Placental and fetal contraindications of dexamethasone administration to pregnant rats

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Summary. Dexamethasone (DEXA) given to pregnant rats for either the last 3 or 6 days of gestation lowered placental, fetal body and adrenal weights. Histologically, DEXA-treated placentas appeared smaller than controls and showed signs of necrosis and pyknosis. Treated animals that were permitted to carry their litters to term did not deliver naturally, and most of their fetuses were dead when excised 1 day postmaturely.

Synthetic corticosteroids have been used for several years to treat a variety of conditions that complicate pregnancy[1-6]. Dexamethasone (DEXA), a relatively potent synthetic glucocorticoid which is becoming more frequently used as a therapeutic agent in pregnancy, has recently been proven effective in promoting labor in prolonged pregnancies[7] and preventing respiratory distress syndrome in premature infants[8]. However, such therapy during pregnancy is not without risk to the conceptus. Recent animal studies[9-11] demonstrate that DEXA exposure during pregnancy increases the incidence of fetal death, cleft palate and sex organ anomalies, and decreases fetal lung and liver weights. To add to these previous works the present study examines specific effects of pharmacologic doses of DEXA given during pregnancy on placental growth and morphology, fetal body and adrenal weights, and length of gestation in the rat.

Materials and methods. Timed pregnant Long Evans rats were divided into 3 groups. 1 group (DEXA-3d) received DEXA (Sigma Chem. Co.) in drinking water (5 µg/ml) from day 18 to day 21 of gestation. The 2nd group (DEXA-6d) received the same dosage of DEXA from day 15 to day 21 of gestation. The 3rd group served as control and received plain tap water ad libitum. Experimental groups were limited to 40 ml of drugged water per day supplemented with tap water ad libitum, thus setting the drug intake at approximately 200 µg/rat/day. Conditions of nutrition, caging, temperature and illumination were the same for all groups. On gestation day 21, dams were killed by decapitation; fetuses and placentas were quickly removed, blotted on filter paper and weighed; fetal adrenals were removed, cleaned of surrounding connective tissue and weighed. Only live fetuses and their placentas and adrenals were studied. Midsections of randomly chosen placentas from each dam were fixed in Helly's solution or 10% buffered formalin, processed for paraffin embedding, sectioned at 7 µm and stained with hematoxylin and eosin (H&E) or periodic acid-Schiff (PAS) reagent. The remaining placentas of each dam were pooled, homogenized in ice-cold 0.25 M sucrose (20%, w/v), and analyzed in duplicate for DNA and RNA[12] and in triplicate for protein[13]. Statistical differences among groups were evaluated by analysis of variance and Duncan's multiple range tests. 4 pregnant animals in the DEXA-6d group were not sacrificed on day 21, but were permitted to carry their litters to term and beyond. Parturition in this strain usually occurs between 21.5 and 22 days.

Results. Fetal mortality was higher, but not significantly, in DEXA-treated groups compared to controls (table). The average body weights of DEXA-3d and -6d fetuses were 22 and 39% smaller than control values, respectively. The average placenta weights for the respective groups wer 13 and 31% less than controls, however, when calculated with reference to fetal body weights (placenta:body ratio), placenta weights were similar for all groups. No significant differences among groups in placental content of DNA, RNA and protein were found, although the levels of all 3 substances tended to be lower in DEXA-treated placentas. Adrenal weights were 44 and 68% lower than controls in DEXA-3d and -6d fetuses, respectively, and these differences remained significant when expressed relative to body weight. No gross morphologic signs of abnormal development were detected in the DEXA-exposed fetuses.

Histologically, placentas of DEXA-treated animals showed structural differences from controls. Although changes were similar in both treatment groups, those of DEXA-6d placentas were more pronounced. The overall size of both labyrinthine and junctional zones were markedly reduced in DEXA-exposed placentas (figs 1 and 2). A greater reduction occurred in the junctional zone, from an apparent loss of cytotrophoblasts and giant cells. No differences among groups were observed in the number or distribution of glycogen (clear) cells. Also, in the junctional zone of DEXA placentas, areas of hemorrhage and leukocytic invasion were frequently found (fig. 3). In the labyrinth of DEXA placentas, syncytical trophoblasts appeared atrophic and their nuclei pyknotic; maternal blood spaces were often distended and engorged with blood, whereas fetal vessels appeared constricted (fig. 4).

