Effect of Betamethasone on the Expression of Dopamine D₁ Receptor mRNA in the Developing Rabbit Adrenal Gland

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ABSTRACT

Background: Antenatal corticotherapy is widely used to enhance pulmonary fetal maturation in cases in which premature birth is likely. The adrenal gland, which has a key role in controlling fetal and neonatal adaptation, appears to be a target organ of glucocorticoids.

Objectives: The aim of this study was first to determine the ontogenic profile of dopamine D₁ receptor (DA D₁-R) messenger RNA (mRNA) in the rabbit. The effects of antenatal exposure to exogenous corticoids on levels of adrenal DA D₁-R mRNA expression were examined.

Methods: Pregnant rabbits free of any treatment or handling before delivery were chosen for the study of DA D₁-R mRNA ontogenic profile. For the study of the antenatal exposure to exogenous corticoids, pregnant rabbits were given 2 injections of either betamethasone 0.1 mg/kg or saline 0.1 mL/kg. DA D₁-R mRNA expression was determined using northern blot analysis at 4 developmental ages: fetus (at 27 days of gestation), 1 day of age, 25 days of age, and adulthood. Rabbits were allocated to their respective group (treated or untreated) depending on maternal treatment (betamethasone or saline, respectively).

Results: Four pregnant rabbits were used for the ontogenic-profile group, which comprised 11 fetuses, seven 1-day-old rabbits, four 25-day-old rabbits, and 2 adults. Six other pregnant rabbits received betamethasone; 6 saline. The treated group comprised 12 fetuses, twelve 1-day-old rabbits, four 25-day-old rabbits, and 3 adults. The untreated group comprised 12 fetuses, fifteen 1-day-old rabbits, five 25-day-old rabbits, and 3 adults. DA D₁-R mRNA was expressed in rabbit adrenal glands from the fetal period to adulthood and this expression was not age dependent. Moreover, antenatal corticotherapy induced a significant increase in respective DA D₁-R mRNA levels of 20%, 15%, and 8% in

treated fetuses, 1-day-old rabbits, and 25-day-old rabbits compared with the untreated groups ($P < 0.003$, 0.003, and 0.005, respectively). This increase was not observed in adulthood.

Conclusions: In the rabbits in this study, DA D₁-R mRNA expression in the adrenal gland began during gestation. Its expression was not age dependent but was rapidly modified by antenatal exposure to betamethasone. These corticoid-induced changes, observed until late infancy, did not occur in adulthood. (*Curr Ther Res Clin Exp.* 2003;64:568–579) Copyright © 2003 Excerpta Medica, Inc.

Key words: dopamine D₁ receptor, antenatal corticotherapy, development, adrenal gland.

INTRODUCTION

Active pharmacotherapeutic management of the fetus has been developed during the past 2 decades to enhance the maturation of fetal physiologic functions in cases in which an increased risk for premature delivery is suspected. For example, the widespread use of antenatal corticotherapy to accelerate fetal pulmonary maturation¹ has resulted in a decrease in pulmonary morbidity.^{2,3} Although this therapy has a good safety profile,⁴ the safety has not been verified in many natural targets of corticoids, particularly the adrenal gland.

The adrenal gland has an essential role at birth in controlling the adaptive response to the perinatal stress that occurs during the switch from intrauterine to extrauterine life, mainly by the release of catecholamines. The cellular mechanism for secretion is present in the adrenal gland early in gestation. Maturation of the catecholamine biosynthesis pathway depends on endogenous cortisol.^{5,6} However, the functional maturity of splanchnic innervation controlling catecholamine exocytosis is not complete at birth in many species, including humans.⁶ Local regulation of the adrenal endocrine response is of vital importance late in the gestational period and in the first weeks after birth.

