Undersea Biomedical Research, Vol. 2, No. 3, September 1975

BRIEF COMMUNICATIONS

Effects of age and magnesium ions on oxygen toxicity in the neonate chicken

L. W. TORLEY and H. S. WEISS

Department of Physiology, College of Medicine, The Ohio State University
312 Hamilton Hall, 1645 Neil Avenue, Columbus, Ohio 43210

Torley, L. W., and H.S. Weiss. 1975. *Effects of age and magnesium ions on oxygen toxicity in the neonate chicken.* Undersea Biomed. Res. 2(3):223-227.– White Leghorn male chicks, 2-6 days and 4 weeks old, were injected with $MgCl_2$ (2mM Mg/kg body weight) and exposed to 100% O_2 at 75 lb/in^2 (approximately 5 atm). Compared to saline-injected controls, average time to onset of convulsions was delayed 37% in the younger group with no significant difference in the protective effect during days 2-6. The delay in onset of convulsions was 66% in the 4-week-old group. The protective action of Mg^{++} in the chick agrees closely with that reported for the mature rat. Chicks treated with Mg^{++} did not appear depressed. Susceptibility to hyperbaric O_2 convulsions increased uniformly between the 2nd and 6th day, with time to onset of convulsions decreasing by 1.4 min/day from the 19.3 ± 1.01 (SE) min observed at day 2 for saline controls. Between the 6th and 28th day, susceptibility was relatively stable with time to onset decreasing by about 0.2 min/day in controls.

hyperoxia	oxygen toxicity and age
hyperbaric oxygen	oxygen toxicity in chickens
convulsions	oxygen toxicity and Mg
chickens	protection against O_2 toxicity

Magnesium has been shown to be effective in mature rats against the convulsive effects of O_2 at high pressure (OHP) but to our knowledge Mg has not been tested in other species (Radomski and Wood 1970; Radomski and Watson 1973). The chick seemed to be a worthwhile test model because it would extend the observations into another class of vertebrates and it might supply some information on the role of age on the Mg^{++} effect, since susceptibility to OHP increases considerably during the first few weeks of the chick's life (Wood 1970; Wood, Radomski, and Watson 1971).

METHODS

Day-old White Leghorn male chicks were obtained from a commercial hatchery and housed in a standard brooder with food and water ad lib. The early phase of the experiment commenced the following day (2 days of age) and continued for 5 consecutive days. The first week after hatch was chosen because it is the interval during which the most striking increase in susceptibility to OHP occurs (Wood 1970; Wood et al. 1971). To determine whether the change during the first week of age was gradual or abrupt, a sample of the chicks was tested daily. The later phase of the experiment was conducted on 28-day-old chicks. Chicks were randomly selected, weighed, individually color coded, and injected ip with 0.01 ml/g body weight of either standard saline (0.9%), or 200 mM $MgCl_2$ in saline.

The injected Mg^{++} dose was 2 mM/kg, corresponding to the upper level found effective in rats (Radomski and Wood 1970). The injection volume was kept as small as possible to minimize volume-loading effects. The Mg^{++} solution was, therefore, hypertonic in order to

maintain the same dose of sodium as for the controls. The injected chicks were placed in cages in groups of 4 to 5 and examined for injection reactions prior to O_2 exposure. At least 20 min were allowed for distribution of the ions.

Pressurization was accomplished in a Bethlehem model H hyperbaric chamber kept at a temperature between 30 and 35°C. The chamber was flushed with O_2 for 10 min prior to insertion of the cages and for an additional 5 min afterwards. The chamber was then pressurized to 75 lb/in^2 with O_2 (approximately 5 atm) in 4-5 min. When the desired pressure was reached, the O_2 flow through the chamber was adjusted to approximately 3 liters/min. Timing commenced when the chamber reached 75 lb/in^2. The chicks were under constant surveillance through the glass ports in the chamber wall. Time of convulsion was recorded for each bird. Convulsions were defined as the onset of a generalized series of colonic and tonic seizures which were quite unmistakable. After all birds had convulsed, the chamber was decompressed and the animals were sacrificed with a chloroform overdose.

