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Evidence for a Role of Arachidonic Acid in Glucocorticoid-Induced Cleft Palate in Rats (41067)

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Abstract. We have shown that arachidonic acid significantly reduces the production of cleft palate in rats by dexamethasone and that this corrective effect of arachidonic acid is blocked by indomethacin, an inhibitor of cyclooxygenase. Moreover, by using [^{3}H]-arachidonic acid as a tracer we have shown that dexamethasone treatment depresses significantly the free [^{3}H]arachidonic acid available to the microsomal cyclooxygenase in the fetal upper and lower jaws including the palate at the critical period of development. These observations suggest that glucocorticoids produce their palatal teratogenicity by limiting the release and consequently the availability of arachidonic acid at the critical period of development.

Glucocorticoids are well known anti-inflammatory hormones and a positive correlation has been demonstrated between their anti-inflammatory potencies and their ability to inhibit prostaglandin synthesis (1). Recently, it has been shown that the mechanism of this inhibition involves a blockade of the release of the rate-limiting prostaglandin or thromboxane precursor, arachidonic acid (2) (Fig. 1). Indeed, exogenous arachidonic acid reverses the glucocorticoid-induced depression of prostaglandin production in anaphylactic lungs (3) and inflamed synovia (4), and reverses the inhibition by glucocorticoids of rat paw edema induced by carrageenin (5). Glucocorticoids are also well-known teratogens producing cleft palate in rodents in accordance with their anti-inflammatory potency (6, 7). Thus, we have hypothesized that glucocorticoids may also produce their palatal teratogenic effect by blocking the release of arachidonic acid. We have tested this hypothesis: first, by studying the effects of exogenous arachidonic acid on dexamethasone-induced cleft palate in rats. Second, by investigating whether the corrective effect of arachidonic acid on dexamethasone-induced clefting can be blocked by indomethacin, an inhibitor of prostaglandin and thromboxane production at the level of cyclooxygenase (8, 9). Last, by using [^{3}H]arachidonic acid as a tracer we have studied whether dexamethasone depresses the free [^{3}H]arachidonic acid in the fetal palates at the critical period of development. The results suggest that glucocorticoids produce palatal clefting by reducing the availability of arachidonic acid in the fetal palates during their differentiation.

Materials and Methods. Virgin outbred Sprague–Dawley rats were caged overnight with fertile males. The presence of sperm in the vagina the following morning was regarded as evidence of mating and this day was considered Day 0 of gestation. On Days 12 to 15 of pregnancy the animals were injected with dexamethasone at 3.75 mg/kg in dimethylsulfoxide vehicle alone subcutaneously (Group 1), or in combination at a different site with arachidonic acid at 200 mg/kg (Group 2), or in a third site on Days 13 to 15 with indomethacin at 10 mg/kg (Group 3). On Day 19 fetuses were obtained by cesarian section and palates were examined by inspection. Significance was determined among Groups 1, 2, and 3 by the Standard χ^2 analysis and Mann–Whitney U test.

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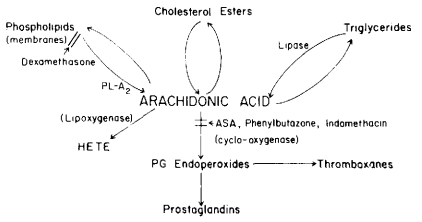

FIG. 1. Scheme of arachidonic acid metabolism. Acetylsalicylic acid (ASA), 12-hydroxy-5,8,10,14-eicosatetraenoic acid (HETE), phospholipase A2 ($PL\text{-}A_2$).