The effects of antenatal exposure to exogenous corticoids on the adrenal gland have been assessed in various ways, and experimental studies have produced contradictory data. Excessive exposure to corticoids may lead to intrauterine growth retardation.⁷ A single dose of dexamethasone may even induce severe adrenal atrophy and lead to a decrease in the birth weight of rat pups.⁸ This significant reduction in fetal weight also has been observed in rabbits.⁹ Similarly, reductions in the weight of the placenta and the fetal brain, lungs, and liver⁹ have been noted, but no data were given about adrenal growth. Another study¹⁰ showed an increase in adrenal catecholamine level in newborn rabbits after antenatal administration of a very low dose of dexamethasone (0.01 mg/kg). This increase in adrenal catecholamine level may be the consequence of the overexpression of tyrosine hydroxylase, which is the key enzyme in catecholamine synthesis.

In humans, antenatal corticoid exposure has not been observed to have severe adverse effects on fetal growth.³ Transient suppression of adrenocortical activity may be observed without suppression of activation induced by the stress of birth.¹¹ However, in preterm infants the birth-related increase in plasma catecholamine levels seems less pronounced in treated than in untreated infants.¹² Nevertheless, a case of Cushing's syndrome was described when corticotherapy was administered 7 times before delivery at 34.5 weeks' gestational age.¹³

Dopamine (DA) is 1 of the 3 catecholamines synthesized and released by the adrenal gland. It was first known to be a neurotransmitter at central and peripheral levels,¹⁴ and it was later shown to be a regulating factor at the adrenal level.¹⁵ This regulating action is mediated by the activation of specific receptors,¹⁶ including the DA D₁ receptor (D₁-R), which is found in the adrenal medulla¹⁵⁻¹⁹ and the cortex^{20,21} in different animal species. However, its presence remains controversial.²²⁻²⁴

As a result, we attempted to indirectly test the presence of DA D₁-R in the adrenal gland by analyzing its messenger RNA (mRNA). The aim of this study was first to determine the ontogenic profile of DA D₁-R mRNA in the rabbit because, according to a MEDLINE search for articles published from 1974 to 2002 and including the key terms *dopamine receptor*, *adrenal gland*, and *rabbit*, this has not yet been described in the rabbit adrenal gland. The effects of antenatal exposure to exogenous corticoids on levels of adrenal DA D₁-R mRNA expression were examined.

MATERIALS AND METHODS

Animals

We used New Zealand white rabbits obtained from a local breeder. Experimental procedures were performed according to the guidelines of the Canadian Council for Animal Care and were approved by the local animal care ethics committee at Laval University (Laval, Quebec, Canada).

Protocols

DA D₁-R mRNA expression was investigated in the adrenal glands and striatum at 4 developmental ages: fetus, 1 day of age, 25 days of age, and adulthood. Fetuses were delivered by cesarean section at 27 days of gestation. Spontaneous delivery took place at 30 or 31 days of gestation. The striatum was used as a positive control for validation of our northern blot analysis technique because this organ contains high levels of DA D₁-R mRNA.²⁵ Pregnant rabbits, reared without any treatment or handling until delivery, were used for the ontogenic-profile study. The effect of antenatal exposure to corticoids on the expression level of DA D₁-R mRNA in the adrenal glands was investigated in the offspring of pregnant rabbits, which were given 2 injections of either betamethasone 0.1 mg/kg*

*Trademark: Celestone Soluspan® (Schering Corp., Kenilworth, New Jersey).

or saline 0.9% 0.1 mL/kg at days 25 and 26 of gestation for the fetal analysis and at days 27 and 28 of gestation for the postnatal analysis. Treatments were blinded and encoded, as were the examination and the performance of northern blots. Litters were reared in the institution's animal house and were allocated to their respective group (treated or untreated) according to maternal treatment (betamethasone or saline, respectively). Because the size of the adrenal glands was sufficient to perform the analytical procedures with a minimal risk for error, and because northern blot data were obtained from 6 experiments with total RNA from 2 independent extractions for each animal, the number of adults was reduced, in keeping with the recommendations of the ethics committee.

All procedures and analyses were performed by the primary author (J.M.L.) and supervised by the secondary authors (M.J.B. and A.B.).

