Intrauterine Growth Restriction Induces Up-Regulation of Cerebral Aromatic Amino Acid Decarboxylase Activity in Newborn Piglets: [^{18}F]Fluorodopa Positron Emission Tomographic Study

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ABSTRACT

There is evidence that intrauterine growth restriction (IUGR) is associated with altered dopaminergic function in the immature brain. However, the relevant enzyme activities have not been measured in the living neonatal brain together with brain oxidative metabolism. Therefore, fluorine-18-labeled 6-fluoro-L-3,4-dihydroxyphenylalanine (FDOPA) was used together with positron emission tomography to estimate the activity of the aromatic amino acid decarboxylase in the brain of 10 newborn IUGR piglets (2 to 5 d old; body weight, 908 ± 109 g) and in 10 normal-weight (3 to 5 d old; body weight, 2142 ± 373 g) newborn piglets. The regional transport of FDOPA to the brain and the clearance rate of labeled metabolites from brain tissue were broadly similar in the two groups. However, the regional rate constant for back flux from the brain was markedly increased in IUGR piglets for striatum (72%) and frontal cortex (83%) ($p < 0.05$). Furthermore, the rate constant for conversion of FDOPA to fluorodopamine was markedly increased (between 48% in cerebellum and 91% in mesencephalon, $p < 0.05$) in all brain regions of IUGR piglets studied. Thus, it is suggested that IUGR induces an up-regulation of aromatic amino acid decar-

boxylase activity that is not related to alterations in brain oxidative metabolism. (*Pediatr Res* 49: 474–480, 2001)

Abbreviations

IUGR, intrauterine growth restriction
 ^{18}F , fluorine-18
FDOPA, ^{18}F -labeled 6-fluoro-L-3,4-dihydroxyphenylalanine
PET, positron emission tomography
AADC, aromatic amino acid decarboxylase (EC 4.1.1.28)
NW, normal weight
 K_1^{FDOPA} , unidirectional clearance of FDOPA
 k_2^{FDOPA} , regional rate constant for FDOPA back flux from the brain
 k_3^{FDOPA} , apparent AADC activity
 $k_{\text{cl}}^{\text{FDA+acids}}$, clearance rate constant for FDOPA metabolites
CBF, cerebral blood flow
CMRO₂, cerebral metabolic rate of oxygen
FDA, fluorodopamine
PS^{FDOPA}, permeability surface area product of FDOPA
ADHD, attention-deficit hyperactivity disorder

An inadequate nutritional supply due to uteroplacental insufficiency or restricted maternal protein intake late in gestation is largely responsible for asymmetrical IUGR (1). Reduced fetal growth can be viewed as a compensation for the reduced supply as long as fetal demand is not critically restricted, leading to decompensation with asphyxia and even death (2). The compromised nutritional state has adverse effects on fetal physiology and metabolism including changes of

hormonal homeostasis (3), which are thought to be associated with postnatal growth failure and a greater propensity to develop cardiovascular metabolic disease or behavioral abnormalities later in life (4). Therefore, the period of fetal adaptation, characterized by reduced growth due to restricted glucose and amino acid availability but largely compensated placental respiratory function (5), reflects a functional state that may lead to both acceleration or delay in organ maturation (6, 7).

The importance of the intrauterine environment for fetal brain development has been stressed by studies showing persistent behavioral abnormalities in prenatally stressed animals (8). Rats exposed to noise and light stress during the last trimester of pregnancy produce offspring with altered locomo-

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tor or exploratory activity. Prenatal stress also appears to modify the levels of the monoamines in various brain regions, indicating that the observed behavioral abnormalities are associated with alterations in central dopaminergic activity. This is thought to contribute to the etiology of ADHD, a common neurodevelopmental disorder with prefrontal (9) and striatonigral (10, 11) dysfunction.

However, the effects of IUGR on regional brain dopamine metabolism *in vivo* have not yet been determined. Therefore, we estimated the activity of AADC, the ultimate enzyme in dopamine synthesis, together with regional CBF, brain tissue Po_2 , and CMRO_2 in newborn NW and IUGR piglets. We used a morphometrically well-characterized state of asymmetrical IUGR in newborn piglets (12) and included animals with optimal vital conditions early after birth. We postulate that IUGR activates brain dopamine turnover.

