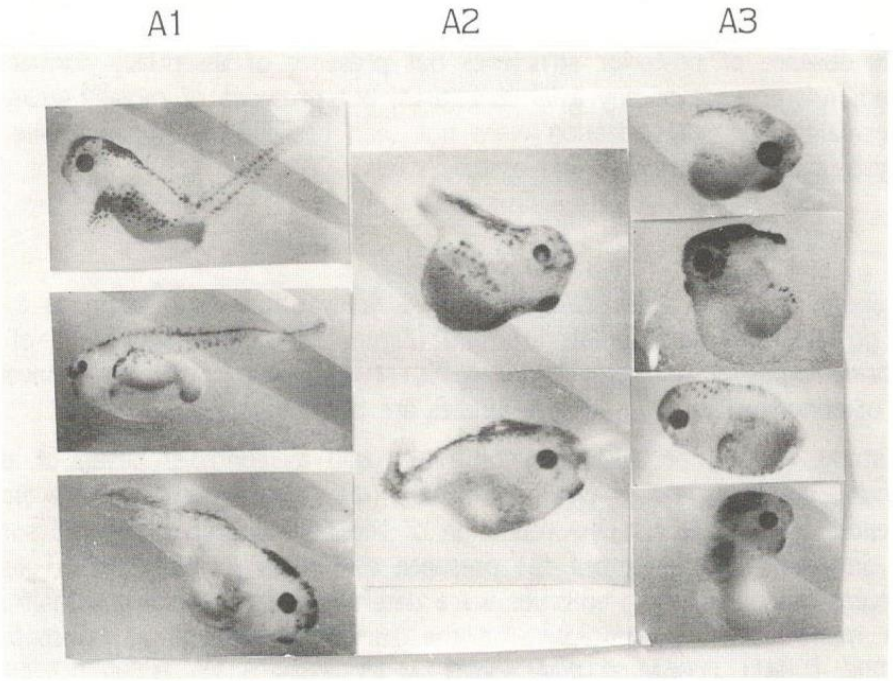

Figure 2. Abnormalities (anteriozation; A1, A2, A3) of Xenopus laevis embryos after 4 hours of exposure to an LiCl concentration of 0.04M.

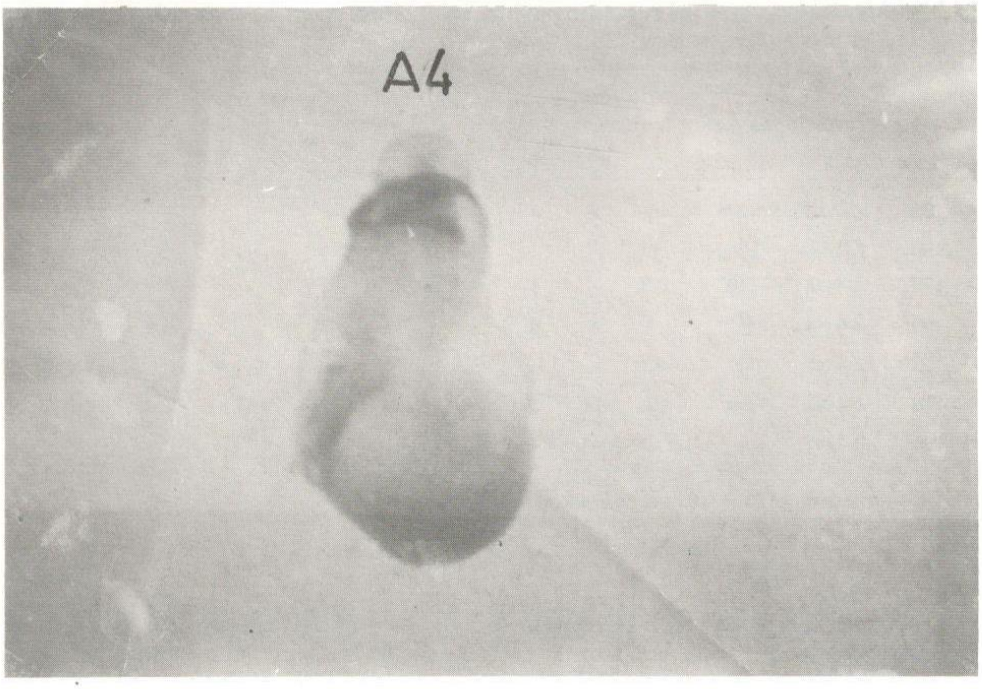

Figure 3. Abnormalities (anteriozation; A4) of Xenopus leavis embryos after 4 hours of expose to an LiCl concentration of 0.08M.