RESULTS

The chicks did not appear anesthetized or depressed following Mg^{++} injection. For the 1-week-old chicks Mg^{++} significantly delayed convulsion time on all days tested (Table 1) with the protective effect ranging from 33% to 46%. In both the Mg^{++} treatment and control groups, time to convulsion occurred sooner as the birds aged. Analysis of variance confirmed that the protective effect of Mg^{++} was highly significant ($P \leq .01$). The earlier onset of convulsions with age was also highly significant ($P \leq .01$). However, interaction between the Mg^{++} and age effects was not significant, indicating the protective effect of Mg^{++} was essentially the same between 2 and 6 days of age. The increase in time to

TABLE 1

Time to convulsions of White Leghorn male chickens exposed to hyperbaric oxygen (75 lb/in^2) and treated with magnesium chloride

		Means ± SE at various ages					
		15 chicks per treatment per day					*8 chicks*
		2 days	3 days	4 days	5 days	6 days	28 days
Body weight (g)		35.5 ±0.8	37.5 ±0.8	40.9 ±0.9	41.1 ±0.8	46.9 ±0.9	244.0 ± 1.4
Time to convulsion (min)	$MgCl_2$†	28.3* ±2.1	24.4* ±1.0	22.6* ±1.5	21.8* ±1.1	20.3* ±1.5	19.6* ±1.9
	Saline control	19.3 ±1.0	18.0 ±0.9	16.3 ±1.1	16.2 ±0.8	15.3 ±0.9	11.8 ±0.8
% change in time to convulsions due to Mg		45.9	35.6	39.0	34.8	32.8	66.1

* Significantly longer than saline control, $P \leq .01$.

† Injection (ip) of 0.01 ml/g of 200-mM solution in 0.9% saline, giving 2 mM Mg/kg.

convulsion, averaged over this 5-day period, was 37.6%, for an improvement ratio over controls of 1.4 (range 1.3 to 1.5).

Convulsions occurred sooner in 28- than in 6-day-old birds but the rate of earlier onset of 0.1 to 0.2 min/day was far less than during the first week. The delay in onset due to Mg^{++} was more evident in the older birds, where the improvement ratio was 1.7.

The first week data was subjected to a more detailed analysis of covariance by taking into consideration differences in chick body weights. This was based on preliminary studies which had suggested that heavier birds tended to convulse sooner than presumably equal aged but lighter birds. However, adjusting convulsion time for body weight differences had very little effect on the means and did not alter the statistical results. Regression equations for the corrected first-week data are

$$\text{for } Mg^{++}: Y = -1.91X + 31.30 \ (S_b = 0.45, r = 0.44), \quad (1)$$

$$\text{and for saline: } Y = -0.88X + 20.21 \ (S_b = 0.27 \text{ and } r = 0.36) \quad (2)$$

where S_b = standard error of the regression coefficient (see e.g. Chap. 6 *in* Snedecor and Cochran, Statistical methods 6th ed., 1967, Iowa State University Press, Ames, Iowa) and r = regression coefficient (values based on the 75 individual chicks per treatment and not on the mean of the 15 chicks used each of the 5 days). The earlier onset of convulsions with increasing age is significant for both groups. Although the regression coefficient for Mg^{++} was almost twice that for controls, the difference between them did not quite reach the 0.05 level of significance. Averaging of the two regression coefficients is therefore assumed appropriate, and provides a decrease in time to convulsions of 1.4 min/day over the first 6 days. The regression equations based on first-week data grossly underestimate time to convulsion in the 28-day-old chicks, which further indicates that the age effect levels off after the first week.

DISCUSSION

These results suggest that the protective action of Mg^{++} against OHP convulsions is probably a general phenomenon in that it can be demonstrated in representatives of Aves as well as Mammals. Our 33-66% delay in onset of convulsions in 2- to 28-day-old chicks injected with 2.0 mM/kg $MgCl_2$ and exposed to 75 lb/in^2 (approximately 5 atm) O_2 appears comparable to the 30-51% longer time to reach 50% convulsions (CT_{50}) in 190-220 g rats treated with from 0.5 to 2.0 mM/kg (Radomski and Wood 1970). Similarly, our improvement ratio of 1.3 to 1.7 seems comparable to the convulsion reduction factor (CRF) of 1.5 for mature rats treated with 1.0 mM/kg (Radomski and Watson 1973). In view of these similarities in response between the young chick and the mature rat over a 4-fold dose range, the 1.3-1.7 improvement ratio in the chick may reflect the maximum to be expected with Mg^{++} in adult chickens as well.

