TERATOLOGY 23:397–401 (1981)

Genetic Aspects of the Effects of Methylmercury in Mice: The Incidence of Cleft Palate and Concentrations of Adenosine 3′:5′ Cyclic Monophosphate in Tongue and Palatal Shelf

KENNETH HARPER, RISA BURNS, AND ROBERT P. ERICKSON
Department of Human Genetics and Pediatrics, University of Michigan School of Medicine, Ann Arbor, Michigan 48109

ABSTRACT Concentrations of adenosine 3′:5′ cyclic monophosphate (cAMP) were measured in the tongues and palates of 14.5-day-old fetuses from control and methylmercury-treated mothers of four inbred lines of mice which represent the four possible combinations of two *H-2* alleles and two residual genetic backgrounds. The incidence of cleft palate in fetuses from control and methylmercury-treated mothers was also examined. The *H-2* alleles significantly affected the degree of reduction of cAMP concentration in palates seen in fetuses from mothers treated with methylmercury. Neither the *H-2* allele nor the residual genetic background played a role in the effect of methylmercury on cAMP concentrations in fetal tongues. The magnitude of increase in the incidence of cleft palate with methylmercury treatment was approximately the same for all lines. Thus, methylmercury-induced cleft palate may not be mediated by the reduction of cAMP. Finally, fetuses with cleft lip had increased palatal cAMP levels, whether or not they were from control or methylmercury treated mothers.

Methylmercury is a known teratogen in mice. Its effects include induction of secondary cleft palate (Spyker and Smithberg, '72; Olson and Massaro, '77; Fuyuta et al., '78). Recently, Olson and Massaro ('80) have demonstrated an effect of methylmercury (MeHg) on levels of cAMP in mouse palatal shelves. We have studied the concentration of cAMP in palatal shelves and tongues in four inbred lines of mice which represent the four possible combinations of two *H-2* alleles and two residual genetic backgrounds (Erickson et al., '79b). One-way analyses of variance of these data revealed that both the *H-2* region and the residual genetic background determine cAMP concentrations in palatal shelves and tongues on days 14.5 and 15.5 gestation. Variation at the *H-2* locus has been shown to be a part of the genetic susceptibility to steroid-induced cleft palate (Bonner and Slavkin, '75; Biddle and Fraser, '77; Tyan and Miller, '78; Erickson et al., '79b) and diphenylhydantoin-induced cleft palate (Goldman et al., '78). Therefore, we sought to determine if *H-2* played a role in genetic susceptibility to methylmercury-induced cleft palate and to determine whether *H-2* or non-*H-2* background effects on palatal shelf cAMP would interact with methylmercury's effects on cAMP in this tissue.

MATERIALS AND METHODS

A/J (A background, *H-2*^{a}), C57Bl/10J (B background, *H-2*^{b}), and their congenic pairs B10.A (B background, *H-2*^{a}), and ABY.Sn (A background, *H-2*^{b})were raised from stock obtained from the Jackson Laboratory. Mice were bedded on pine shavings, and Purina mouse chow #5020 and water were available *ad lib.* The photoperiod was 14 hours light and 10 hours dark. The room temperature was maintained at 22°C. Females were caged overnight with males and checked daily for vaginal plugs. The day on which a plug was

Received April 30, 1980; accepted October 29, 1980.

found was designated as day 0 of pregnancy. On day 12 of pregnancy, experimental females were injected subcutaneously on the back with 0.01 ml of methylmercury solution (0.75 mg methylmercury chloride per ml of 50% ethanol and 50% Dulbecco's phosphate buffered saline (PBS) per gram body weight, while control females received the same dosage of 50% ethanol and 50% Dulbecco's PBS. Mothers were killed on the eighteenth day of pregnancy and fetuses were examined, after removal of the tongue and mandible, to determine the incidence of cleft palate in fetuses of experimental and control females. A minimum of two litters were used per strain per condition.

cAMP was measured in the tongues and palates of 14.5-day fetuses by the method of Erickson et al. ('79b). The tongue and palatal shelves of each fetus were boiled (separately) for 10 minutes in 200-μl aliquots of distilled water. The tissue was pelleted and protein content determined by the method of Lowry et al. ('51). The supernatant was assayed for cAMP by radioimmunoassay (using a kit purchased from New England Nuclear).

Pairwise comparison of means for statistical significance were performed with Student t test, and strain effects were analyzed by a one-way analysis of variance.

