Changes in 5α-Pregnane Steroids and Neurosteroidogenic Enzyme Expression in Fetal Sheep with Umbilicoplacental Embolization

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ABSTRACT

Pregnane steroids have sedative and neuroprotective effects on the brain, due to interactions with the steroid-binding site of the $GABA_A$ receptor. In the adult brain, synthesis of the pregnane steroids is increased in response to stress. Therefore, we have used umbilicoplacental embolization to mimic chronic placental insufficiency during late gestation in sheep, to investigate the expression of the steroidogenic enzymes P450scc, 5α-reductase type I (5αRI), 5α-reductase type II (5αRII), and allopregnanolone (AP) content in the fetal brain. Umbilicoplacental embolization was induced from 114 d gestation (term ~147 d) by daily injection of inert microspheres into the umbilical artery and continued for 17–23 d. Fetal arterial oxygen saturation was reduced to ~60% of the preembolization value in each fetus, with a significant reduction in blood arterial Po_2, pH, and plasma glucose concentrations ($p < 0.05$) and a significant increase in blood arterial Pco_2 and plasma lactate concentrations ($p < 0.05$). At postmortem at 131–137 d gestation, embolized fetuses were growth-restricted (2.10 ± 0.14 kg, $n = 5$) compared with age-matched controls (4.43 ± 0.56 kg, $n = 7$, $p < 0.05$). Umbilicoplacental embolized fetuses showed increased P450scc expression in the primary motor cortex; 5αRI expression was not changed in any of the regions examined, whereas 5αRII expression was markedly increased in all brain regions. Brain AP content did not significantly change, whereas plasma concentrations were increased. These findings suggest that the increased expression of P450scc and 5αRII may be a response that maintains AP concentration in the fetal brain after compromised placental function and/or intrauterine stress. (*Pediatr Res* **54: 840–847, 2003**)

Abbreviations

AP, allopregnanolone, 5α-pregnan-3α-ol-20-one
P450scc, P450 side chain cleavage enzyme
5αRI, 5α-reductase type I enzyme
5αRII, 5α-reductase type II enzyme
PMC, primary motor cortex
GABA_A, γ-aminobutyric acid/benzodiazepine
5α-DHP, 5α-dihydroprogesterone
SaO_2, oxygen saturation of Hb in arterial blood
PaCO_2, arterial Pco_2
PaO_2, arterial Po_2
EMG, electromyogram

Chronic hypoxemia and placental insufficiency are associated with fetal growth retardation (1). Generally, the brains of these growth-retarded fetuses are either not smaller, or are reduced to a lesser degree compared with the overall size of the fetus, suggesting that the brain is able to adapt to the intrauterine conditions that compromise fetal growth. On the other hand, growth-retarded and premature infants are at a greater risk of perinatal brain damage (2), indicating that some of these adaptive changes leave the brain more vulnerable to cytotoxic damage arising from a range of insults, such as acute hypoxia, asphyxia, or infection-induced inflammation.

Pregnane steroids, including AP, modify the excitability of the CNS by interaction with the $GABA_A$ receptor at the steroid binding site. In the adult, AP has been shown to have potent anxiolytic (3, 4), anticonvulsant (5, 6), sedative/hypnotic, and anesthetic effects (7) on behavior. A constitutive role for neuroactive steroids in the developing brain was proposed after the observation that progesterone metabolites such as AP appear to maintain the low level of arousal-like behavior that typifies fetal life (8, 9). In the rat, stressful stimuli of handling or swim stress have been shown to increase plasma AP content (10, 11), suggesting that modulation of the $GABA_A$ receptor by AP may limit CNS excitation in such circumstances. The presence of elevated concentrations of AP in the fetal brain may also prevent excessive excitation after periods of hypoxia or asphyxia.

AP can be synthesized *de novo* from cholesterol in the brain, but a proportion may be derived from precursors in blood, including progesterone and pregnenolone that may enter the brain across the blood-brain barrier (12, 13). The cytochrome P450 side-chain cleavage enzyme (P450scc) catalyzes the conversion of cholesterol to pregnenolone on the inner surface of the mitochondrial membrane (14). The 5α-reductase enzyme exists as two isoforms—type I and II—which both catalyze the conversion of progesterone to the 5α-reduced derivative 5α-DHP. The enzyme 3α-hydroxysteroid dehydrogenase, which catalyzes the subsequent conversion of 5α-DHP to AP, is also strongly expressed throughout the brain (15).

Hypoxemia resulting from chronic placental insufficiency has been shown to increase adrenal gland growth and function (16) and may provide a major source of metabolites that are subsequently converted to pregnane steroids in the brain. The aim of this study was to determine the effects of induced chronic placental insufficiency on the expression of the neurosteroid synthesizing enzymes, P450scc, 5α-reductases I and II, and on the content of AP in various regions of the brain and adrenal glands.

MATERIALS AND METHODS

All procedures were conducted in accordance with the Code of Practice for the Care and Use of Animals for Scientific Purposes of the National Health and Medical Research Council and had received prior approval from the Monash University Standing Committee on Ethics in Animal Experimentation.

