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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

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SUMMARY

The concentration of cAMP was measured in palatal shelves and tongues of 14·5-day old foetuses, 14·5-day old foetuses from steroid treated mothers, and 15·5-day old foetuses from four inbred lines of mice which represent the four possible combinations of two *H-2* alleles and two residual genetic backgrounds. The incidence of spontaneous and steroid-induced cleft palate in these four strains was also determined. Analyses of variance of the cAMP data reveal that both the *H-2* region and residual genetic background determine cAMP concentrations in both tissues and on both days of development. Similar analyses of cAMP concentrations after steroid treatments of the mother indicate that the interaction between *H-2* and residual genetic background is significantly different in the injected than in the uninjected mice in both palatal shelves and tongues. The incidence of steroid-induced cleft palate parallels the palatal shelf concentration of cAMP before steroid treatment of the mother with one exception. These data suggest that a portion of the *H-2* controlled component of susceptibility to steroid-induced cleft palate is mediated through alterations in the metabolism of cAMP.

INTRODUCTION

Cleft palate induced by steroid injections of the pregnant mouse is a well-studied model of teratogenesis (Fraser & Fainstat, 1951). In analogy to spontaneous cleft palate of man and mice, it is multifactorially determined, i.e., affected by multiple genetic loci and environmental factors (Kalter, 1954). Recently, it has been shown that the major histocompatibility locus of mice, *H-2*, plays a role in the etiology of cleft palate. This was demonstrated by the use of congenic strains (Bonner & Slavkin, 1975) and by the analysis

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of backcross data (Biddle & Fraser, 1977). There are strain variations in the concentration of glucocorticoid receptors (Salomon & Pratt, 1976) but this difference is apparently not due to allelic variation at the *H-2* locus (Butley *et al.*, 1978). Thus, the biochemical basis for an *H-2* effect on susceptibility to steroid-induced cleft palate is unknown.

We know of two biological properties of the *H-2* locus which could play a role in the causation of cleft palate; (a) its direct effect on cell-to-cell adhesiveness (Bartlett & Edidin, 1978; Zelený *et al.*, 1978) and (b) its role in the determination of cellular levels of 3′:5′ adenosine cyclic monophosphate (cAMP) (Meruelo & Edidin, 1975). The latter seemed more likely to be the decisive factor since it has been demonstrated that two events related to the preparation of palatal shelves for fusion can be induced prematurely with dibutyrl cAMP (Pratt & Martin, 1975). The normal stimulus for increases of cAMP in palatal shelves is unknown but it might conceivably be a mesenchymal inducer. Glucocorticoids might have a permissive effect on palatal shelf cAMP, as they do for the induction of cAMP by glucagon in liver where pre-treatment with these steroids results in greater cAMP responses to this protein hormone. Thus, we hypothesized that the effects of *H-2* on the levels of palatal shelf cAMP might increase the sensitivity of some strains to a permissive action of glucocorticoids because of initially higher cAMP levels. Glucocorticoid receptor levels determined by other parts of the genome would interact with the *H-2* effect on levels of palatal shelf cAMP.

Our data provide evidence for an effect of *H-2* genotype on levels of cAMP in palatal shelves, but the residual genetic background also plays a major role in this respect. Comparisons of palatal shelf cAMP in foetuses from steroid-injected mothers with controls reveal a very significant interaction of *H-2* genotype, the rest of the genetic background, and steroid injections in determining the levels of palatal shelf cAMP. The pretreatment levels correspond to the incidence of cleft palate in the offspring of steroid injected mothers from different cleft palate strains with one exception.

MATERIALS AND METHODS

Mice

A/J (A background, *H-2*^{a}), C57Bl10/J (B background, *H-2*^{b}), and their congenic pairs B10.A (B background, *H-2*^{a}) and A.BY (A background, *H-2*^{b}) were purchased from the Jackson Laboratory. They were maintained under a 14 hr light, 10 hr dark cycle and fed Purina mouse chow and water *ad libitum*. The bedding was two-thirds pine shavings and one third cedar shavings and they were dipped to control mites in creosate every 2–3 weeks.