METHODS

Animals. All surgical and experimental procedures were approved by the Committee of the Saxon State Government on Animal Research. Animals were obtained from a breeding farm. Delivery was observed and the viability of neonatal piglets assessed immediately after birth, so that only animals with a viability score ≥ 7 (13) were included in the study. Immediately before the onset of the experiments, animals were carried to the laboratory in a climatized transport incubator (environmental temperature, 33–34°C; time for transportation, 30 to 60 min). Animals were divided into NW piglets ($n = 10$; aged 3 to 5 d old; body weight, 2142 ± 373 g) and IUGR piglets ($n = 10$; aged 2 to 5 d old; body weight, 908 ± 109 g) according to their birth weight. The birth weight distribution of the breed of piglets used here (German Landrace) has been described previously (12).

Anesthesia and surgical preparation. The piglets were initially anesthetized with 1.5% isoflurane in 70% nitrous oxide and 30% oxygen by mask. The anesthesia was maintained throughout the surgical procedure with 0.8% isoflurane. A central venous catheter was introduced through the left external jugular vein and was used for the administration of drugs and for volume substitution (lactated Ringer's solution, 5 mL/h). An endotracheal tube was inserted through a tracheotomy. After immobilization with pancuronium bromide (0.2 mg/kg body weight/h, i.v.), the piglets were artificially ventilated (Servo Ventilator 900C, Siemens-Elema, Sweden). The artificial ventilation was adjusted to maintain normoxic and normocapnic blood gas values. Polyurethane catheters (inner diameter, 0.5 mm) were advanced through both umbilical arteries into the abdominal aorta to record the arterial blood pressure and to withdraw reference samples for the colored microsphere technique. A further polyurethane catheter (inner diameter, 0.3 mm) was inserted into the superior sagittal sinus through a midline burr hole (3 mm in diameter and located 4 mm caudal to the bregma) and advanced to the confluence sinuum to obtain brain venous blood samples. The left ventricle was cannulated retrogradely via the right common carotid artery with a polyurethane catheter (inner diameter, 0.5 mm). The arterial, left ventricular, and central venous catheters were

connected with pressure transducers (P23Db, Statham Instruments Inc., Hato Rey, Puerto Rico). Correct positioning of the catheter tips was checked by continuous pressure trace recordings and by autopsy at the end of the experiment. Body temperature was monitored by a rectal temperature probe and was maintained throughout the general instrumentation at $38 \pm 0.3^\circ\text{C}$ by use of a warmed pad and a feedback-controlled heating lamp. A hole was drilled into the left frontal bone (2 mm in diameter) to implant a Clark-type Po_2 electrode 3–5 mm into the brain cortex together with a thermocouple catheter to serve as a temperature probe (LICOX Po_2 monitor, GMS mbH, Kiel-Mielkendorf, Germany). Physiologic parameters were recorded on a multichannel polygraph (Gould, U.S.A.). The arterial blood pressure was monitored continuously, and arterial blood samples were withdrawn and analyzed at regular intervals to monitor blood gases and whole blood acid-base parameters.

Experimental protocol. After the surgical preparation had been completed, the anesthesia was reduced to 0.25% isoflurane in 70% nitrous oxide and 30% oxygen, and the piglets were allowed to stabilize for 1 h. The piglets were studied lying prone in the PET scanner with the head in a custom-made head-holder. The position of the head was checked throughout the experiment with laser markers. Baseline measurements of physiologic parameters including CBF and brain oxidative metabolism were performed immediately before FDOPA infusion. Emission scanning began simultaneously with the start of the FDOPA infusion (for details, see "PET studies"). A second series of physiologic parameter measurements was performed 1 h after the radiotracer injection (PET). Blood volume replacement was given after each blood withdrawal by using stored heparinized blood obtained from a donor sibling piglet.