Organ Collection

Pregnant rabbits were anesthetized with an injectable mixture of ketamine 50 mg/kg and xylazine 10 mg/kg. Fetuses were removed by cesarean section and decapitated immediately. Pups and adults were sacrificed with a lethal dose of anesthetic. The adrenal glands and the striatum of each animal were quickly removed, immediately frozen on dry ice, and stored at -80°C for subsequent analysis.

RNA Studies

Total RNA was extracted from frozen organs using RNeasy[®] kits (QIAGEN, Hilden, Germany). Quantitation of the purified RNA was made by ultraviolet spectrophotometry (absorbency, 260 nm) by staining with ethidium bromide after 1% agarose gel migration and comparing with known quantities of standard 28S and 18S ribosomal RNA (rRNA) (Pharmacia Corp., Uppsala, Sweden).

The probe preparation for rabbit DA D₁-R was similar to that described previously.²⁶ First, we generated a fragment by using rabbit complementary DNA as a template. This fragment was amplified by polymerase chain reaction (PCR) using nucleotides 151–175 and 738–761 as primers.²⁶ The PCR was purified on a 1% agarose gel, and the band at ~600 base pairs was excised from the gel. The DNA was extracted using a Prep-A-Gene[®] kit (BioRad Laboratories, Inc., Richmond, California), ³²P-labeled by random priming,²⁷ and used at $\sim 1 \times 10^6$ cpm/mL of hybridization solution.

Northern blot analysis was performed as previously described²⁸ using 5 μg of total RNA. After hybridization and washing, the membranes were exposed to a BAS 1000[®] phosphorimager plate (Fuji Medical Systems USA, Inc., Stanford, Connecticut) for 36 hours and then exposed to X-OMAT AR[®] film (Eastman Kodak Co., Minneapolis, Minnesota) at -80°C with an intensifying screen for 7 days.

mRNA Studies

Northern blot analyses were performed 6 times with total RNA obtained from 2 independent extractions. The tissue samples were analyzed together on a

single DA D₁-R mRNA northern blot hybridization. Afterward, an 18S rRNA hybridization was used on the same membranes to confirm equal loading and transfer of RNA. The amount of DA D₁-R mRNA during development was determined for each age by phosphorimager signal intensity using BAS version 2.5 (Fuji Medical Systems USA, Inc.). The amounts of DA D₁-R mRNA and 18S rRNA were expressed in pixel intensities per square millimeter (psi/mm²), after subtracting the background count for each membrane, and DA D₁-R ³²P counts were normalized using the 18S rRNA net count. We used the same procedure with adrenal samples for assessment of relative mRNA modulation after antenatal corticotherapy.

Statistical Analysis

Changes in DA D₁-R mRNA were given as a ratio of DA D₁-R mRNA to the fetuses for ontogenesis data and to the untreated animals for the corticotherapy data. The expression level was arbitrarily set at 1 for the reference groups (fetus and untreated). Differences in DA D₁-R mRNA levels were compared using 2-way analysis of variance followed by the Fisher post hoc test or Student *t* test, as appropriate (Statview®, SAS Institute Inc., Cary, North Carolina). *P* < 0.05 was considered significant.

RESULTS

Four pregnant rabbits free of any treatment or handling until delivery were used for the ontogenic-profile study: 11 fetuses, seven 1-day-old rabbits, four 25-day-old rabbits, and 2 adults. Six other pregnant rabbits received betamethasone and 6 saline for the study of antenatal exposure to exogenous corticoids. The treated group (born to does given betamethasone) comprised 12 fetuses, twelve 1-day-old rabbits, four 25-day-old rabbits, and 3 adults. The untreated group (born to does given saline) comprised 12 fetuses, fifteen 1-day-old rabbits, five 25-day-old rabbits, and 3 adults.