Table 1. The survival and malformation percentages of embryos after 96 hours of exposure to different LiCl concentrations at the 2- to 4-cell stage (Conc. = Concentration, N = Normal, A = Abnormal).

Conc. (g/L)	Group I	Group II	Group III	Group IV	Group V	Total no of abnormal normal embryos	Percent of Survivors	Percent of Malformed	Total no of Dead
0.01M (n=150)	27 N 1 A	24 N 3 A	26 N 1 A	25 N 2 A	25 N 1 A	127 N 8 A	90.0	5.3	15
0.02M (n=150)	26 N 2 A	23 N 2 A	25 N 2 A	22 N 4 A	22 N 3 A	118 N 13 A	87.3	8.6	19
0.04M (n=150)	18 N 6 A	17 N 8 A	18 N 7 A	16 N 4 A	23 N 0 A	92 N 26 A	78.6	17.3	33
0.06M (n=150)	0 N 13 A	14 N 1 A	0 N 14 A	12 N 2 A	2 N 9 A	5 N 62 A	44.6	41.3	83
0.08M (n=150)	0 N 9 A	0 N 5 A	0 N 9 A	0 N 10 A	0 N 1 A	34 A	22.6	22.6	116
0.1M (n-150)	0 0	0 0	0 0	0 0	0 0	0 0	0	0	0
Control (n=105)	27 N 1 A	27 N 3 A	27 N 1 A	27 N 0 A	29 N 1 A	137 N 3 A	93.3	2.0	7

Table 2. Anomaly scaling of embryos exposed to LiCl concentrations from the 2- to 4-cell stage to the 7-cell stage for 4 hours (Conc. = Concentration, Cont. = Control).

Conc. No.	Embryos	Dev. Stage	Normal	Anteriozation				Posteriozation*				% Anomaly
				A1	A2	A3	A4	P1	P2	P3	P4	
Cont.	150	2-4 cell	127	3	–	–	–	–	–	–	–	2.3 A1
0.01	150	2-4 cell	127	8	–	–	–	–	–	–	–	5.9 A1
0.02	150	2-4 cell	118	13	–	–	–	–	–	–	–	9.9 A1
0.04	150	2-4 cell	92	7	5	4	–	–	–	–	–	5.9 A1, 12.7 A2, 3.58 A3
0.06	150	2-4 cell	62	23	–	–	–	–	–	–	–	2.9 A1, a.4 A2
0.08	150	2-4 cell	34	6	12	6	–	–	–	–	–	8.8 A2, 17.6 A3, 23.5 A4
0.1	150	2-4 cell	–	–	–	–	–	–	–	–	–	–

* Anomalies were not seen at the P1, P2, P3 and P4 phases of posteriozation.

Table 3. The mean body length (cm) of the surviving embryos after exposure to Lithium Chloride concentrations in the same experiment (Conc. = Concentration, Cont. = Control, M = Mole).

Conc. g/mg	Body Length	Cont. (n=150)	0.01M (n=150)	0.02M (n=150)	0.04M (n=150)	0.06M (n=150)	0.08M (n=150)
Cont.	1.09±0.002	–	p<0.001	p<0.001	–	p<0.001	p<0.001
0.01M	079±0.027	p<0.001	–	p>0.05	p<0.05	p<0.001	p<0.001
0.02M	0.74±0.015	p<0.001	p>0.05	–	p>0.05	p<0.001	p<0.001
0.04M	0.67±0.51	p<0.001	p<0.001	p>0.05	–	p>0.001	p<0.001
0.06M	0.42±0.056	p<0.001	p<0.001	p<0.001	p<0.001	–	p<0.01
0.08M	0.28±0.036	p<0.001	p<0.001	p<0.001	p<0.001	p<0.01	

44.6%, 22.6% and 0%, respectively. Thus, the rate of embryo survival was sharply reduced at a concentration of 0.06M LiCl (44.6%).