Measurements. The regional CBF was measured by means of the reference sample color-labeled microsphere (Dye-Trak, Triton Technology, San Diego, CA, U.S.A.) technique, which represents a valid alternative to the radionuclide-labeled microsphere method for organ blood flow measurement in newborn piglets without the disadvantages arising from radioactive labeling (14). Application of this technique in piglets and methodologic considerations have been presented and discussed in detail elsewhere (14, 15). Briefly, in random color sequence, a known amount of colored polystyrene microspheres was injected into the left ventricle. A blood sample was withdrawn from the thoracic aorta as the reference sample. At the end of each experiment, the piglet brains were obtained. To retrieve the microspheres, each tissue sample was digested and then filtered under vacuum suction through an 8- μm -pore polyester-membrane filter. Colored microspheres were quantified by their dye content. The dye was recovered from the microspheres by adding dimethylformamide. The photometric absorption of each dye solution was measured by a diode-array UV/visible spectrophotometer (model 7500, Beckman Instruments, Fullerton, CA, U.S.A.). Calculations were performed using MISS software (Triton Technology, San Diego, CA, U.S.A.). The number of microspheres was calculated using the specific absorbance value of the different dyes. All reference and tissue samples contained >400 microspheres.

The heart rate, arterial blood pressure, arterial and brain venous pH, P_{CO_2} , P_{O_2} , oxygen saturation, and Hb values were measured immediately before the microsphere injection. Blood pH, P_{CO_2} , and P_{O_2} were measured with a blood gas analyzer (model ABL50, Radiometer, Copenhagen, Denmark), and blood Hb and oxygen saturation were measured using a hemoximeter (model OSM2, Radiometer, Copenhagen, Denmark) and corrected to the body temperature of the animal at the time of sampling.

The absolute flows to the tissues measured by the colored microspheres were calculated by the following formula: $\text{flow}_{\text{tissue}} = \text{number of microspheres}_{\text{tissue}} \cdot (\text{flow}_{\text{reference}} / \text{number of microspheres}_{\text{reference}})$. Flows are expressed in milliliters per minute per 100 g tissue by normalizing for tissue weight. Blood O_2 content (cO_2) was calculated using the equation

$$cO_2[\text{mL} \cdot \text{dL}^{-1}] = \text{cHb}[\text{g} \cdot \text{dL}^{-1}] \times sO_2[\text{mmol} \cdot \text{mmol}^{-1}] \times 1.39[\text{mL} \cdot \text{g}^{-1}] + (P_{O_2}[\text{mm Hg}] \times \alpha O_2[\text{mL} \cdot \text{dL}^{-1}; \text{mm Hg}^{-1}]) \quad (1)$$

to obtain the sum of oxygen that is physically dissolved and chemically bound to Hb [cHb indicates Hb concentration; sO_2 , O_2 saturation; 1.39 (mL/g), theoretical oxygen capacity of Hb; αO_2 , solubility of O_2 in blood = $\text{cHb} \times 0.000054$ (Hb-dependent O_2 solubility) + 0.0029 (solubility of O_2 in plasma)]. Because the sagittal sinus drains the cerebral cortex, the cerebral white matter, and some deep gray structures (basal ganglia, thalamus, and hippocampus) (16), the blood flow measured to the forebrain included these structures. The $CMRO_2$ was obtained by multiplying the blood flow to the forebrain by the cerebral arteriovenous cO_2 difference.

PET studies. FDOPA was produced according to the desaturation method by direct fluorination of the tin precursor with $[^{18}\text{F}]\text{F}_2$ (17), simplifying the procedure (18). The piglets were studied lying prone in the scanner (CTI/Siemens ECAT EXACT HR+; dynamic scans, 35 frames between 30 and 600 s each; total length, 120 min). In each case, 50 to 150 MBq FDOPA was infused within 60 s into the upper caval vein, followed immediately by heparinized isotonic saline (1 IU heparin/mL) to flush the catheter. Fifty-two arterial blood samples were obtained at intervals between 15 s and 30 min, stored on ice, and centrifuged for plasma sampling. Plasma activity (100 μL) was measured in a well counter (COBRA II) cross-calibrated with the tomograph. Additionally, nine blood samples (at 2, 4, 8, 12, 16, 25, 50, 90, 120 min) were withdrawn for HPLC analysis to correct the plasma input function for the presence of FDOPA metabolites (19).