Animals. Surgery was performed on 12 pregnant Border Leicester/Merino crossbred ewes carrying a single fetus at 108–112 d gestation using aseptic techniques. General anesthesia was induced by i.v. injection of thiopentone sodium (20 mg/kg; Pentothal, Bomac Laboratories Ltd. Asquith, NSW, Australia) and maintained by inhalation of 1–2% halothane (Fluothane; ICI, Villawood, Australia) in O_2. Polyvinyl catheters (outer diameter, 1.50 mm; inner diameter, 1.00 mm) were implanted into a femoral artery and femoral vein, with the tip of the femoral artery catheter advanced to the level of umbilical artery. An EMG electrode (Cooner Wire Co., Chatsworth, CA, U.S.A.) was fixed onto the uterus to monitor uterine electrical activity. The fetus was returned to the uterus, and the catheters and electrodes were exteriorized through a maternal flank incision. The ewe was allowed to recover for 2–3 d before beginning the experimental procedures.

Determination of blood gases, pH, and glucose and lactate concentrations. Arterial blood gas parameters, Pa_{CO_2}, Pa_{O_2}, Sa_{O_2}, and pH were determined using an ABL520 blood gas analyzer (Radiometer, Copenhagen Denmark). Fetal blood gas measurements were corrected to the expected fetal temperature of 39°C. Blood glucose and lactate concentrations were measured by using a YSI 2300GL glucose and lactate analyzer (YSI Inc., Yellow Springs, OH, U.S.A.).

Umbilicoplacental embolization. At 114 d of gestation, fetal hypoxemia was induced by partial embolization of the umbilicoplacental circulation in five fetuses from singleton pregnancies as described previously (17). Briefly, unlabelled mucopolysaccharide microspheres (Sephadex G25, superfine, diameter 40–70 μM, Pharmacia, Uppsala, Sweden) suspended in sterile heparinized saline containing 0.02% Tween 80 were injected into the fetal femoral catheter to induce umbilicoplacental embolization. Repeated injections of microspheres (0.1–0.2 million) were given every 10–20 min until a fall in Sa_{O_2} occurred. After the initial fall in Sa_{O_2}, hourly injections of microspheres were given until Sa_{O_2} reached the desired level of 20–30% (approximately 60% reduction of normal levels). After the onset of embolization, no further microspheres were given if the Sa_{O_2} was <30%. This treatment was performed daily until postmortem. EMG activity was continuously monitored in all ewes as the number of EMG burst recorded for each 2 h interval as previously described (18). Ewes were killed when there was an indication of the approach of labor onset based on a rise in EMG burst frequency that reached twice the basal frequency recorded between 114 and 125 d of gestation. Thus, all ewes with embolized fetuses were killed between 131 and 137 d of gestation before there was a change to the contracture pattern of EMG activity that is associated with the onset of active labor. Seven additional fetuses from singleton pregnancies received a daily injection of saline, were killed at 132–134 d gestation, and were used to provide control samples.

Tissue collection. Ewes and fetuses were killed by injection of an overdose of sodium pentobarbitone (130 mg/kg i.v.) given to the ewe. The brains of the fetuses were immediately removed and divided into blocks corresponding to the following regions: cerebellum; hippocampus; medulla; midbrain; pons; hypothalamus/thalamus; and primary motor (PMC), frontal, parietal, occipital, and temporal cortex. Fetal adrenal glands were also collected. All tissue was frozen in liquid nitrogen and stored at −70°C.

$5\alpha RI$ antisera. A polyclonal antipeptide antibody against a synthetic peptide corresponding to amino acids 234–249 of the enzyme deduced from the cDNA sequence (19) was prepared in three New Zealand White rabbits following standard protocols (20). The peptide was synthesized as previously described (21) and with a 94% purity assessed by ion spray mass spectrometry. Rabbits were immunized and serum collected as previously described using standard protocols (20). Specificity of the antibody was assessed by immunoblotting and ELISA (22). The antisera raised against $5\alpha RI$ showed no cross-reactivity with $5\alpha RII$ and showed a band at 23 kD (the expected size of human $5\alpha RI$). Preimmune sera did not show any bands, and no bands were observed on immunoblots when antisera were preabsorbed with 15 μg of antigen.

Immunoblotting. Cytochrome P450scc, $5\alpha RII$, and $5\alpha RI$ expression in the brain and adrenal glands was determined by Western immunoblotting. Immunoblotting was performed as previously described (21). Briefly, frozen samples (~0.1 g) from each brain or adrenal glands were homogenized and then concentrated by ammonium sulfate precipitation. Protein content was determined in an aliquot according to the method of Bradford (23) using BSA as a standard. Fifteen micrograms protein was separated by SDS-PAGE on 15% separating gel and transferred onto 0.2 μM polyvinylidene difluoride membranes (Osmonics, Westborough, MA, U.S.A.) by electroblotting. Membranes were blocked for 1 h at 22°C in TBST (25