Observations on spontaneous and steroid-induced incidence of cleft palate

Females placed with males were inspected twice daily for vaginal plugs. The day on which a vaginal plug was found was considered day 0 of pregnancy. Several timed pregnant females were dissected on day 18 or 19 to observe the spontaneous incidence of cleft palate (either primary or secondary) in foetuses of the strains, but most of our data are derived from dissections of newborns. No significant difference in the incidence of cleft palate was detected between foetuses and newborns checked daily (e.g., A.BY, $\chi^2 = 2\cdot76$, $P > 0\cdot05$, data not included). Steroid-induced cleft palate frequencies were determined after 2·5 mg of cortisol in 0·05 ml volume were injected intramuscularly into the mother during the morning of days 11–14. These females were then dissected on day 18 or 19 and the foetuses examined for cleft palate.

Palatal shelf and tongue cAMP determinations

Timed females were dissected after 14·5 or 15·5 days of pregnancy with or without steroid injections as described in the preceding paragraph. The uterine horns were quickly removed and placed in media with a high glucose content (0·2 M glucose, 0·05 M NaCl, 0·02 M Na_2HPO_4, and 0·0026 M KH_2PO_4). All the foetuses were then dissected out, leaving the umbilical cord attached. Subsequently, each foetus was removed from the uterine horn, decapitated, and the palatal shelves and tongue dissected out. The accumulated pairs of palatal shelves and single tongues were separately placed into 200 μl of boiling water in a boiling water bath for 10 min. This method of deproteinizing tissue has been found to be superior to perchloric and trichloracetic acid precipitations (Cooper *et al.*, 1972); its use also avoids difficulties encountered in removing the organic acids from the sample. At the end of this time they were quickly cooled on ice, spun at 1300 ***g*** at 4°C and the supernatants removed for radioimmunoassay of cAMP. Usually the supernatants and the palates or tongues were separately frozen at −70°C until the determinations could be performed. The pair of palates and the tongue were homogenized in 1·0 ml 0·1 N NaOH prior to determination of protein by the method of Lowry. Fifty microlitre aliquots of the supernatant were assayed for cAMP by the radioimmunoassay of Steiner *et al.* (1972) with the kit purchased from New England Nuclear. Phosphodiesterase treatment was performed on a number of samples in order to demonstrate that only cAMP was being measured. Aliquots of samples were diluted 50:50 with 0·5 M Tris, pH 7·5; 10 μg of 3′:5′ cyclic nucleotide phosphodiesterase (0·21 units/mg, Sigma Chemical Co., #P.O. 134) were added per 90 μl sample; and digestion was performed for 30′ at 30°C. These samples were then deproteinized at 100°C and assayed as above. Phosphodiesterase treatment reduced the concentration of cAMP to nearly undetectable levels; this represented a 90% or more decrease in cAMP.

Statistical analyses

Analysis of variance procedures were employed which take into account modifications for unequal and disproportionate subclass numbers. Scheffe's method for computation of multiple contrasts (Bancroft, 1968) was used to obtain tests of hypotheses concerning the effects of strain, developmental time, and steroid injection. Separate analyses were carried out on the cAMP levels determined in palates and tongues. For each tissue an analysis of the effects of strain and time in uninjected mice and of those of strain and injection on 14·5-day old foetuses were conducted independently.

RESULTS

The cAMP means of palatal shelves are presented in Table 1 for the uninjected mice assayed on each of the two days studied. A one-way analysis of variance of data collected on 14·5-day old foetuses showed that all four strains were not the same at the $\alpha = 0{\cdot}02$ level of probability. Scheffe's multiple comparisons identified B10.A as significantly lower ($\alpha = 0{\cdot}01$) than the average of the other three strains and these three were not judged to be significantly different on this day. We then asked if the strain differences persisted on day 15·5. The two-way analysis of variability of cAMP due to strain and time (day 14·5 *v* day 15·5) difference is given with the summary of mean values in Table 1. The differences among the strains are similar at the two times in foetal development since the *H-2* × time,

TABLE 1. (a) Palatal shelf cAMP (pmol/mg protein) in uninjected animals (mean and s.e.). (b) Analysis of variance. Numbers in parentheses indicate number of samples

	Strain	Genetic background	*H-2* allele	Day 14·5 uninjected	Day 15·5 uninjected
(a)	A/J	*A*	*a*	(11) 27·2 ± 1·23	(14) 38·4 ± 1·95
	A.BY	*A*	*b*	(10) 25·6 ± 1·45	(26) 39·4 ± 1·35
	C57Bl10/J	*B*	*b*	(7) 23·1 ± 2·99	(25) 38·7 ± 1·98
	B10.A	*B*	*a*	(9) 19·2 ± 2·18	(22) 30·9 ± 1·31
	Total			(37) 24·1 ± 1·05	(87) 36·9 ± 0·91