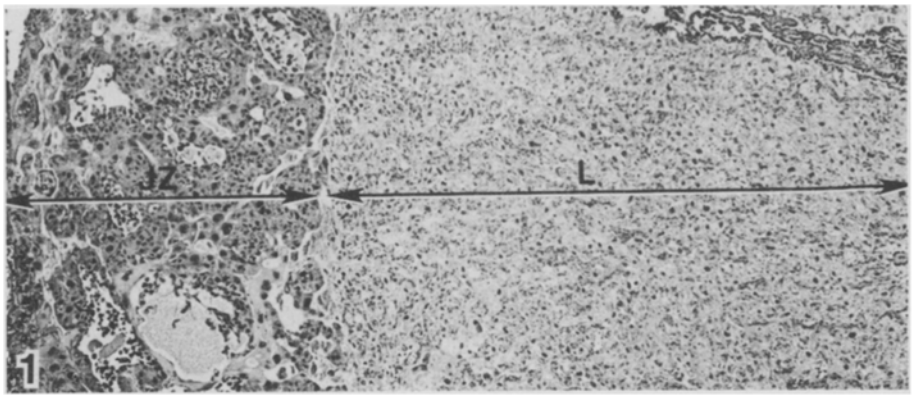
Of the 4 pregnant animals that received DEXA from day 15 but were not sacrificed on day 21, none delivered their litters at normal term. When sacrificed on day 23, only 3 animals still carried fetuses of which 68% (19/28) were dead. The average body, placental and adrenal weights of the live fetuses were 4.9 ± 0.1 g (mean ± SEM), 403.8 ± 23.1 mg and 0.9 ± 0.05 mg, respectively. Compared to 21-day fetuses (table), body and placenta weights fall between control and DEXA-3d values, whereas adrenal weights are still markedly depressed and similar to those of DEXA-6d animals. The remaining animal aborted its entire litter between the 22nd and 23rd day of pregnancy.

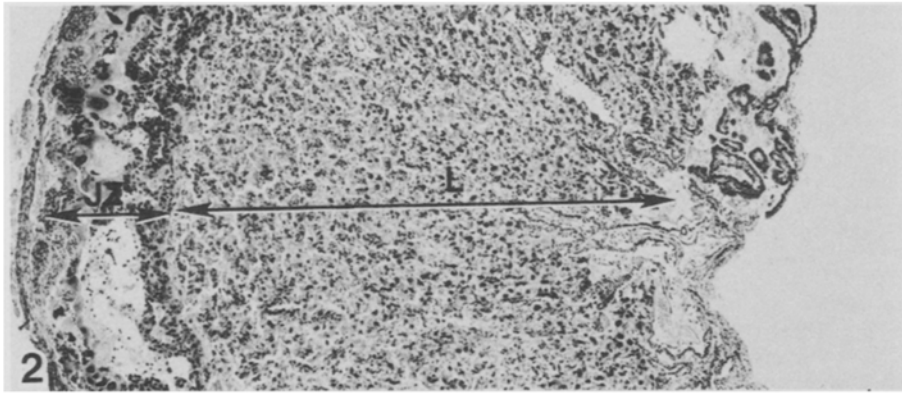
Discussion. As described here and by others[9-11,14,15], DEXA administration to pregnant rats can have profound effects on fetal development. From these studies, however, it is still uncertain whether the adverse effects of adrenal steroids on fetal development are direct, on the fetus, or indirect, through placental dysfunction. In view of the present findings, we suggest that both mechanisms may be

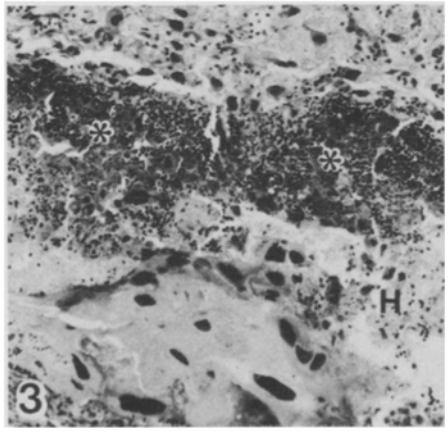
Effects on the fetus and placenta of DEXA (200 µg/day) given to pregnant rats for the last 3 or 6 days of gestation