mM Tris, 14 mM sodium chloride, 0.2% (vol/vol) Tween-20, pH 7.4) containing 5% (wt/vol) skim milk powder and incubated with a 1:3000 dilution of either the P450scc, 5αRI, or 5αRII antibody in TBST, for 1 h at 22°C. Specificity of the P450scc and 5αRII antibodies have been previously reported (21). The membranes were washed with TBST, followed by 1:3000 dilution of a horse radish peroxidase–conjugated goat anti-rabbit IgG (DAKO, Glostrup, Denmark) for 30 min, followed by washes in TBST. The immune complexes were visualized by chemiluminescence using the Amersham ECL detection system (Amersham, Buckinghamshire, UK) for 1 min and captured using BioMax ML autoradiograph film (Eastman Kodak, Rochester, NY, U.S.A.). The autoradiographs were scanned and the images were imported into ImageQuaNT (Amersham). The intensities of the bands were quantified using ImageQuaNT and were individually corrected for background by subtracting the density of the blank background area immediately below each band.

Neurosteroid RIA. Neurosteroids were extracted from brain or adrenal tissue and plasma by a modification of the method of Barbaccia *et al.* (24) as previously described (21). Briefly, brain or adrenal gland tissue were homogenized three times with 50% methanol containing 1% acetic acid and the supernatants pooled. The supernatant was then applied to preprimed Sep-Pak C18 cartridges (Waters, Milford, MA, U.S.A.) and steroids were eluted with 100% methanol and the collected fractions dried under N_2, and the steroids resuspended in 1.0 mL assay buffer (0.1 M PBS, pH 7.0). The recovery of steroids was monitored by adding radiolabeled steroid (5000 c.p.m) to two homogenates run in parallel with each extraction run. The recovery for the progesterone extraction was 86.5% $\pm$ 4.5% (n = 3 extractions) and for the pregnenolone extraction was 57.8% $\pm$ 4.1% (n = 3 extractions), respectively, and corrections for losses during extraction were included in final calculations.

AP was measured by specific RIA, as previously described (21) using a polyclonal antibody purchased from Dr. R. H. Purdy (Department of Psychiatry, Veterans Administration Hospital, San Diego, CA, U.S.A.), which has been previously characterized (25). The limit of detection for AP was 0.19 $\pm$ 0.03 pmol/tube (n = 3) and the intra- and interassay coefficients of variance were 5% and 9%, respectively. Pregnenolone and progesterone were measured by specific RIA using antibodies purchased from ICN Biomedicals (Seven Hills, NSW, Australia) and provided by Dr. J. Malecki (Bairnsdale, Victoria, Australia), respectively, as previously described (26). The limit of detection for pregnenolone was 0.09 $\pm$ 0.02 pmol/tube (n = 3) and progesterone was 0.12 $\pm$ 0.02 pmol/tube (n = 3). The intra- and interassay coefficients of variance were 5% and 16%, respectively, for pregnenolone and 8% and 19%, respectively, for progesterone.

Cortisol RIA. Cortisol was extracted from plasma with dichloromethane and was measured by RIA as previously described (27). Intra- and interassay coefficients were 4% and 15%, respectively. The sensitivity of the assay was 0.33 pmol/ tube (n = 2).

Statistical analysis. Data are shown as mean $\pm$ SEM. All data were analyzed using statistical software (SPSS Version 9.0, SPSS Inc., Chicago, IL, U.S.A.). Significance of changes in brain neurosteroidogenic enzyme content was determined by t test. The Holm's step-down modification of Bonferroni's correction for multiple comparisons was used to avoid the potential for the introduction of type-I errors (28). Changes in brain neurosteroid content were examined using two-way ANOVA with treatment and brain region the factors in the analysis. Changes in fetal blood parameters, glucose and lactate, were examined using two-way ANOVA for repeated measurements. Differences between individual means were determined using by Fisher's least significant difference posthoc test. Adrenal steroid content and plasma steroid concentrations values were compared using t test. A p value <0.05 was considered to be statistically significant.

RESULTS

Fetal body and brain weights. The gestational age at postmortem examination was 132–134 d in control fetuses and 131–137 d in umbilicoplacental embolized fetuses. All fetuses were obtained before there was a change in uterine EMG activity from the gestational-associated contracture pattern and, thus, before the onset of active labor. Embolization resulted in a significant reduction in body weight (embolization, 2.10 $\pm$ 0.14 kg, n = 5, *versus* control, 4.43 $\pm$ 0.56 kg, n = 7; $p < 0.05$), but there was no change in brain weight (embolization, 37.59 $\pm$ 1.84 g, n = 5, *versus* control, 40.05 $\pm$ 2.35 g, n = 7).

Fetal blood gases analysis. Placental embolization for 17–23 d resulted in a significant reduction in fetal Sao_2, Pao_2, and pH (Table 1). Mean arterial glucose concentrations were significantly lower in the embolized fetuses whereas $Paco_2$ and lactate concentrations were significantly increased (Table 1). The total number of spheres delivered to the fetuses to maintain the desired level of hypoxemia ranged from 0.1 to 2 million per day with the total number of spheres administered to each fetus over the study period ranged from 1.25 to 6.45 million.