PET image data were reconstructed using a Hanning filter with a cutoff frequency of 0.5. The spatial resolution was 4–5 mm. Transmission scans were performed using three rotating germanium (^{68}Ge) sources to correct for attenuation. Regions of interest were set by hand according to piglet brain stereotaxic coordinates (20, 21).

PET data analysis was performed using a compartmental model of transport and metabolism of FDOPA in the brain,

which was described previously (18). A correction was included for the transport of the metabolite 3-*O*-methyl-FDOPA across the blood-brain barrier. The fate of FDOPA transported into the brain is described by the following processes. K_1^{FDOPA} and k_2^{FDOPA} characterize the reversible transfer of FDOPA across the blood-brain barrier. Within the brain, FDOPA is decarboxylated to FDA by AADC with the rate constant k_3^{FDOPA} . FDA is stored in vesicles or further metabolized by the enzymes monoamine oxidase and catechol-*O*-methyltransferase to acidic metabolites that are transported back to the blood ($k_{cl}^{\text{FDA+acids}}$). The rate constants for blood-brain and brain-blood transfer (K_1^{FDOPA} and k_2^{FDOPA}), the apparent AADC activity (k_3^{FDOPA}), and the clearance rate constant ($k_{cl}^{\text{FDA+acids}}$) were estimated from a double integral form of the compartmental model [analogous to (22)].

Statistical analysis. Data are reported as mean $\pm$ SD. Comparisons between groups were made with unpaired *t* tests. Comparisons between baseline and PET measurements within the groups were made with paired *t* tests. One-way ANOVA with repeated measures was used to compare CBF values and PET data of different brain regions. *Post hoc* comparisons were made with Tukey's test for all pairwise multiple comparisons. Differences were considered significant when $p < 0.05$.

RESULTS

In IUGR piglets, body weight was greatly reduced (42% of NW group, Table 1). Naturally occurring growth restriction in swine is asymmetrical with an increase in the mean ratio of brain weight to liver weight from 0.61 ± 0.16 to 1.42 ± 0.24 ($p < 0.01$). The reduction in brain weight was quite small (83% of NW group). In contrast, the decrease in liver weight (35% of NW group) was similar to that in body weight (42% of NW group). All differences in organ weight were significant ($p < 0.01$).

Table 2 summarizes physiologic values for newborn NW and IUGR piglets, which were consistent with other data obtained from slightly anesthetized and artificially ventilated newborn piglets (23, 24). The arterial blood pressure, heart rate, and arterial glucose content were mildly but significantly lower in IUGR piglets ($p < 0.05$). Other physiologic values such as $CMRO_2$, brain tissue P_{O_2} , and regional CBF were similar in NW and IUGR piglets (Table 2). However, the regional CBF distribution showed marked differences. In NW piglets, the mesencephalic and cerebellar blood flows were increased compared with the blood flows of frontal cortex and striatum. In addition, during baseline, striatal blood flow was distinctly higher than the blood flow of the frontal cortex ($p <$

Table 1. Organ weights of newborn piglets after normal growth or IUGR

	NW piglets	IUGR piglets
	<i>n</i> = 10	<i>n</i> = 10
Body weight (g)	2142 $\pm$ 373	908 $\pm$ 109**
Brain weight (g)	36 $\pm$ 2	30 $\pm$ 1**
Liver weight (g)	63 $\pm$ 15	22 $\pm$ 5**
Brain/liver ratio	0.61 $\pm$ 0.16	1.42 $\pm$ 0.24**

Values are presented as mean $\pm$ SD; ** $p < 0.01$, comparison between NW newborn piglets and IUGR ones.