In the last series of experiments, timed pregnant rats were treated with either dexamethasone (3.75 mg/kg) or vehicle, from Day 12 to 15 of pregnancy. On Day 14, 20 μCi of [5,6,8,9,11,12,14,15-^{3}H(N)]-arachidonic acid (62.2 Ci/mmole) in 20 μl ethanol were administered intravenously. On Day 15, 1 hr after the last dose of dexamethasone or vehicle fetuses were removed by cesarian section. Fetal upper and lower jaws including palatal shelves were dissected, weighed, homogenized in 4 ml isotonic citric acid–saline buffer (pH 5), and centrifuged at 10,000g for 10 min. Each supernatant fraction and residual pellet was extracted with chloroform/methanol (2:1) (10). After counting an aliquot of each extract for total radioactivity, the remainder of the extract of each supernatant and pellet was chromatographed as follows: (a), dioxane/benzene/acetic acid (20:20:1) on an Eastman chromogram silica gel (11); (b), chloroform/methanol/acetic acid/water (60:30:7:4) on silica gel (500 μm), (12) and (c), ethyl acetate/isooctane/acetic acid/water (90:50:20:100) upper phase on an Eastman chromogram silica gel (13). Chromatograms were scraped or cut into 0.5-cm segments, placed in scintillation vials, and counted with automatic external standardization at an efficiency for ^{3}H of 33%. Each ^{3}H peak cochromatographing with authentic arachidonic acid was identified in at least two of the three thin-layer systems according to the method of Needleman *et al.* slightly modified as to composition of the mobile phase (14). Significance was determined by the Student t test.

Results. A standard dosage regimen of dexamethasone produces an 82.9% incidence of cleft palate (Table I). Concurrent administration of arachidonic acid reduces this incidence to 40%. The reduction in palatal clefting is highly significant whether analyzed as number of clefts per total number of fetuses by standard χ^2 analysis or by rank–score method of percentage clefting per litter by the Mann–Whitney U test. Indomethacin, an inhibitor of prostaglandin and/or thromboxane biosynthesis from arachidonic acid by cyclooxygenase (8, 9), administered concurrently with dexamethasone and arachidonic acid blocks the corrective effect of arachidonic acid, since it increases the incidence of cleft palate to a level 90% not significantly different from that of dexamethasone alone.

Distribution of ^{3}H-arachidonic acid in the fetal palates. In the experiment using ^{3}H-arachidonic acid as a tracer, after separating labeled phospholipids, prostaglandins, thromboxanes, and triglycerides from arachidonic acid in three different thin-layer chromatographic systems, it was apparent that dexamethasone treatment depressed significantly the amount of free ^{3}H-arachidonic acid in the supernatant fraction from 76.8%, control, to 42.4% treated (Table II). There was a highly significant reciprocal increase in the amount of free arachidonic acid associated with the 10,000g pellet from 24.6 to 57.6%, respectively (Table II). There were no significant differences in any fraction of the phospholipids or triglycerides and the amount of label in the prostaglandin or thromboxane fractions was too small for statistical comparisons.

Discussion. The present study demonstrates that the production of cleft palate in rats by dexamethasone is markedly reduced by arachidonic acid suggesting that the teratogenic lesion produced by glucocorticoids may involve a depression of arachidonic acid in the palate. This is supported by the observation that dexamethasone inhibits the release of labeled free arachidonic acid from the precipitable fraction of the fetal jaws which presumably means less is available to the microsomal cyclooxygenase in the supernatant fraction.

Prevention of the corrective effect of ex-

TABLE I. EFFECT OF ARACHIDONIC ACID AND INDOMETHACIN ON DEXAMETHASONE-INDUCED CLEFT PALATE

Treatment[a]	Dose (mg/kg)	Litters (No.)	Fetuses (No.)	Clefts	
				No.	%
Group 1					
Dexamethasone (Days 12–15)	3.75	10	76	63	82.9*
Group 2					
Dexamethasone (Days 12–15) plus	3.75	14	85	34	40.0**
Arachidonic acid (Days 12–15)	200				
Group 3					
Dexamethasone (Days 12–15) plus	3.75	5	30	27	90.0***
Arachidonic acid (Days 12–15) plus	200				
Indomethacin (Days 13–15)	10				