Relative expression of P450scc, 5αRII, and 5αRI in the different brain regions. The relative abundance of P450scc, 5αRII, and 5αRI expression in the different brain regions was

Table 1. *Arterial blood measurements in the control and umbilicoplacental embolized fetuses*

	pH	Pco_2 (mmHg)	Po_2 (mmHg)	HCO_3^- (mmol/L)	Sao_2 (%)	Lactate (mmol/L)	Glucose (mmol/L)
Control (n = 7)	7.35 $\pm$ 0.01	45.33 $\pm$ 1.17	21.42 $\pm$ 0.63	24.02 $\pm$ 0.79	64.88 $\pm$ 2.08	0.68 $\pm$ 0.07	0.74 $\pm$ 0.06
IUGR (n = 5)	7.31 $\pm$ 0.01*	53.49 $\pm$ 0.49*	11.12 $\pm$ 0.21*	25.57 $\pm$ 0.30	19.22 $\pm$ 0.76*	1.73 $\pm$ 0.26*	0.42 $\pm$ 0.05*

Values shown are $Paco_2$, Pao_2, Sao_2, pH, plasma glucose, and lactate concentrations at the time of tissue collection. Umbilicoplacental embolized fetuses were hypoxemic and hypoglycemic. Data presented as mean $\pm$ SEM. Asterisks indicate significant differences between control and embolized fetuses ($p < 0.05$).

assessed with two age-matched fetuses, a control and a umbilicoplacental embolized fetus at 134 d gestation (Fig. 1). As shown in Figure 1, all regions in either the control or embolized fetus were examined on individual blots. In both the umbilicoplacental embolized fetus and control fetus, the highest relative expression of P450scc and 5αRII occurred in the medulla, midbrain, and pons (Fig. 1, *lanes 3, 4, 5*). 5αRI was detected throughout the brain at relatively low levels, however, no differences between brain regions in the relative expression of 5αRI were apparent (data not shown).

Effect of placental embolization on the expression of P450scc, 5αRII, and 5αRI . Expression of P450scc, 5αRII, and 5αRI in the cerebellum, hippocampus, medulla, hypothalamus/thalamus, and PMC were compared directly between the control and umbilicoplacental embolized fetuses on individual immunoblots for each area as described previously (21), and densitometry was then used to compare between all of the control and embolized fetuses. P450scc expression was significantly increased in the PMC of the embolized fetuses compared with controls, with no differences in expression in the other regions examined ($p < 0.05$; Fig. 2A). There was no change in 5αRI expression in the embolized fetal brain, although there was a trend toward higher expression in the hypothalamus/thalamus (Fig. 2B). In contrast, 5αRII expression was significantly increased after embolization in all the brain regions examined ($p < 0.05$; Fig. 2C).

Brain and adrenal steroid content. Whereas both AP and progesterone content appeared lower in most region of the brain after embolization, there were no significant changes in AP content (Fig. 3A). Progesterone content was significantly reduced in the hypothalamus/thalamus of embolized fetuses, but not in the other regions examined (Fig. 3B). There were also no significant between-region differences in AP and progesterone content in either the control or embolized fetuses. There were no differences in pregnenolone content in any of the brain regions examined between control and embolized groups of fetuses, although content appeared more variable in

Figure 1. Expression of (*A*) P450scc and (*B*) 5αRII in brains of a control fetus at 134 d gestation and a umbilicoplacental embolized fetus at 134 d gestation. Lanes marks indicate the following regions; 1: cerebellum, 2: hippocampus, 3: medulla, 4: midbrain, 5: pons, 6: hypothalamus/thalamus, 7: PMC, 8: frontal cortex, 9: occipital cortex, 10: parietal cortex, and 11: temperol cortex. Protein (15 μg) was separated on 10% polyacrylamide gels, transferred to PVDF membranes, and incubated with either P450scc or 5αRII antisera. Expression of both P450scc and 5αRII was found to be highest in the brain stem (medulla and pons) and midbrain in both the control and umbilicoplacental embolized fetus.

the embolized group (Fig. 3C). Pregnenolone and progesterone content was significantly increased in the adrenal gland of the embolized fetuses (Fig. 4, *B* and *C*); the trend toward increased AP in these fetuses did not reach significance ($p = 0.069$).

Plasma steroid concentrations. Plasma AP and cortisol concentrations were significantly higher after embolization compared with control fetuses (Fig. 5, *A* and *B*). However, plasma pregnenolone and progesterone concentrations were not different in the two groups of fetuses (Fig. 5, *C* and *D*).

DISCUSSION

The principal finding of this study was that chronic embolization of part of the fetal side of the placenta resulted in elevated expression of P450scc in the primary motor cortex and 5αRII in all regions of the fetal brain examined. In this study, we used a previously established technique of umbilicoplacental embolization (1, 17) to induce changes in fetal blood parameters and fetal weight that was consistent with a severe and chronic reduction of placental function. We found that significant fetal hypoxemia, acidosis, and hypoglycemia was maintained during the embolization period, whereas plasma lactate concentrations were increased. Consistent with previous studies, this treatment reduced fetal body weight but not brain weight (29, 30). These findings show that this method mimics the changes in fetal growth associated with chronic placental insufficiency (1).