Dopamine D₁ Receptor Transcripts During Development

The autoradiographic films showed low levels of DA D₁-R mRNA in the adrenal gland and the striatum in all of the fetuses. The mean level increased from the fetal to the adult stage only in the striatum (Figure 1A). Densitometric analysis of phosphorimager signals (Figure 1B) showed a 1.2-fold increase in mean DA D₁-R transcript level from day 1 after birth to the adult stage, reaching the highest increase at day 25 (2.1-fold). Mean values were significantly higher in the 1-day-old group, the 25-day-old group, and adults than in fetuses (*P* < 0.01, 0.001, and 0.001, respectively). In contrast, in the adrenal gland, DA D₁-R mRNA level did not change significantly (Figure 1). These patterns suggest that the expression of DA D₁-R mRNA is age-modulated in the striatum but not in the adrenal gland.

Figure 1. Dopamine D₁ receptor (DA D₁-R) messenger RNA expression in striatum and adrenal gland (A) as shown on a representative northern blot analysis and (B) the relative phosphorimager densitometric mean (SD) values. * $P < 0.01$ versus fetuses. † $P < 0.001$ versus fetuses. F = fetuses; 1d = 1 day of age; 25d = 25 days of age; Ad = adults; rRNA = ribosomal RNA.

Dopamine D₁ Receptor Transcripts After Antenatal Corticotherapy

The autoradiographic films of adrenal samples showed higher mean levels of DA D₁-R transcripts in treated fetuses and 1-day-old animals (Figure 2A). Densitometric analysis of phosphorimager signals showed that mean adrenal mRNA levels were significantly higher in treated fetuses and 1- and 25-day-old rabbits than in untreated rabbits ($P < 0.003$, 0.003, and 0.005, respectively). The increase was 20% in fetuses, 15% in 1-day-old rabbits, and 8% in 25-day-old rabbits. No significant difference in mean adrenal mRNA levels was found between treated and untreated adults (Figure 2B). These data show an increase of DA D₁-R mRNA in the adrenal gland following antenatal exposure to betamethasone.

DISCUSSION

Using northern blot analyses, our data show that DA D₁-R mRNA is expressed in the rabbit adrenal gland from the fetal stage to adulthood and that its expression

Figure 2. Dopamine D₁ receptor (DA D₁-R) messenger RNA expression in adrenal gland after antenatal corticotherapy (A) as shown on a representative northern blot analysis and (B) the relative phosphorimager densitometric mean (SD) values. * $P < 0.003$ versus untreated. † $P < 0.005$ versus untreated. rRNA = ribosomal RNA; F = fetuses; 1d = 1 day of age; 25d = 25 days of age; Ad = adults.

level may be affected by antenatal treatment with corticoids. The expression of DA D₁-R mRNA in the adrenal gland had not previously been described in rabbits.

DA D₁-R is localized to the brain and peripheral tissues by blood vessels, postganglionic sympathetic nerve terminals, and adrenal glands. In the brain, DA D₁-R is expressed at higher levels than any other catecholamine receptor,^{29,30} and its functional role has been extensively reviewed.^{31,32} However, this is only a first step in improving our knowledge of the adrenal glands. These glands are responsible for an intranatal catecholamine surge.⁶ DA D₁-R seems to be the basis of a positive feedback for catecholamine release. Its stimulation in bovine chromaffin cells facilitates Ca²⁺ channel currents by involving adenylate cyclase and protein kinase A.¹⁹ This increase in Ca²⁺ currents stimulates rapid catecholamine secretion in response to stress.¹⁹ Thus, we have come full circle:

plasma DA released from the adrenals with other catecholamines stimulates DA D₁-R adenylate cyclase protein kinase A facilitation of Ca²⁺ channels in adrenal chromaffin cells catecholamine release in plasma.³³ Circulating chemical signals associated with states such as hypoxemia or hypoglycemia also can directly stimulate adrenal catecholamine receptors, specifically, dopamine receptors (DA-Rs), until sympathetic innervation reaches complete maturity, several days or even weeks after birth.^{34,35}