	Contrasts	d.f.	F	$P_r > F$
(b)	Time contrast	1	80·95	0·00
	Strain contrasts:			
	H-2	1	3·67	0·06
	Background	1	10·45	0·00
	H-2 × background interaction	1	4·51	0·04
	Strain × time interaction contrast:			
	H-2 × time	1	1·26	0·26
	Background × time	1	0·16	0·69
	H-2 × background × time	1	0·05	0·83

the background × time and the *H-2* × background × time interactions were not statistically significant. Stated in terms of time, the statistically significant increase in cAMP levels from day 14·5 to day 15·5 occurred independently of the strain being studied. The average cAMP increase in each strain was 12·8 pmol/mg protein. Since the palatal shelf cAMPs increased in parallel between days 14·5 and 15·5 in all four strains, each strain could be averaged across the 2 days to give a larger number of samples. The weighted averages of the strains for the 2 days are presented in Table 2 with the contributions of various genetic components to the values indicated. The strains homozygous for $H\text{-}2^a$ have lower levels of palatal shelf cAMP than those homozygous for $H\text{-}2^b$. The strains with the B background have lower levels, on the average, than the strains with the A background. The F ratio of 4·51 (probability of a larger F by chance = 0·04) for the test of the *H-2* by background interaction argues that the effects of $H\text{-}2^a$ and $H\text{-}2^b$ do not combine additively with the effects of genetic background in these strains. The

TABLE 2. Weighted averages of palatal shelf cAMP (pmol/mg protein, mean ± sd) for uninjected mice at days 14·5 and 15·5 (calculated from data in Table 1). Numbers in parentheses indicate the number of samples

Strain	Genetic background	*H-2* allele	Mean palatal shelf cAMP	Strain effects*		
				H-2	Background	Interaction
A/J	*A*	*a*	(25) 33·48 ± 1·64	−2·47	1·67	1·42
A.BY	*A*	*b*	(36) 35·57 ± 1·48	+2·47	1·67	−1·42
C57Bl10/J	*B*	*b*	(32) 35·28 ± 2·02	+2·47	−1·67	1·42
B10.A	*B*	*a*	(31) 27·50 ± 1·47	−2·47	−1·67	−1·42
Total			(124) 33·06 ± 0·88			

* Assuming equal subclass numbers, values are pmol/mg protein.

strain (B10.A) representing the *H-2*^{a} and B background has a lower cAMP level than predicted if the *H-2*^{a} and B genetic background are considered separately while *H-2*^{a} with the A genetic background (A/J) had a higher level of cAMP than predicted by the separate effects of *H-2*^{a} and the A background.

The comparisons of data from uninjected mice with data on steroid injected mice on day 14·5 (Table 3) revealed that steroids affect different strains differently, i.e., cAMP was increased in some strains and decreased in others. The two-way analysis of the effects of strain and steroid injections on the levels of cAMP clearly indicates that the interaction between *H-2* and background is significantly different in the injected than in the uninjected

TABLE 3. (a) Palatal shelf cAMP (pmol/mg protein, mean ±s.c.) in steroid injected and uninjected animals. Numbers in parentheses indicate the number of samples. (b) Analysis of variance

Strain	Genetic background	*H-2* allele	Day 14·5 uninjected	Day 14·5 injected	Change induced by steroids
(a) A/J	*A*	*a*	(11) 27·2 ± 1·23	(17) 23·9 ± 2·32	↓
A.BY	*A*	*b*	(10) 25·6 ± 1·45	(11) 33·4 ± 3·40	↑
C57BL10/J	*B*	*b*	(7) 23·1 ± 2·99	(12) 20·4 ± 1·35	↓
B10.A	*B*	*a*	(9) 19·2 ± 2·18	(12) 29·2 ± 2·22	↑
Total			(37) 24·1 ± 1·05	(52) 25·9 ± 1·32	

Contrasts	d.f.	*F*	$P_r > F$
(b) Injection contrast	1	2·14	0·15
Strain contrasts:			
H-2	1	0·03	0·87
Background	1	5·94	0·02
H-2 × background interaction	1	2·65	0·11
Strain × injection contrasts:			
H-2 × injection	1	0·28	0·60
Background × injection	1	0·52	0·47
H-2 × background × injection	1	10·84	0·00

mice (probability of a larger *F* by chance = 0·0015). Stated otherwise, the interaction of *H-2* allele and background effects determined in day 14·5 and 15·5 foetuses from uninjected mothers (Table 1) was significantly different from the interaction observed in fetuses of injected mice. Steroid injection resulted in an increased level of cAMP in the A.BY (the *H-2*^{b}, A background genotype) and the B10.A (*H-2*^{a}, B background genotype) strains but depressed the levels in the A/J and C57Bl10/J strains (Table 3).