[a] Virgin timed pregnant outbred CD Sprague–Dawley rats were treated with daily doses of the drugs as indicated (sperm = Day 0). On Day 19 fetuses were obtained by cesarian section and palates were examined.
* 1 vs 2 $P < 0.0005$ by χ^2; $P < 0.0014$ by Mann–Whitney.
** 2 vs 3 $P < 0.0005$ by χ^2; $P < 0.0026$ by Mann–Whitney.
*** 1 vs 3 N.S.

ogenous arachidonic acid on dexamethasone-induced palatal teratogenesis by indomethacin, an inhibitor of cyclo-oxygenase, suggests a teratogenic role of inhibition of the production of prostaglandin(s) and/or thromboxane(s) by dexamethasone. The postulation that the clefting action of dexamethasone leads to a reduction of the products of cyclo-oxygenase activity, raises the question whether prostaglandins

TABLE II. EFFECT OF DEXAMETHASONE ON DISTRIBUTION OF FREE [^{3}H]ARACHIDONIC ACID IN FETAL PALATES

Animals[a]	Litters (No.)	^{3}H Total (fmole/g)	[^{3}H]Arachidonic acid 10,000g supernatant (% total)	10,000g Pellet (% total)
Controls DMSO Days 12–15	7	13.1 ± 3.2	76.8 ± 8.5	24.6 ± 8.8
Dexamethasone 3.75 mg/kg Days 12–15	8	13.5 ± 1.8	42.4 ± 19.8*	57.6 ± 19.8*

[a] Timed pregnant CD Sprague–Dawley rats were injected subcutaneously with either dexamethasone or vehicle once daily from Day 12 to 15. On Day 14, 20 μCi of [5, 6, 8, 9, 11, 12, 14, 15-^{3}H(N)]arachidonic acid (62.2 Ci/mmole) in 20 μl ethanol were administered intravenously. On Day 15, 1 hr after the last dose of dexamethasone, fetuses were obtained by cesarian section. Fetal upper and lower jaws including palatal shelves were dissected, weighed, homogenized in 4 ml isotonic citric acid–saline buffer (pH 5), and centrifuged at 10,000g for 10 min. Each supernatant fraction and pellet was extracted with organic solvents and chromatographed as described under Materials and Methods.
* $P = 0.001$, Student's t; ± SD.

and/or thromboxanes may be involved in normal palatal development. If this were true, one would expect palatal clefting to be produced by indomethacin and other nonsteroidal and anti-inflammatory inhibitors of cyclo-oxygenase, such as, aspirin and phenylbutazone (8, 9). Indomethacin does not produce cleft palate (15), but aspirin and phenlybutazone do (16, 17). Moreover, aspirin increases the clefting induced by cortisone (18). Recently, Klein, *et al.* (19) have shown that both aspirin and indomethacin inhibit cyclooxygenase on Day 11 rat embryos *in vitro,* but only aspirin produces a potent and long inhibition *in vivo.* Aspirin at this time is teratogenic (polydactyly, cleft palate, etc.), but indomethacin is not (personal communication). Thus, indomethacin produces an inhibition of fetal cyclooxygenase sufficient to block the effects of exogenous arachidonic acid under the conditions of the present study, but not sufficient to produce cleft palate by itself. Therefore, it is possible that prostaglandins and/or thromboxanes participate in normal palatal development.

Our data as a whole suggest that the mechanism considered to be the basis for the hormonal anti-inflammatory action of glucocorticoids is probably also the basis for their teratogenic action. This is further supported by several parallels between the two mechanisms. The hormonal action involves a cytosolic hormone-receptor complex and uptake into the nucleus with induction of a protein which inhibits the release of arachidonic acid (20–23). This mechanism can be blocked by inhibitors of protein synthesis and the action is reversed by exogenous arachidonic acid (3–5, 20–23). The teratogenic action of glucocorticoids is also dependent upon formation and nuclear uptake of a cytosolic hormone-receptor complex with ensuing transcription and translation of specific protein which can be blocked by inhibitors of protein synthesis (24–27). The present report shows that teratogenic action can also be reversed by arachidonic acid.

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