Table 2. Physiologic values for newborn NW and IUGR piglets

	NW piglets	IUGR piglets
Mean arterial blood pressure (mm Hg)		
BL	75 ± 12	65 ± 6*
PET	79 ± 13	66 ± 12*
Heart rate (min ⁻¹)		
BL	232 ± 34	194 ± 36*
PET	234 ± 47	183 ± 40*
Arterial Pco ₂ (mm Hg)		
BL	39 ± 2	40 ± 3
PET	38 ± 1	39 ± 2
Arterial pH		
BL	7.45 ± 0.04	7.47 ± 0.06
PET	7.47 ± 0.03	7.48 ± 0.06
Arterial Po ₂ (mm Hg)		
BL	126 ± 23	125 ± 21
PET	130 ± 28	119 ± 30
Arterial O ₂ content (mL · dL ⁻¹)		
BL	7.9 ± 1.4	9.1 ± 1.8
PET	7.8 ± 1.5	8.2 ± 1.9
Arterial glucose content (mmol · L ⁻¹)		
BL	7.0 ± 0.9	5.1 ± 2.3*
PET	7.3 ± 1.1	5.3 ± 2.2*
Cerebral blood flow (mL · 100 g ⁻¹ · min ⁻¹)		
BL	59 ± 14	48 ± 13
PET	60 ± 14	50 ± 17
Cerebral metabolic rate of oxygen (mL · 100 g ⁻¹ · min ⁻¹)		
BL	2.2 ± 0.5	2.1 ± 0.5
PET	2.1 ± 0.5	1.9 ± 0.7
Brain tissue Po ₂ (mm Hg)		
BL	22 ± 5	24 ± 11
PET	19 ± 5	23 ± 10

Values are presented as mean ± SD; BL indicates baseline; PET, measurement at 1 h of PET scanning. * Indicates significant differences between both groups, $p < 0.05$.

0.05). In IUGR piglets, the mesencephalic and cerebellar blood flows were increased compared with the blood flow of frontal cortex ($p < 0.05$).

Figure 1 shows the time course of ¹⁸F activity accumulated

Figure 1. Measured ¹⁸F activity (mean ± SD) in the striatum of NW ($n = 10$, open squares) and IUGR piglets ($n = 10$, filled circles). The coincidence counts detected by PET were divided by the injected activity and corrected for differences in body weight. Note the higher amount of ¹⁸F activity accumulated in striatum of IUGR piglets (* $p < 0.05$, significant difference of ¹⁸F activity in striatum between NW and IUGR on end of acquisition).

in the striatum as measured by PET. The data are normalized to the injected activity and corrected for differences in body weight. There was a distinctly greater amount of ¹⁸F activity accumulated in the striatum of IUGR piglets ($p < 0.05$). The regional transport of FDOPA to the brain indicated by K_1^{FDOPA} and PS^{FDOPA} and the clearance rate of labeled metabolites from brain tissue ($k_{\text{cl}}^{\text{FDA+acids}}$) were similar in both groups (Table 3). However, the regional rate constant for back flux from the brain (k_2^{FDOPA}) was markedly increased in IUGR piglets in the striatum (72%) and frontal cortex (83%) ($p < 0.05$). Furthermore, the rate constant for FDA production (k_3^{FDOPA}) was markedly increased in all brain regions of IUGR piglets studied by between 48 (cerebellum) and 91% (mesencephalon) (Fig. 2, $p < 0.05$), indicating a distinct up-regulation of AADC activity. Cerebellar k_3^{FDOPA} was significantly lower compared with the mesencephalic and striatal k_3^{FDOPA} in the IUGR piglets.

DISCUSSION

The main new finding in this study is that IUGR induces a marked increase in dopamine production within the mesencephalon (91%) and in two major projection areas of the mesotelencephalic dopaminergic system, indicated by an increased AADC activity of 85% in the striatum and 93% in the frontal cortex ($p < 0.05$). These changes in AADC activity are not linked to brain oxygen delivery or brain tissue Po₂. This is noteworthy because the immature cerebral dopaminergic system is sensitive to altered brain oxidative metabolism. There is no oxygen reserve to protect dopamine release and metabolism from a decrease in oxygen pressure because in newborn piglet brain, a reduction of brain tissue Po₂ causes a significant increase in striatal extracellular dopamine content in a dose-dependent fashion (25). We have recently found that the synthesis rate of FDA from FDOPA is also increased under those circumstances (26).