In summary, there is evidence for an epistatic effect of unidentified genes in the genetic background with *H-2* alleles to determine the level of palatal shelf cAMP. This epistatic effect is consistent over an important period of palatal shelf development (days 14 and 15), and seems to be altered by steroid injection.

The analysis of cAMP in the tongues of uninjected animals at day 14·5 and day 15·5 is presented in Table IV. Levels in the tongue were measured primarily to serve as a control in the study of cAMP in the palatal shelf. However, genetic and steroid injection effects on levels of tongue cAMP were observed which suggest that the tongue could be involved in the etiology of steroid-induced cleft palate. The level of cAMP in the tongue is

approximately one quarter of those values measured in the palatal shelf. The 21% increase from day 14·5 to day 15·5 in the tongue is not as large as the 50% increase observed in the palatal shelf but is also highly significant ($F = 37 \cdot 37$, probability of a greater F by chance $= 0$). On the other hand, instead of the consistent increase of palatal shelf cAMP observed in all strains, the levels in the tongue show statistically significant differences depending on the interaction of *H-2* allele and foetal age. In tongues, the differences of cAMP levels in different *H-2* strains are not the same on day 15·5 as on day 14·5. The A.BY strain increased by 3·0 pmol/mg protein during this period of development, whereas the other strains increased by approximately 1·5 pmol/mg protein.

TABLE 4. (a) Tongue cAMP (pmol/mg protein, mean ±s.e.) in uninjected and injected animals. Numbers in parentheses indicate number of samples. (b) Analysis of variance

Strain	Genetic background	*H-2* allele	Day 14·5 uninjected	Day 14·5 injected	Change induced by steroids
(a) A/J	*A*	*a*	(11) 8·51 ± 0·37	(17) 6·76 ± 0·31	↓
A.BY	*A*	*b*	(10) 6·23 ± 0·33	(11) 8·68 ± 0·28	↑
C57Bl10/J	*B*	*b*	(6) 7·09 ± 0·32	(12) 6·01 ± 0·14	↓
B10.A	*B*	*a*	(9) 6·75 ± 0·34	(12) 8·43 ± 0·28	↑
Total			(36) 7·20 ± 0·23	(52) 7·38 ± 0·20	

Contrasts	d.f.	F	$P_r > F$
(b) Injection contrast	1	2·01	0·16
Strain contrasts:			
H-2	1	7·19	0·01
Background	1	4·33	0·04
H-2 × background	1	3·58	0·06
Strain × injection contrasts:			
H-2 × injection	1	2·54	0·11
Background × injection	1	0·01	0·91
H-2 × background × injection	1	58·38	0·00

A comparison of tongue cAMP values in foetuses from steroid injected with those from uninjected mothers at day 14·5 is given in Table 4. Again, as with the palate, there is an interaction of strain with injection. A.BY and B10.A strains have increased levels of cAMP in steroid injected animals whereas A/J and C57Bl10/J react with depressed levels. On the average, steroid injection did not have an identical effect on cAMP levels, a finding which parallels that observed for palates (Table 3). In summary, it is noteworthy that both palatal shelf and tongue tissue express similar effects of interaction of *H-2* with genetic background and injection.

The data seem to support two conclusions. Interaction of the *H-2* allele with the genetic background produces an effect on cAMP which differs in tongues but not palates between foetal ages of 14·5 and 15·5 days. Secondly, the effect of steroid injection is strain dependent in both palate and tongue. A.BY and B10.A strains present significantly increased cAMP levels after steroid injection. This effect is contrasted by a decrease in the average levels of cAMP in the A/J and C57Bl10/J strains.

In the original report by Bonner & Slavkin (1975) the A/J and C57Bl10/J strains and one congenic pair, the B10.A strain, were used to study the *H-2* effect on steroid-induced

TABLE 5. Incidence of spontaneous and steroid-induced cleft lip with cleft palate and cleft palate (fraction affected ±s.c.