RESULTS

In order to achieve the 7.5 mg/kg of methylmercury which had previously been shown to be teratogenetic in mice, we found that the methylmercury had to be given in a 50% ethanol solution (with PBS). Thus, it was important to determine whether or not maternal injections with 50% ethanol:50% Dulbecco's PBS resulted in effects on levels of cAMP in palatal shelves or tongue. There was no difference in mean cAMP levels for either tongue or palate between 50% ethanol-injected and control ABY. Sn mice (Table 1). Therefore, the ethanol-PBS solution was considered not to have an effect on palatal shelf and tongue cAMP levels. Control values for these levels for the other strains were taken from the publication of Erickson et al. ('79b). Since the data were homogenous, we pooled the injected and noninjected data for the ABY.Sn control. Injection of females on day 12 of gestation with methylmercury resulted in a decrease in fetal palatal shelf cAMP levels when measured on day 14.5 gestation (Table 2). As we have previously shown, the four strains differ in their palatal shelf cAMP levels; a one-way analysis of variance showed that the four strains were not the same at $P = 0.036$ (reanalyzed as ABY.Sn values include new controls; see above). The B background strains have, on the average, lower cAMP levels than the A background strains. As before (i.e., despite the inclusion of more data on ABY.Sn) a pair-wise comparison of the four strains showed that B10.A was significantly lower than A/J, while the other three strains were not significantly different. The decrease in palatal shelf cAMP following methylmercury injection is statistically significant for the A/J, ABY.Sn and B10.A strains ($P < 0.05$). The palatal shelf cAMP concentrations decreased to a greater extent in the strains with the *H-2*^{a} allele (A/J, B10.A); one-way analysis of variance of the *H-2*^{a} against *H-2*^{b} methylmercury-induced decreases in palatal shelf cAMP showed that the effect of methylmercury on the two *H-2* types was significantly different ($F = 38.43$, $P < 0.0001$). Thus, genetic background has a greater effect on normal palatal shelf cAMP levels than does *H-2* haplotype, but *H-2* has a stronger effect when interacting with methylmercury to lower palatal shelf cAMP.

The levels of cAMP in tongues of fetuses from nontreated mothers were lower than those found in palatal shelves and showed little strain variation (Table 3). The four strains were statistically different by one-way analysis of variance ($P < 0.0001$). A pair-wise comparison showed A/J to be significantly higher than the other strains, while the other three strains were not significantly different from each other. Methylmercury treatment of the mother on day 12 of gestation reduced the cAMP concentrations of tongue on day 14.5 by about 50% in A/J and C57Bl/10J. However, cAMP was only reduced by about 30% in B10.A and was not reduced in ABY.Sn. Thus, genetic background and the *H-2* locus have different effects in tongue than in palatal shelves on the interaction of methylmercury treatment with cAMP levels. The genetic background seems to have a slight effect on methylmercury's reduction in concentration of cAMP in tongues whereas, *H-2* showed a more predominant role in palatal shelves.

Although we only had data on a few fetuses, we also examined palatal shelf and tongue cAMP concentrations in fetuses from control and methylmercury-injected mice when the fetus has cleft lip (Table 4). This spontaneous facial defect only occurs with a significant frequency in the A/J and ABY. Sn lines and,

TABLE 1. Comparison of mean 14.5-day palate and tongue cAMP levels in fetuses from injected and uninjected control ABY.Sn mothers[1]

	pmole/mg protein	
	injected[2]	uninjected[3]
Palate cAMP	(7) 25.19 ± 3.30[4]	(10) 25.62 ± 1.45
Tongue cAMP	(7) 6.26 ± 0.40	(10) 6.23 ± 0.33

[1]Unless otherwise stated all cAMP levels were measured on fetuses with unfused palatal shelves and without cleft lip.
[2]Mothers received 0.01 ml/gm body weight of 50% ethanol – 50% Dulbecco's PBS.
[3]From Erickson et al. ('79b).
[4](No. of fetuses) mean ± S.E.

TABLE 2. Levels of palatal cAMP present on day 14.5 gestation in control and methylmercury-treated fetuses

Strain	Genetic background	*H-2* allele	pmole/mg protein		P[1]
			Control	MeHg	
A/J	A	a	(11) 27.2 ± 1.23[2]	(16) 7.9 ± .71	<0.0001
C57Bl/10J	B	b	(7) 23.1 ± 2.99	(2) 14.8 ± 3.8	n.s.
ABY.Sn	A	b	(17) 25.5 ± 1.54	(27) 17.3 ± 1.5	<0.0009
B10.A	B	a	(9) 19.2 ± 2.18	(17) 7.8 ± 1.07	<0.0001

[1]Probability of this difference by chance using Student's 2-tailed t test.
[2](No. of fetuses) mean ± S.E.