The up-regulation of AADC activity found in this study may indicate an accelerated maturation of the dopaminergic system. The AADC is mainly localized in the presynaptic dopaminergic neurons. An increased expression of both the D₁ and D₂ dopamine receptors occurs during postnatal development (27, 28). The cause of AADC up-regulation in the dopaminergic system in IUGR cannot be determined from this study. However, evidence is available that an altered glucocorticoid metabolism in IUGR fetuses due to disturbed placental clearance of maternal glucocorticoids may influence brain dopaminergic activity in IUGR offspring. Because the concentration of circulating corticosteroid is several-fold higher in the sow than in the fetus (29), fetal protection against maternal corticosteroid intoxication is normally effected by an appropriate placental 11 β -hydroxysteroid dehydrogenase activity, which rapidly converts physiologic glucocorticoids to inactive products (30–32). This may be altered during IUGR pregnancies. Placental 11 β -hydroxysteroid dehydrogenase activity was markedly reduced in the late gestational period of maternal protein malnutrition sufficient to cause IUGR in rats (33). Recently, a significant association was found in pigs between fetal or placental size and placental 11 β -hydroxysteroid net dehydro-

Table 3. Transfer coefficients and PS product of FDOPA of different brain regions calculated from the measured tracer activities in arterial blood and brain of NW and IUGR piglets

	K_1^{FDOPA} ($\text{mL} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$)		k_2^{FDOPA} (min^{-1})	
	NW	IUGR	NW	IUGR
Mesencephalon	0.070 ± 0.020	0.068 ± 0.029	0.087 ± 0.045	0.100 ± 0.068
Cerebellum	0.086 ± 0.028	$0.076 \pm 0.029^{\dagger}\S$	0.096 ± 0.025	0.104 ± 0.075
Frontal cortex	$0.062 \pm 0.018^{\dagger}\S$	0.056 ± 0.018	$0.057 \pm 0.026^{\dagger}$	$0.104 \pm 0.058^*$
Striatum	0.072 ± 0.016	0.064 ± 0.022	0.072 ± 0.020	$0.124 \pm 0.076^*$
	$k_{\text{cl}}^{\text{FDA+acid}}$ (min^{-1})		PS^{FDOPA} ($\text{mL} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$)	
	NW	IUGR	NW	IUGR
Mesencephalon	0.009 ± 0.014	0.012 ± 0.007	0.074 ± 0.022	0.073 ± 0.033
Cerebellum	0.012 ± 0.006	0.014 ± 0.007	$0.093 \pm 0.031^{\dagger}\S$	0.081 ± 0.033
Frontal cortex	0.006 ± 0.014	0.013 ± 0.008	0.066 ± 0.020	$0.060 \pm 0.020^{\dagger}\S $
Striatum	0.011 ± 0.012	0.011 ± 0.007	0.079 ± 0.019	0.068 ± 0.025

Values are mean $\pm$ SD; $^{\dagger}\S||p < 0.05$; * indicates significant differences between NW and IUGR piglets; $^{\dagger}\S||$ indicate significant differences within the NW and the IUGR piglet group to mesencephalon † , cerebellum † , frontal cortex † , striatum $||$, respectively.

Figure 2. Marked increase of rate constants for FDA production (k_3^{FDOPA}) in different brain regions of IUGR piglets ($n = 10$) compared with those of NW piglets ($n = 10$). Values are mean $\pm$ SD.

$^{\dagger}p < 0.05$.

*Indicates significant differences between NW and IUGR piglets.

† Indicates differences within the IUGR piglet group to cerebellum.

genase activity (34). In addition, IUGR rats exhibited elevated liver and brain activities of specific glucocorticoid-inducible marker enzymes (35), suggesting an increased glucocorticoid action in brain and liver. Furthermore, Diaz *et al.* (36, 37) have shown that prenatal glucocorticoid administration induces a disturbed dopamine metabolism in juvenile rats with concomitant short-term and long-term neurobehavioral alterations. Indeed, ADHD is obviously associated with abnormalities in the dopaminergic system. This is suggested by the main symptoms characterizing this highly prevalent and disabling psychiatric disorder, *i.e.* hyperactivity, impulsivity, and impaired attention,

as well as the therapeutic efficacy of stimulants. Direct evidence for the major involvement of the dopamine network came from [^{18}F]FDOPA-PET studies on children and adults with ADHD. Ernst *et al.* (11) found recently an abnormally high accumulation of [^{18}F]FDOPA in the right midbrain, caudate, and putamen of children with ADHD, indicating a unilateral increase in AADC activity of these brain regions. These findings suggest that cerebral regions functionally dependent on midbrain dopamine input (striatum, prefrontal cortex, limbic structures) may be affected during their development. In contrast, adults with ADHD showed a reduced prefrontal

AADC activity, whereas dopaminergic midbrain structures exhibited normal findings (38). This is in line with a change in characteristic symptoms of adult ADHD, with less hyperactivity but unchanged impairment in attention, and could be the result of an adaptive response of down-regulation of AADC activity owing to long-term midbrain hyperactivity (39).