The present study examined the expression of two key rate-limiting steroidogenic enzymes, P450scc and 5αRII, in the steroidogenic pathway that leads to AP production in the brain. P450scc is responsible for the production of pregnenolone, the obligate precursor for neuroactive steroid production, and 5αRII catalyzes the synthesis of 5α-DHP, the immediate precursor for AP synthesis. The finding that both enzymes are strongly expressed in fetal brain in late gestation, with marked regional differences in the level of expression between regions, is consistent with our previous studies and supports the contention that AP may be synthesized *de novo* from cholesterol in the fetal brain (21, 31).

The finding that P450scc content in the brain was significantly increased in the PMC, showed a trend toward higher expression in the hippocampus, but was unchanged in the other regions examined suggests the mechanisms by which embolization affects the expression of this enzyme differs markedly between regions. The mechanisms that control the expression of the CYP11A1 gene, which encodes P450scc in the brain, are unclear. These mechanisms have been shown to be independent of SF-1 (steroidogenic factor 1), the transcription factor that regulates P450scc expression in the adrenal glands. These observations indicate that the region-specific control of P450scc expression in the brain differs from the adrenal glands, however, the nature of this regulation awaits further investigation.

The finding that 5αRII content was markedly increased in all regions of the brain after umbilicoplacental embolization suggests similar regulatory pathways may be activated throughout the brain. Although the mechanisms that control of 5αRII expression in the brain also remains uncertain. Analysis of the
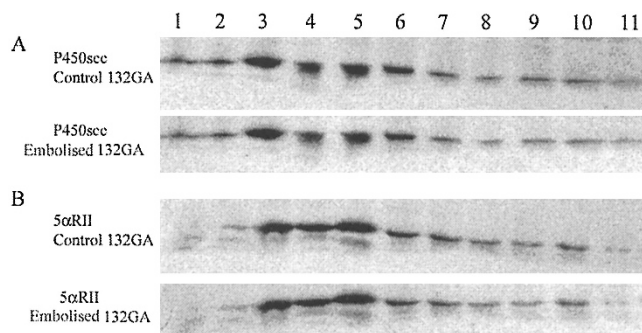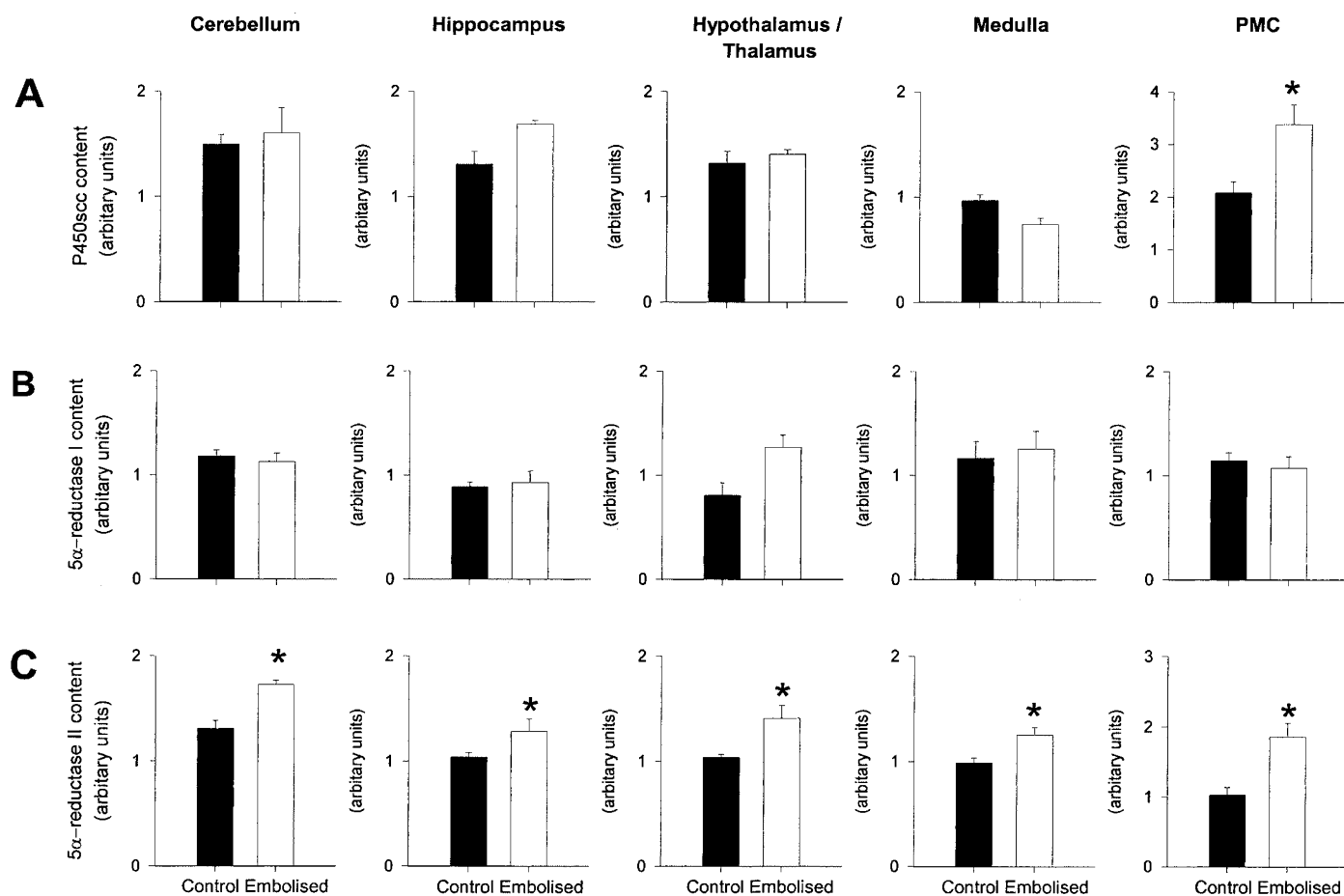
Figure 2. Densitometry values of (*A*) P450scc, (*B*) 5αRI, and (*C*) 5αRII expression in the cerebellum, hippocampus, hypothalamus/thalamus, medulla, and PMC of control fetuses at 132–134 d gestation (*n* = 7, *filled columns*) and umbilicoplacental embolized fetuses (*n* = 5, *open columns*) at 131–137 d gestation. Each bar represents the mean ± SEM of expression in arbitrary units. Asterisks indicate significant differences between control and embolized fetuses (*p* < 0.05).