TABLE 3. Levels of tongue cAMP present on day 14.5 of gestation in control and methylmercury-treated fetuses

Strain	Genetic background	*H-2* allele	pmole/mg protein		P[1]
			Control	MeHG	
A/J	A	a	(11) 8.51 ± 0.376[2]	(15) 4.11 ± 0.151	<0.0001
C57Bl/10J	B	b	(6) 7.09 ± 0.318	(2) 3.35 ± 0.050	0.007
ABY.Sn	A	b	(17) 6.25 ± 0.246	(28) 6.48 ± 0.397	NS
B10.A	B	a	(9) 6.76 ± 0.344	(18) 4.62 ± 0.593	0.0198

[1]Probability of this difference by chance using Student's 2-tailed t test.
[2](No. of fetuses) mean ± S.E.

thus, the data are only represented for them. The presence of cleft lip had no effect on the manner in which MeHg reduced the cAMP levels of both tongue and palatal shelves, nor did it affect the mean tongue cAMP levels. However, in both strains mean palatal shelf cAMP levels were almost double that of fetuses without cleft lip (i.e., compared to Table 2).

The incidence of cleft palate (without cleft lip) is presented in Table 5 for both control (ethanol-PBS) and experimental (methyl-

mercury) animals. The spontaneous rate, i.e., background, for all strains is quite low. A/J has the highest rate and is the only strain with a control rate greater than 5%. (ABY.Sn has a high incidence of cleft lip with cleft palate (28%) but we did not detect any isolated cleft palate.) All strains showed a roughly threefold increase in the incidence of cleft palate with methylmercury treatment (assuming that the lack of any cleft palate in the ABY.Sn is merely a sampling error). *H-2* or genetic background effects on the incidence of cleft palate were not noted.

We also studied the effects of methylmercury on the number of resorbed embryos in the four strains (Table 6). The A background

TABLE 4. Levels of cAMP of tongue and palate in control and methylmercury-treated fetuses with cleft lip on day 14.5 of gestation

Strain	pmole/mg protein	
	Control	MeHg
Palate		
A/J	(2) 42.35 ± 6.34[1]	(4) 14.925 ± 2.025
ABY.Sn	(4) 56.08 ± 18.17[1]	(2) 28.05 ± 4.95
Tongue		
A/J	(2) 8.35 ± 0.2496	(4) 4.15 ± 0.3175
ABY.Sn	(4) 6.55 ± 0.698	(5) 6.14 ± 0.4597

[1](No. of fetuses) mean ± S.E.

TABLE 5. Incidence of cleft palate without cleft lip in fetuses from control and methylmercury-treated females

Strain	Genetic background	*H-2* allele	Control	MeHg
A/J	A	a	(2/39) 5.1[1]	(6/37) 16.2
C57Bl/10J	B	b	(1/60) 1.6	(3/50) 6.0
ABY.Sn	A	b	(0/49) 0.0	(3/40) 7.5
B10.A	B	a	(1/55) 1.8	(3/56) 5.3

[1](Affected/total) percentage.

TABLE 6. Incidence of resorbed fetuses from control and methylmercury-treated females

Strain	Genetic background	*H-2* allele	Control	MeHg
A/J	A	a	(18/66) 27.3[1]	(27/72) 39.5
C57Bl/10J	B	b	(10/70) 14.3	(23/77) 29.9
ABY.Sn	A	b	(15/88) 17.0	(12/69) 17.4
B10.A	B	a	(5/61) 8.2	(13/72) 18.1

[1](Number resorbed/total) percentage.

strains have a higher spontaneous resorption rate than the B background strains. However, after treatment with methylmercury, the rate of resorptions is doubled in the black background strains (C57Bl/10J, B10.A), while the rate of resorption increased to a lesser extent in the A/J strain, and no effect was found for the ABY.Sn strain.