Treatment	No. of litters	Dead fetuses (%)	Fetal body weight (g)	Placenta weight (mg)	Placental content (mg/placenta) DNA	RNA	Protein	Fetal adrenal weight (mg/2)
Control	6	3 (2/68)	5.40 ± 0.2	451.5 ± 17.3	0.98 ± 0.06	1.19 ± 0.10	36.5 ± 3.4	2.5 ± 0.1
DEXA-3d	5	12 (6/52)	4.2 ± 0.2*	393.0 ± 8.2*	0.89 ± 0.06	0.89 ± 0.14	29.5 ± 3.8	1.4 ± 0.1*
DEXA-6d	6	20 (10/51)	3.3 ± 0.3**	311.2 ± 13.2**	0.78 ± 0.09	0.82 ± 0.16	29.8 ± 1.4	0.8 ± 0.1**

Values given as mean ± SEM. * Significantly different from controls at $p < 0.001$. ** Significantly different from other experimental group at $p < 0.05$.







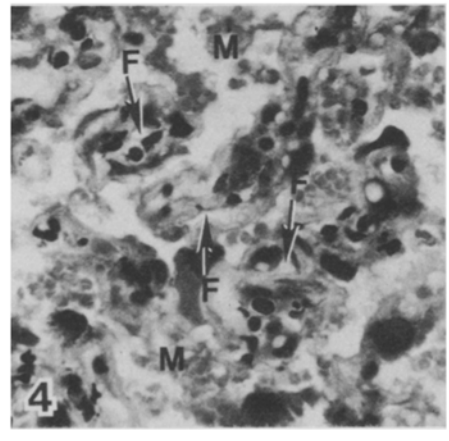


Fig. 1. Control placenta (day 21 showing a well-developed junctional zone (JZ) and labyrinth (L). PAS, ×35.
Fig. 2. Experimental placenta (day 21) exposed to DEXA for 6 days. Widths of the junctional zone (JZ) and labyrinth (L) are reduced. PAS, ×35.
Fig. 3. Junctional zone in a 6-day DEXA-treated placenta. Areas of leukocytic invasion (*) and hemorrhage (H) are prominent. H & E, ×300.
Fig. 4. Labyrinth in a 6-day DEXA-treated placenta. Fetal capillaries (F) are surrounded by aggregates of pyknotic trophoblast cells. Maternal channels (M) appear enlarged. H & E, ×300.

involved, i.e., the impaired growth of the adrenal glands resulting from a direct inhibition by DEXA on the hypothalamo-pituitary-adrenal axis of the fetus[14,23] and placental alterations by glucocorticoid effects on placental growth and metabolism[16–18,24]. The morphologic changes in the placenta following DEXA treatment are similar in scope and nature to those found by other investigators using different adrenocorticoids, such as prednisolone, hydrocortisone, deoxycorticosterone and cortisone[16–18], and seem to suggest premature placental aging, particularly in the loss of cytotrophoblasts and giant cells in the junctional zone and atrophy and pyknosis in the syncytial layers of the labyrinth.

The results of DEXA administration on parturition in the rat were somewhat surprising in that corticosteriod treatment is known to shorten gestation in other animal species[19–22]. The cause of the failure of DEXA-treated animals to deliver was not determined in this study. However, because of the high fetal mortality associated with the delayed parturition, this phenomenon and its underlying mechanisms should be further investigated.

In conclusion, our results suggest that although DEXA is an effective therapeutic agent in a number of conditions that trouble pregnancy, benefits of its use should be carefully weighed against potential risks to the fetus. In the past, fetal and newborn responses have been emphasized in the study of maternal exposure to drugs, however, effects on the placenta and the direct consequences of these effects on fetal well-being cannot be overlooked. Although no teratogenic effects were observed in our study, the prolongation of gestation, high fetal mortality, retardation of growth and potential long-term deleterious effects associated with DEXA treatment warrant concern if this drug is to be used in human pregnancy therapy.

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Further support for the postsynaptic action of substance P and its blockade with baclofen in neurons of the guinea-pig hypothalamus in vitro

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Summary. Effects of substance P on neurons of the guinea-pig hypothalamus in vitro and antagonism between substance P and baclofen were investigated. Substance P increased the firing rate of neurons in the medium containing 0 mM Ca^{2+} and 12 mM Mg^{2+}. The excitatory action of stubstance P was antagonized by a low dose of baclofen whereas that of acetylcholine was not antagonized even by much higher doses of baclofen.