In Table 2, the embryos were graded according to Yamaguchi (1), as anteriozation 1, 2, 3, 4 and posteriozation 1, 2, 3, 4. The malformation percentages for control groups at concentrations of 0.01M, 0.02M, 0.04M, 0.06M, 0.08M and 0.1M were 2.3% A1, 5.9% A1, 9.9% A1, 5.9% A1, 12.7% A2, 3.58% A3, 2.9% A1, 4.4% A2, 8.8% A2, 17.6% A3, 23.5% A4, respectively. As seen above, while LiCl concentrations 0.01M and 0.02M yielded only grade A1, a concentration of 0.04M yielded grades A1, A2, and A3, and a concentration of 0.8M yielded grades A2, A3, and A4 (Table 2, Figure 1, 2, 3).

In Table 2, body length as measured at 4 days is presented. When the control group (1.09±0.002cm) was compared to groups with other concentrations (0.01M, 0.79±0.027; 0.02M, 0.74±0.005; 0.04M, 0.67±0.51; 0.06M, 0.42±0.056; 0.08M, 0.28±0.036) the differences were found to be statistically significant ($p<0.001$). When the 0.01M LiCl group was compared to other groups, it was found to be no different from the 0..02M LiCl group, but significantly different from the 0.04M ($p<0.05$), 0.06M, 0.08M LiCl groups ($p<0.001$).

The 0.02M LiCl group was not significantly different from the 0.01M and 0.04M LiCl groups, but the differences between it and the 0.06M and 0.08M LiCl ($p<0.001$) groups were significant. on the other hand, when the 0.04M LiCl group was compared to the 0.02M LiCl group the differences were not significant, but when the 0.01M LiCl group :0.05) was compared to the 0.06M and 0.08M LiCl groups, the differences were significant ($p<0.001$). Also, when 0.06M and 0.08M LiCl was analyzed wiht other groups there were statistical differences ($p<0.001$).

Discussion

The observations suggest that early blastular lithium treatment of whole embryos could transform and restrict body pattern solely by modulation of the responses to inductive signals in the tissue of the animal hemisphere (1). In experiments with amphibians many different kinds of malformations have been produced. In our experiment we graded the abnormalities according to Yamaguchi (1).

There are no reports on the precise stage at which alteration in the effect of lithium occurs. This may be attributed to the fact that in previous studies, embryos have been exposed to somewhat low concentrations of lithium (about 0.1M) for rather longer periods (1-4 hours). During such long periods of exposure, most amphibian embryos normally undergo two or more cycles of cleavage, and so the exact time of alteration was not determinable (1).

As Backström reported, two distinct syndromes of deficiency, reduction of posterior structures such as the tail and the segmented somite, and of anterior structure such as the tail, eyes and cement gland were observed to various extents with exposure of early and late-stage embryos to lithium (1). Yamaguchi, for the sake of convenience, called the former syndrome anteriozation and the latter posteriozation. However, in our study, embryos were exposed to LiCl only at early stages. Yamaguchi graded larvae showing anteriozation as A1 to A4, according to Cooke and Smith, and those showing posteriozations a P1 to P4 (1, 5). The criteria used for each grade of deficiency were as follows: A1, slight reduction of the tail; A2, extreme reduction of posterior structures such as the tail fin and segmented somites; A3, the absence of posterior structures but the presence of essentially normal eyes and cement gland; A4, the absence of any axial structures, but the presence of a radially located cement gland; P1, slight reduction of anterior structures; P2, the absence of eyes and cement gland, but the presence of essentially normal posterior struc-

tures; P3; the absence of both head and tail structures, but the presence of segmented somites; P4, round shape without any distinct external organs, such as a cement gland.

Most of these abnormalities were found at a concentration of 0.08M, and the percentage of survivors was reduced. At a concentration of 0.06M LiCl, abnormalities were seen, and the survival rate of embryos was 41.3% and 44.6%, respectively. As seen in Table 1, when the concentrations were high, the grade of malformation increased. At an LiCl concentration of LiCl, grade A2, and A4 malformation were seen, while at a concentration of 0.1M the eggs did not develop because they had been inhibited at early stages. This showed that when the concentration was high, then the abnormalities grade of the abnormalities increased, the percentage of survivors was reduced and the percentage of malformation was increased.

Body lengths were reduced parallel to increased concentration (Table 3). It was thought that when the concentration was increased, the malformation rate was increased. Furthermore, certain abnormalities e.g., edema, Mesodermalization of notochord, kinked tail caused reduction of body lengths.

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