5αRII gene in the mouse brain indicates the presence of a progesterone regulatory element (32). However, although this suggests that this steroid may have a direct role in the regulation of 5αRII enzyme expression, the results of the present study are not consistent with such a mechanism because 5αRII expression was increased throughout the brain after embolization, despite pregnenolone and progesterone concentrations either remaining unchanged or decreasing.

Previous studies using this model of placental insufficiency from mid-gestation to near term in sheep have shown a number of effects on the fetal brain, such as selective neuronal loss and cerebral infarction (29, 30, 33). Studies have found that a 12-h episode of placental underperfusion in the fetal sheep at mid-gestation results in the mild focal damage in the cortical white matter and a reduction in the number of cerebellar Purkinje neurons, the width of the molecular layer, and the area of the inner granule cell layer (33). Shorter periods of hypoxemia (8 h) result in predominately white matter injury with some adjacent cortical necrosis but no selective neuronal injury (34). In the growth-retarded guinea pig and rat, myelination was found to be both reduced and retarded by chronic hypoxemia (35, 36). Together, these findings indicate that a severe and chronic reduction in placental function results in a major alteration in the composition of cell types and myelination in

many areas of the brain. These changes may result in a compensatory mechanism(s) that could account for the differences in steroidogenic enzyme expression after embolization and neurosteroid content in particular regions of the brain.