There are several lines of evidence to suggest that the undecapeptide, substance P (SP), is involved in synaptic transmission in the brain[2]. As the hypothalamus is one of the areas in the brain rich in SP[3], we investigated the effect of SP on neurons in the hypothalamus of the guinea-pig brain. In addition, we examined the effect of baclofen, a derivative of γ-aminobutyric acid (GABA) and reportedly a specific antagonist of SP[4].

Materials and methods. Adult guinea-pigs of either sex were used. Details of the preparation of the hypothalamic slices (400-600 µm thick), incubation and recording procedures were as described elsewhere[5]. The slices were continuously perfused with Krebs solution, and extracellular unit discharges were recorded with conventional glass microelectrodes. To obtain antidromically as well as orthodromically evoked spikes, an area adjacent to the tip of the recording electrode (about 1 mm distance) was stimulated by single electrical pulses (50 µsec duration) through an electrode consisting of a pair of tungsten wires. After control records were taken, the normal Krebs solution was gradually replaced by the Krebs solution containing the chemical.

Results and discussion. Bath-applied SP markedly increased the spontaneous firing of hypothalamic neurons. Figure 1a illustrates the typical dose-response relations in application of SP in the silent cell of the anterior hypothalamus. To determine whether or not the excitatory action of SP on the hypothalamic neuron was direct, the effect of SP was studied using a Krebs solution containing 12 mM Mg^{2+} but not Ca^{2+}(Ca-free medium). The excitatory action of SP persisted even in the Ca-free medium in 29 out of 30 units tested (see figs. 1b and 3c). Acetylcholine (ACh) in doses of 5.5×10^{-6}–5.5×10^{-8} M also increased the firing rate dose-dependently in both the normal (fig. 1c) and Ca-free (fig. 3a and d) media. All the SP-sensitive neurons were ACh-sensitive, but not for all the ACh-sensitive neurons. As shown in figure 1c, atropine (1.4×10^{-6} M) markedly suppressed the effect of ACh whereas it did not affect the action of SP.

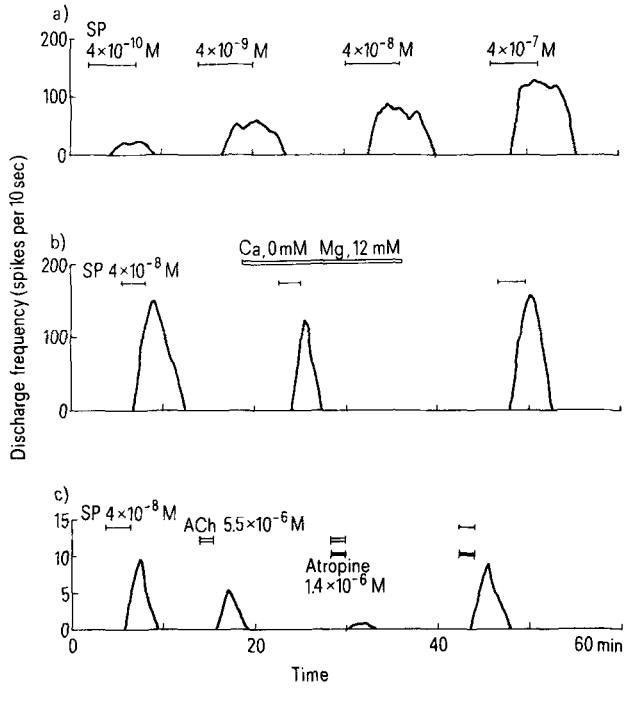

Fig. 1. Effects of substance P (SP) and acetylcholine (ACh) on the spontaneous firing rate of neurons in the anterior hypothalamus. Spontaneous unit discharges were recorded on magnetic tape, digitized by an A-D converter, processed by a general purpose computer Nihon-Kohden ATAC 1200 for compilation of the spontaneous firing rate of the neurons, and recorded on a pen-recorder in this and in subsequent illustrations. Drugs were applied at periods indicated by bars. The bar in *b* entitled 'Ca, 0 mM Mg, 12 mM' represents perfusion with the medium which was calcium free and in which the magnesium was raised to 12 mM. In *c*, antagonism of atropine with ACh is shown.