Although we saw no depression, these results do not clarify whether the action of Mg^{++} is peripheral or central (Radomski and Wood 1970; Radomski and Watson 1973) because of the controversey over the existence of a blood-brain barrier in the neonate chick (Hanig, Aiello, and Seifter 1970; Lajtha 1970; Svanberg 1970; Wood et al. 1971; Osuide 1972).

Average time to convulsion in our 2-day-old control White Leghorn male chicks exposed to 5 atm O_2 (19.2 min) appears quite similar to experiments (Wood 1970; Wood et al. 1971)

where the CT_{50} was 21.2-22.5 min. We find that the increase in susceptibility to O_2 convulsions with age (Wood 1970; Wood et al. 1971) develops quite uniformly during the first week (1.4 min/day) and also confirm that, after the first week, there is relatively little change in convulsion time, at least up to the 4th week (Table 1).

The increasing susceptibility to convulsions with age in the chick has been linked to a progressive lowering by OHP of brain GABA levels (Wood 1970; Wood et al. 1971). The chicken also exhibits increased susceptibility with age to the 1-atm or *pulmonary* type of O_2 toxicity (Weiss, Beckman, and Wright 1965) but this is presumably via a different mechanism since at least 3 atm O_2 is said to be needed to affect GABA (Wood, Watson, and Murray 1969). Species differences in susceptibility to O_2 toxicity seen at 1 atm also do not carry over to 5 atm. Thus, the unusual resistance of the young chick compared to the mature rodent to 1 atm O_2 (Weiss, Wright, and Hiatt 1965) appears reversed at 5 atm. At 5 atm O_2 the 21 min to CT_{50} in the adult mouse (Wood et al. 1969) and the 21-42 min to CT_{50} in the mature rat (Radomski and Wood 1970; Radomski and Watson 1973) are considerably longer than the 8-9 min CT_{50} in 22-day-old chicks (Wood 1970; Wood et al. 1971) or our 12-min average in 28-day-old chicks (Table 1).

Received for publication February 1975; revision received July 1975.

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Torley, L. W., and H. S. Weiss. 1975. Influence de l'âge et des ions de magnésium sur la toxicité de l'oxygène chez le poulet nouveau-né. Undersea Biomed. Res. 2(3):223-227.–Des poulets mâles blancs Leghorn, agés de 2-6 jours et de 4 semaines, ont reçu des injections de $MgCl_2$ (2 mM Mg/kg poids corporel) et ont été exposés à 5 atm d'oxygène à 100%. (Les poulets témoins ont reçu des

injections de solution saline physiologique.) Le temps moyen jusqu' au commencement des crises convulsives a été retardé par 37% chez les poulets plus jeunes; aucune différence significative quant à l'effet protecteur au cours des journées 2-6 n'a été observée. Le délai jusqu'au commencement des crises était de 66% chez les poulets âgés de 4 semaines. L'effet protecteur des ions de magnésium chez le poulet ressemble fort à celui rapporté pour le rat adulte. Les poulets traités avec le Mg^{++} ne semblaient pas déprimés. L'incidence de convulsions dues à l'oxygène hyperbarique a augmenté d'une facon regulière entre le 2^e et la 6^e journée. Le délai jusqu'au commencement des crises a diminué de 1.4 minutes par jour, du délai de 19.3 ± 1.01 (SE) minutes noté au 2^e journée pour les animaux témoins. Entre le 6^e le 28^e jour, le délai jusqu'au commencement des crises a diminué de 0.2 min/jour chez les témoins.

hyperoxie	toxicité de l'oxygène et l'âge
oxygène hyperbarique	toxicité de l'oxygène chez le poulet
crises convulsives	toxicité de l'oxygène et Mg
poulets	protection contré la toxicite de l'oxygène