Strain	Genetic background	*H-2* allele	Spontaneous (predominantly cleft lip with cleft palate)	Steroid-induced	Steroid-induced—spontaneous (predominantly cleft palate)
A/J	*A*	*a*	(6/207) 0·03 ± 0·01	(28/76) 0·37 ± 0·13	0·34
A/HeJ	*A*	*a*	(12/147) 0·082 ± 0·02	—	
A.BY	*A*	*b*	(37/194) 0·19 ± 0·028	(39/95) 0·41 ± 0·05	0·22
C57Bl10/J	*B*	*b*	(0/151) 0	(2/69) 0·029 ± 0·020	0·029
B10.A	*B*	*a*	(0/231) 0	(14/176) 0·085 ± 0·021	0·085

Numbers in parentheses indicate affected/total.

cleft palate susceptibility. However, the other congenic pair, A.BY, was not investigated. Our data with all four strains and one substrain of A/J show lower incidences of steroid-induced cleft palate, perhaps due to differences in diet (Miller, 1977), but the rankings among strains parallel those observed by Bonner & Slavkin (1975). As seen in Table 5, all A strains had high levels of spontaneous cleft palate with the highest in the A.BY strain (this is mostly cleft lip with cleft palate). Thus, *H-2* seems to have no or little effect on the spontaneous incidence of cleft palate (and there is significant substrain variation in the incidence of spontaneous cleft palate). However, steroid-induced cleft palate (corrected for spontaneous incidence, i.e., isolated cleft palate) was highest in the A background strains and the introduction of the $H\text{-}2^b$ allele into the A background (the A.BY strain) somewhat decreased the incidence of steroid-induced cleft palate. The introduction of $H\text{-}2^a$ allele from the A/J strain into the C57Bl10/J strain moderately increased the incidence of steroid-induced cleft palate. The rank order of steroid-induced cleft palate corrected for

TABLE 6. (a) Tongue cAMP (pmol/mg protein) in uninjected animals (mean ±s.e.). Numbers in parentheses indicate the number of samples. (b) Analysis of variance

	Strain	Genetic background	*H-2* allele	Day 14·5 uninjected	Day 15·5 uninjected
(a)	A/J	*A*	*a*	(11) 8·51 ± 0·37	(14) 9·07 ± 0·32
	A.BY	*A*	*b*	(10) 6·23 ± 0·33	(26) 9·29 ± 0·35
	C57Bl10/J	*B*	*b*	(7) 7·09 ± 0·32	(24) 8·70 ± 0·17
	B10.A	*B*	*a*	(9) 6·75 ± 0·34	(22) 8·01 ± 0·30
	Total			(36) 7·20 ± 0·23	(86) 8·76 ± 0·16

	Contrasts	d.f.	*F*	$P_r > F$
(b)	Time contrast	1	37·37	0
	Strain contrast:			
	H-2	1	0·96	0·33
	Background	1	5·76	0·02
	H-2 × background	1	8·45	0
	Strain × time contrasts:			
	H-2 × time	1	7·85	0·01
	Background × time	1	0·51	0·48
	H-2 × background × time	1	4·10	0·04

spontaneous incidences and day 14·5 cAMP levels of palatal shelf is the same except for the reverse order of B10.A and C57Bl10/J between the two variables.

DISCUSSION

Many biochemical events occur within palatal shelves prior to, or near the time, of palatal shelf fusion. Two of these, in particular, have been shown to involve cAMP in their timing. These are epithelial glycocalyx formation and programmed cell death at the tip of the shelves both of which were shown by Pratt & Martin (1975) to be preceded by a rise of cAMP; more importantly, both events can be induced prematurely by the addition of dibutyrl cAMP to cultured palatal shelves. A physiological relationship of cAMP to programmed cell death and epithelial glycocalyx formation is supported by the interaction of epithelial growth factor with this system. Epithelial growth factor inhibits the programmed cell death, and the effect can be reversed by dibutyrl cAMP (Hassell & Pratt, 1977). All of these studies are performed *in vitro*.