DISCUSSION

The data presented here confirm and extend Olson and Massaro's ('80) observations on effects of methylmercury on palatal shelf cAMP. We found a marked decrease in palatal shelf cAMP in fetuses from mothers who were injected with methylmercury, while fetuses from mothers injected with the same amount of ethanol were not found to be different than previously published controls for the ABY.Sn strain. *H-2* has been shown to affect the concentration of cAMP in liver (Meruelo and Edidin, '75) but not in sperm (Erickson et al., '79a). The effect on liver cAMP was influenced by *H-2*-mediated changes in amount of glucagon receptor (Lafuse and Edidin, '80). Therefore, it was of interest that the *H-2* type of the treated animals had marked effects on the methylmercury-induced decreases in cAMP. However, neither *H-2* nor genetic background influenced the frequency of cleft palate which resulted from methylmercury treatment. This situation is quite different from the situation in regards to steroid-induced cleft palate where *H-2* and genetic background both interacted strongly with the steroid treatment in determining the levels of cAMP and the frequency of cleft palate. The meaning of the results for genetic effects on the frequency of methylmercury-induced cleft palate is not easily explicable. One explanation might be that the alterations of cAMP are not related to the frequency of cleft palate—glucocorticoids had much smaller effects on palatal shelf cAMP than methylmercury and increased them in some strains but decreased them in others. Thus the large decreases in cAMP induced by methylmercury may not be related to the pathway of methylmercury-induced cleft palate and might be due to cytotoxicity, inhibition of adenylcyclase (Spuhler and Prasad, '80), or other causes.

ACKNOWLEDGMENTS

This work was supported by a grant from The National Foundation—March of Dimes. We thank Mrs. Rena Jones for excellent secretarial assistance with the manuscript.

LITERATURE CITED

Biddle, F.G. and F.C. Fraser (1977) Cortisone-induced cleft palate in the mouse. A search for the genetic control of the embryonic response trait. Genetics, *85*:289–302.

Bonner, J.J. and H.C. Slavkin (1975) Cleft palate susceptibility linked to histocompatibility-2 *(H-2)* in the mouse. Immunogenetics, *2*:213–218.

Erickson, R.P., M.S. Butley, S.R. Martin, and C.J. Betlach (1979a) Variation among inbred strains of mice in adenosine 3′:5′ cyclic monophosphate levels of spermatozoa. Genet. Res., *33*:129–136.

Erickson, R.P., M.S. Butley, and C.F. Sing (1979b) *H-2* and non-*H-2* determined strain variation in palatal shelf and tongue adenosine 3′:5′ cyclic monophosphate: a possible role in the etiology of steroid-induced cleft palate. J. Immunogenet., *6*:253–262.

Fuyuta, M., T. Fujimoto, and S. Hirata (1978) Embryotoxic effects of methylmercuric chloride administered to mice and rats during organogenesis. Teratology, *18*: 353–366.

Goldman, A.S., M. Katsumata, and D.L. Gasser (1978) Fetal hydantoin syndrome: susceptibility to phenyltoin-induced cleft lip and palate linked to *H-2* in mice. Pediatr. Res., *12*:913a.

Lafuse, W. and M. Edidin (1980) Influence of the mouse major histocompatibility complex, *H-2*, on liver adenylate cyclase activity and on glucagon binding to liver cell membranes. Biochemistry, *19*:49–54.

Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall (1951) Protein measurement with Folin reagent. J. Biol. Chem., *193*:265–275.

Meruelo, D. and M. Edidin (1975) Association of mouse liver adenosine 3′:5′-cyclic monophosphate (cyclic AMP) levels with *Histocompatibility-2* genotype. Proc. Natl. Acad. Sci. USA, *72*:2644–2648.

Olson, F.C. and E.J. Massaro (1977) Effects of methylmercury on murine fetal amino acid uptake, protein synthesis and palate closure. Teratology, *16*:187–194.

Olson, F.C. and E.J. Massaro (1980) The effects of methylmercury on palate closure and the developmental patterns of cAMP, adenyl cyclase, and phosphodiesterase in fetal mouse palate, lung and liver. Teratology, *2*:155–166

Spuhler, K. and K.N. Prasad (1980) Inhibition by methylmercuric chloride of prostaglandin E_1-sensitive adenylate cyclase activity in glioma but not in neuroblastoma cells in culture. Biochem. Pharm., *29*:201–204.

Spyker, J.M. and M. Smithberg (1972) Effects of methylmercury on prenatal development in mice. Teratology, *5*:181–190.

Tyan, M.L. and K.K. Miller (1978) Genetic and environmental factors in cortisone induced cleft palate. Proc. Soc. Exp. Biol. Med., *158*:618–621.