The physiologic importance of an increase of AADC activity is unclear considering that tyrosine hydroxylase is postulated to be rate limiting for catecholamine synthesis. It has been hypothesized that it may be related to the rate-limiting step in the synthesis of trace amines (40). For example, 2-phenylethylamine, a putative modulator of dopamine transmission (41), is synthesized from phenylalanine with AADC as the rate-limiting enzyme (42). Furthermore, because a substantial fraction of DOPA formed within the living brain is not used as a precursor for catecholamine synthesis but is exported from the brain, the activity of AADC *in vivo* determines the several pathways of DOPA. Considering this, it appears reasonable to assume that the rate of dopamine synthesis is modulated by AADC activity (43).

In a previous study, we have shown that AADC activity is up-regulated during moderate asphyxia despite an unchanged cerebral oxidative metabolism in newborn NW piglets (26). Because, under similar conditions, a strong increase of extracellular dopamine content occurred in the newborn brain (44), it can be assumed that IUGR piglets are at risk of developing an increased extracellular dopamine content. This is likely to be harmful because an increase in extracellular dopamine content is thought to play an important role in the pathogenesis of neuronal injury in newborn brain. Direct neurotoxic effects of dopamine on cell cultures have been shown (45). Another proposed mechanism for the neurotoxic effect of dopamine is through an increase in the production of free radicals. There are several pathways for free radical generation in the brain in which dopamine may contribute. The excess dopamine released during ischemia is oxidized by molecular oxygen during reperfusion, resulting in the formation of superoxide anion radicals, which may further oxidize dopamine and form covalent bonds with sulfhydryl-containing cellular components (46). Dopamine can react with hydroxyl radicals to form the dopaminergic neurotoxin 6-hydroxydopamine, which generates free radicals during its spontaneous rapid autooxidation (47). Furthermore, the enzymatic oxidation of dopamine by monoamine oxidase results in the formation of hydrogen peroxide, a hydroxyl radical precursor (48).

Although a marked up-regulation of AADC activity occurred in the mesencephalon and in two major projection areas of the mesotelencephalic dopaminergic system of IUGR piglets, regional transport of FDOPA to the brain indicated by K_1^{FDOPA} and PS^{FDOPA} and the clearance rate of dopamine metabolites ($k_{\text{cl}}^{\text{FDA+acids}}$) were unchanged. Therefore, these transport processes across the blood-brain barrier appear to be flow independent because the mesencephalic blood flow was significantly higher compared with that of forebrain structures ($p < 0.05$). We did not find differences in regional CBF or cerebral O_2 delivery between NW and IUGR piglets.

Apparently, a high nonspecific AADC activity indicated by cerebellar AADC activity is present in the developing newborn

brain that may not be related to dopaminergic neurons (49). The biologic significance of this enzyme activity remains unclear. It may reflect the synthesis of trace amines that serve as neurotransmitters. DOPA itself has also been suggested to be a neurotransmitter (50). The PET results with regard to DOPA metabolism outside the regions containing dopaminergic neurons were previously confirmed by studies of metabolite turnover in the newborn piglet brain (18). The up-regulation of AADC activity due to IUGR occurred preferentially in the mesencephalon and in two major projection areas of the mesotelencephalic dopaminergic system, *i.e.* striatum and frontal cortex, which are fully developed at birth, and this suggests a functional role for dopamine already in the early postnatal period (51). However, cerebellar AADC activity was also moderately increased in IUGR piglets, suggesting that nonspecific AADC activity seems to be sensitive to changes occurring with IUGR.

In summary, this study shows that IUGR is associated with a marked increase of AADC activity in the mesostriatal and telencephalic dopaminergic system of immature piglet brain. However, brain oxidative metabolism remains unchanged.

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