Glucocorticoids have a well-known permissive effect on cAMP (Jost & Rickenberg, 1971). Pretreatment with glucocorticoids followed by glucagon causes an augmented response of cAMP in liver. A minimum time (>30 min) must elapse after glucocorticoids are given which suggests that new gene expression must occur (Friedman *et al.*, 1967). However, there is some *in vitro* evidence that cortisol inhibits cAMP phosphodiesterase (Schmidtke *et al.*, 1976) and in the amphibian egg there seems to be a direct effect of steroids on cyclic nucleotide metabolism (Speaker & Butcher, 1977) which occurs at the cell membrane (Goodeau *et al.*, 1978). Thus, it would seem possible that glucocorticoids could directly or indirectly augment palatal shelf cAMP responses to some endogenous inducer. A likely candidate would be the mesenchymal inducer that Filosa *et al.* have studied, implicating cycle nucleotide metabolism in its mode of action (Filosa *et al.*, 1975).

There is a variety of evidence to show that the number and kind of glucocorticoid receptors are probably a rate-limiting step in steroid-induced cleft palate causation (Biddle & Fraser, 1976). Although there has been a suggestion in the literature that *H-2* controls glucocorticoid receptors (Goldman *et al.*, 1977), this seems highly unlikely and we have shown clearly that the *H-2* region cannot include the structural locus for glucocorticoid receptors in liver, a tissue where this receptor is usually studied (Butley *et al.*, 1978). Although our own work suggests that *H-2* does not have any significant effect on cAMP in sperm (Erickson *et al.*, 1978), the data of Meruelo & Edidin (1975) have shown clearly that cAMP levels are affected by *H-2* in some tissues, i.e., liver. Because of this, we hypothesized that an *H-2* effect on endogenous levels of cAMP in palatal shelves might interact with the rest of the genetic background, e.g., genes determining levels of steroid receptors, in the induction of steroid-induced cleft palate. A high endogenous level of cAMP might more easily be increased by steroids past a threshold such that preparation for palatal shelf closure would occur prematurely and palatal shelves would not fuse later, after rotation. In this hypothesis, *H-2* would be expected to exert only part of the genetic control of palatal shelf susceptibility; this is supported by the data presented here as well as those of others (Bonner & Slavkin, 1975; Biddle & Fraser, 1977) concerning incidence of steroid-induced cleft palate in various strains. The data presented here are in accord with the hypothesis as the determination of levels of palatal shelf cAMP is subject to very strong interaction of *H-2* allele, residual genetic background (possibly related to strain variation in levels of glucocorticoid receptors), and steroid injections.

Our hypothesis would predict that pre-steroid treatment levels of palatal shelf cAMP would correlate with the incidence of steroid-induced cleft palate. This was found with one exception. It is possible that the pretreatment cAMP levels actually correlate with the incidence of steroid-induced cleft palate at some other, critical time for the cAMP-induced preparation for palatal fusion. The precise timing of this event is unknown, we only studied cAMP at daily intervals, and cAMP may be fluctuating with much shorter time intervals in palatal shelves.

Studies of cAMP in tongues showed the same interaction of *H-2* and genetic background with steroid injections. This was true even though *H-2* seems to have less of an effect on tongue cAMP than does genetic background (Table 6). This points out that a variety of genetic factors affect tissue levels of cAMP, a not surprising conclusion given the large number of known factors which affect cAMP in a variety of tissues. For instance, it has been noted (Lafuse, 1978) that the effect of the *H-2* region on modifying liver cAMP levels is masked by other loci in the A/J strain. These loci may be part of the non-*H-2* genetic background effects we note on palatal shelf and tongue cAMP. Nonetheless, the finding that tongue cAMP is affected by steroid injection in a manner suggestively parallel to the reaction of palatal shelves raises the possibility that biochemical changes in the tongue altering its rate of movement or size may be a significant factor in the causation of steroid-induced cleft palate in mice. A variety of observations suggest that the tongue blockage to palatal shelf closure plays a role in the causation of spontaneous cleft palate and it has been suggested also that tongue movement plays a role in steroid-induced cleft palate (Walker & Quarles, 1975).

Other *H-2* associated phenomena can provide alternative hypotheses for the role of *H-2* in causing susceptibility to steroid-induced cleft palate. Fibroblast cellular adhesion varies as a function of *H-2* haplotype; however, both a high, $H\text{-}2^{a}$, and a low cleft palate sensitivity haplotype, $H\text{-}2^{b}$, are intermediate in their effects on adhesive rates (Bartlett & Edidin, 1978). Recently it has been shown that *H-2* antigen and actin can be shed from membranes as a complex resistant to detergent (Koch & Smith, 1978). Such an interaction with actin could also lead to a direct effect of *H-2* haplotypes on the contractile elements which rotate the palatal shelves.

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