Despite the increase in 5αRII expression observed in all regions of the brain examined, AP concentrations did not change significantly and, if anything, tended to be lower after embolization. This may be the result of the lower progesterone concentrations after placental embolization and a consequently reduced progesterone precursor availability for AP synthesis. Our previous studies suggest that high brain AP concentrations during gestation are dependent on a supply of placental precursors, possibly progesterone, inasmuch as AP concentrations decline sharply after removal of the placenta at birth (21). In addition, a reduction in placental function may decrease the concentrations of other possible precursors for AP production such as the progesterone metabolite 3α-hydroxyprogesterone that is present in the fetal circulation (37). Importantly, the observed increase in enzyme content that was observed in the present study may not necessarily translate to active enzyme *in vivo*. Therefore, it is possible that other unknown factor(s) resulting from embolization may reduce enzyme activity and suppress AP production. The observed increase in 5αRII expression may serve to maintain AP production to preserve
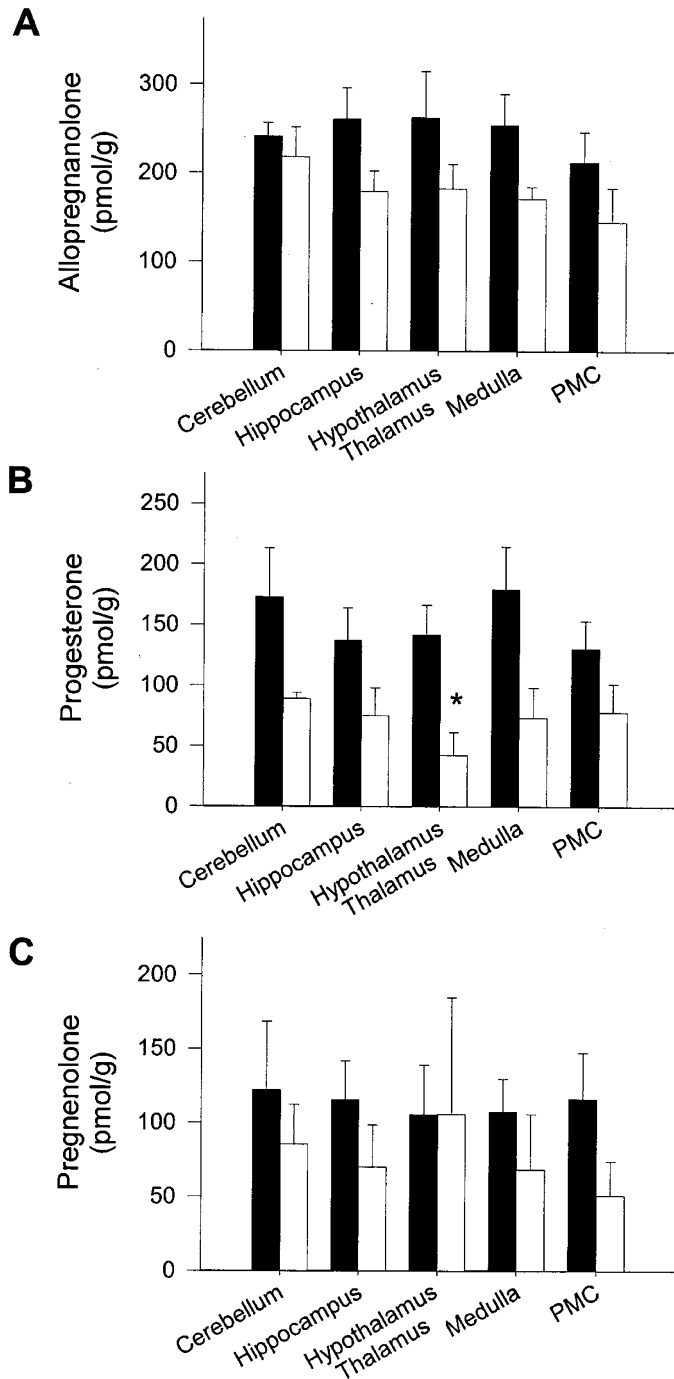
Figure 3. Content of (*A*) AP, (*B*) progesterone, and (*C*) pregnenolone in the cerebellum, hippocampus, hypothalamus/thalamus, medulla, and PMC of control fetuses at 132–134 d gestation (*n* = 7, *filled columns*) and umbilicoplacental embolized fetuses (*n* = 5, *open columns*) at 131–137 d gestation. Each bar represents the mean ± SEM. Asterisks indicate significant difference between control and embolized fetuses (*p* < 0.05).

Figure 4. Adrenal gland content of (*A*) AP, (*B*) progesterone, and (*C*) pregnenolone of control fetuses at 132–134 d gestation (*n* = 7, *black columns*) and umbilicoplacental embolized fetuses (*n* = 5, *open columns*) at 131–137 d gestation. Each bar represents the mean ± SEM. Asterisks indicate significant differences between control and embolized fetuses (*p* < 0.05). Progesterone and pregnenolone content of the adrenal gland were both significantly increased (*p* < 0.05) with umbilicoplacental embolization.

normal gestational AP concentrations during periods when adverse condition reduces the activity of the enzymes.

In contrast to brain content, plasma AP concentrations were markedly increased in the embolized fetuses. Interestingly, these findings indicate that plasma concentrations do not directly relate to brain content after the stress of embolization. This is consistent with our previous findings in sheep (38) and
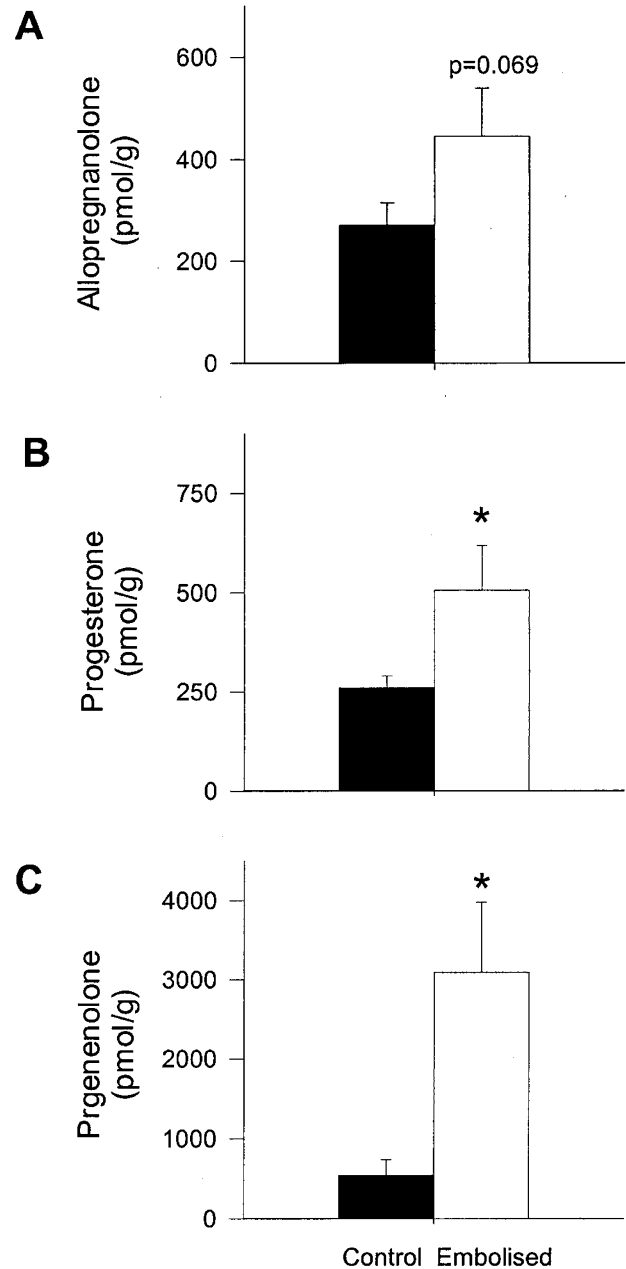
of others in rats (11) that brain neurosteroid concentration is independent of, and is markedly higher than, plasma concentrations. The observation that adrenal progesterone and pregnenolone content were increased and AP content tended to be higher in the embolized fetuses suggests the rise in plasma levels may result from increased adrenal production. However, the present findings suggest that adrenal activation caused by a stress does not lead to increased AP concentrations in the brain.