The difference in developmental patterns observed in the striatum compared with the adrenal glands should be underlined. In striatum, DA-R mRNA expression increases from birth to 25 days after birth, when it is at its highest level. These changes are consistent with those reported by Xu et al³⁶ in rat striatum, where the highest levels were observed at day 30 after birth. This developmental pattern in striatum follows the same time course as growth and maturation of the central nervous system. In the adrenal glands, DA D₁-R mRNA expression does not seem to be age dependent and has been found to be at its highest level at birth, which may be related to the fact that sympathoadrenal activity is higher at birth than under any other physiologic circumstance throughout development or adulthood.⁶

In the second stage of our study, we assessed the effect of a pharmacologic stimulus induced by an exogenous glucocorticoid on DA D₁-R mRNA expression in developing adrenal glands. Antenatal corticotherapy induced changes in DA D₁-R mRNA expression in the adrenal glands, with an early increase followed by sustained overexpression until 25 days of age. The opposite pattern has been described in rabbit striatum,³⁷ suggesting that the effects of antenatal exposure to betamethasone might be tissue specific. For the adrenal gland, endogenous glucocorticoids are closely involved in growth and differentiation. During fetal development and until the early neonatal period, the cortex projects cords into the medulla, making it impossible to reliably distinguish the cortex from the medulla.³⁸ The close relationship between cortical and chromaffin cells should lead us to consider the medulla as the first target for corticoids. Antenatal exposure to exogenous glucocorticoids often leads experimentally to adrenal atrophy^{8,9,39} due to necrosis of the cortical zone.⁹ On the other hand, the effects on the medullary zone are generally beneficial: enlargement of the layer and increase in the trophicity of the cells³⁸; increase in adrenal catecholamine content¹⁰; and enhancement of enzyme gene transcription, including that of tyrosine hydroxylase,⁴⁰ the key enzyme for DA synthesis.

In the current study, we observed an increase in DA D₁-R mRNA levels. As DAD₁-R is involved in the autoregulation of the sympathoadrenal functions linked to postnatal adaptation, an increase in the transcript levels might be beneficial. In any case, it should not cause concern about the neonatal viability in the face of perinatal stress. However, the enhancement of transcription of the gene encoding for DA D₁-R mRNA is not necessarily followed by an increase in receptor density. For other catecholamine receptors such as beta-adrenergic receptors, corticosteroids have significant effects on maturation,⁴¹ but the effects

are species specific. Rabbit and rat fetuses exposed to corticosteroids in utero increase pulmonary beta-receptor density,⁴² but this does not occur in fetal sheep.⁴³ If the increase in DA D₁-R mRNA in rabbit adrenal glands was followed by an increase in receptor density, as is the case for rabbit lungs, one might suspect a positive effect of corticoids. The effects of corticoids could be considered as positive, not only in coping with the physiologic stress of birth, but also in counteracting pathologic circumstances, such as hypoxic episodes, that are responsible for downregulation of DA D₁-R mRNA expression.⁴⁴

Three points should be emphasized: (1) the early occurrence of the increase in the fetus (within 24 hours after the second injection of betamethasone); (2) the very small amounts of drug used (small but comparable to the amounts given in perinatal practice and known to be sufficient to induce pulmonary maturation)¹; and (3) the long-term enhancement of DA D₁-R mRNA expression. Such a long-lasting change in a biologic process, induced by a drug received early in development and found unusual, is classified as a *printing effect*. It may be observed in the pups born to mothers given a drug during gestation. It has been found with phenobarbital,⁴⁵ benzodiazepines,⁴⁶ and glucocorticoids.⁴⁷ The current data suggest a printing effect of betamethasone on the DA D₁-R mRNA expression in the rabbit adrenal gland.

CONCLUSIONS

In the rabbits in this study, DA D₁-R mRNA expression in the adrenal gland began during gestation. Its expression was not age dependent but was rapidly modified by antenatal exposure to betamethasone. These corticoid-induced changes, observed until late infancy, did not occur in adulthood.

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