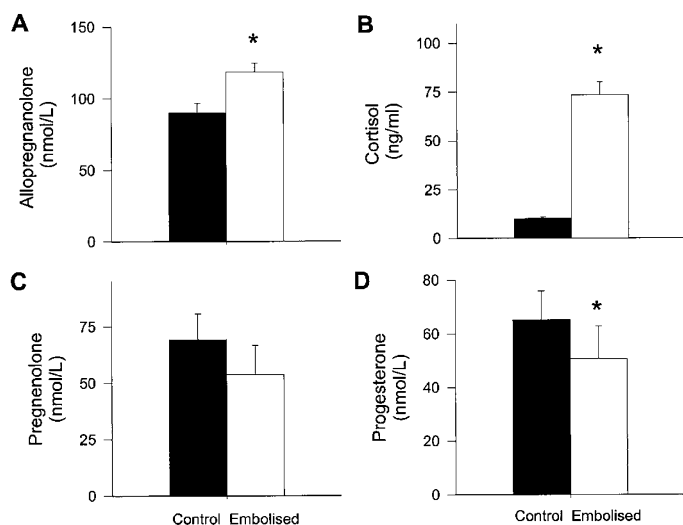Neurosteroids, including AP, have been shown to have neuroprotective functions (39), by modulation of the GABA$_A$

Figure 5. Plasma concentrations of (*A*) AP, (*B*) cortisol, (*C*) pregnenolone, and (*D*) progesterone of control fetuses at 132–134 d gestation (*n* = 7, *filled columns*) and umbilicoplacental embolized fetuses (*n* = 5, *open columns*) at 131–137 d gestation. Each bar represents the mean ± SEM. Asterisks indicate significant differences between control and embolized fetuses (*p* < 0.05). Plasma progesterone and pregnenolone concentrations did not change, whereas cortisol and AP concentrations were significantly increased after umbilicoplacental embolization (*p* < 0.05).

receptor, particularly in the hippocampus. AP exerts a potent neuroprotective effect in cell viability studies with rat hippocampal slices and in primary hippocampal cultures as a result of the attenuation of glutamate-induced increases of intracellular calcium (40). $GABA_A$ receptor density has been shown to increase in the fetal brain between 85 and 128 d gestation such that adult concentrations are attained by 128 d. These receptors are highly sensitive to AP, with concentrations of 50–150 nmol/L causing marked stimulation (41). Additionally, increases in progesterone and AP during pregnancy or after transient stress have been shown to decrease the expression of the $GABA_A$ receptor $\alpha4$ subunit, resulting in a further enhancement of $GABA_A$ receptor steroid sensitivity (42–44) and the possible potentiation of AP action. Therefore, continuous stimulation of the $GABA_A$ receptor in the brain by AP and the possible down-regulation of the $GABA_A$ $\alpha4$ subunit may suppress fetal CNS activity in pregnancy, particularly during adverse conditions caused by a chronic reduction of placental function. The observed increase in both P450scc and 5αRII content may be a compensatory response that enables AP levels to be maintained despite a fall in neurosteroid precursor availability. This would preserve GABAergic inhibition that may be an intrinsic protective mechanism that counteracts the increased risk of hypoxia-induced excitotoxic injury.

Chronic fetal hypoxemia and altered endocrine status associated with intrauterine stresses and placental insufficiency (1) lead to the increased risk of permanent neurologic disability in the neonate (2). *In utero* insults have been shown to alter brain structure and composition, with changes in glial and neuron numbers (33–36). The present findings have shown that there is increased expression of 5αRII in all areas of the brain and P450scc in some areas, without a marked change in brain AP

concentration. We speculate that these changes to the fetal brain may be an adaptation or a compensatory response, brought about through as yet unknown mechanisms, that act to maintain the AP concentrations and the level of GABAergic inhibitory activity, and thus to protect the fetal brain from excitotoxic injury.

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