

Circulating Adrenomedullin Is Increased in Preterm Newborns Developing Intraventricular Hemorrhage

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ABSTRACT

Adrenomedullin is a novel vasoactive peptide that participates in cerebral blood flow regulation and circulates in human plasma. To verify whether plasma adrenomedullin is able to identify preterm newborns at risk of intraventricular hemorrhage (IVH), we performed a case-control study. Plasma samples collected within 6 h after birth in 24 preterm newborns who developed IVH, as diagnosed at 72 h, were assessed for adrenomedullin and compared with those obtained from 48 preterm newborns, matched for gestational age, who did not develop IVH. Cerebral ultrasound and Doppler velocimetry waveform patterns in the middle cerebral artery were also recorded at the time of blood sampling. Adrenomedullin blood concentrations and middle cerebral artery pulsatility index values were significantly higher in infants developing IVH (20.1 ± 4.5 fmol/mL and 1.71 ± 0.21 fmol/mL, respectively) than in controls (7.5 ± 3.0 fmol/mL and 1.49 ± 0.19 fmol/mL, respectively). Adrenomedullin blood concentrations correlated with middle cere-

bral artery pulsatility index ($r = -0.77, p < 0.01$) and with the grade of IVH extension ($r = 0.83, p < 0.01$). This study suggests that adrenomedullin blood concentration might be a promising tool for identifying preterm infants at risk of IVH immediately after birth, when imaging assessment and clinical symptoms of hemorrhage are still silent. (*Pediatr Res* 50: 544–547, 2001)

Abbreviations:

IVH, intraventricular hemorrhage
CGRP, calcitonin gene-related peptide
FHR, fetal heart rate
MCA PI, middle cerebral artery pulsatility index
NICU, neonatal intensive care unit
RDS, respiratory distress syndrome
PvO₂, venous oxygen partial pressure
PvCO₂, venous carbon dioxide partial pressure

IVH is the most common variety of cerebral hemorrhage, affecting about 15 to 20% of preterm infants, which continues to represent one of the major problems in the NICU (1). Perinatal asphyxia constitutes a risk in preterm infants by altering cerebral blood flow regulatory mechanisms (e.g.; hemodynamic, neurogenic, and metabolic mechanisms) and causing loss of cerebral vascular autoregulation. Hypotension, cerebral ischemia, and reperfusion are the main events involved in vascular autoregulation (2–4). Severe and prolonged hypoxia or ischemia result in cell death and tissue damage. Reperfusion could be critical because organ damage, particularly of the brain, may be amplified during this period. An exaggerated release of vasoactive agents, acting in autocrine-paracrine manner, could be responsible for reperfusion injury (5), which, in turn, leads to IVH.

Adrenomedullin is a 52-amino acid, newly discovered vasoactive peptide first isolated from pheochromocytoma, that shares some homology with CGRP (6). Cultured vascular endothelial cells and smooth muscle cells produce adrenomedullin (7), which elicits its vasodilator activity through an increase in intracellular cAMP (8). Adrenomedullin has been identified in a variety of tissues, including the CNS, and adrenomedullin-like immunoreactivity has been also described in human cerebrospinal fluid (9). More recently, it has been reported that adrenomedullin also exerts vasodilator activity in the cerebral circulation (10, 11) and that this peptide is involved in the regulation of systemic and local blood flow during neonatal cardiovascular adaptation (12, 13) and during birth stress (14). In human fetuses, there is evidence that adrenomedullin participates in cerebral fetal hemodynamic modifications (15).

We hypothesized that adrenomedullin plasma levels would increase as a response to ischemic insult and overshoot cerebral blood flow during the reperfusion period. Therefore, the

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present study was aimed to investigate whether plasma adrenomedullin concentrations sampled a few hours after birth may be used to identify preterm newborns with high risk of IVH.

MATERIALS AND METHODS

Population. We performed a case-control study on 24 preterm newborns (27–35 wk of gestation) with IVH. Diagnosis of IVH was performed at 72 h from birth by ultrasound scanning (16); 8 infants were diagnosed to have a grade I IVH, 11 grade II, and 5 grade III. At admission to the NICU, all newborns are routinely sampled for the assessment of clinical parameters (red blood cell count, venous blood pH, ion concentrations, plasma glucose level, arterial blood pressure) and undergo cerebral ultrasound and Doppler examination. After IVH was diagnosed, clinical and laboratory parameters and Doppler velocimetry values, recorded within 6 h after birth, were re-evaluated and compared with those obtained from 48 preterm infants, matched for gestational age, who were admitted consecutively to each IVH case to the NICU and who did not develop IVH (two control infants for each IVH infant). Exclusion criteria were multiple pregnancies, maternal hypertension, diabetes and infections, clinical evidence of chorioamnionitis, premature rupture of membranes (>24 h), fetal malformations, and chromosomal abnormalities.

Clinical characteristics at birth are shown in Table 1. Emergency cesarean section was performed in 11 out of 24 cases in the IVH group and in 24 of 48 cases in the control group; indications included placental abruptio and nonreassuring fetal status as defined by the American College of Obstetricians and Gynecologists (bradycardia, fetal heart rate late decelerations, severe and repetitive fetal heart rate variable decelerations, reduced beat-to-beat variability) (17). Corticosteroid therapy was administered (betamethasone 12 mg/24 h for 2 d i.m., Bentelan, GlaxoWellcome, Verona, Italy) in 14 out of 24 cases in the IVH group and in 29 of 48 cases in the control group. Fourteen out of 32 women who delivered vaginally received tocolytic therapy (ritodrine 30–50 mg/h i.v., Miolene, Luso-farmaco, Milan, Italy). In 28 infants (10 in the IVH group and 18 among the controls) the birth weight was <10th percentile for gestational age. None of the newborns were delivered by forceps.

The study protocol was approved by the Ethics Committee of the “Giannina Gaslini” Children’s Hospital, University of Genoa, and the parents of the subjects examined gave informed consent.

Table 1. Clinical characteristics of study groups at birth

	IVH (n = 24)	Controls (n = 48)
Gestational age at birth (wk)	32.7 ± 2.0	32.5 ± 2.4
Birth wt (g)	1641 ± 395	1671 ± 356
Birth wt < 10th percentile (n)	10 (41%)	18 (37%)
Cesarean section (n)	11 (46%)	24 (50%)
Apgar score, 1 min	7.0 ± 1.6	6.6 ± 2.0
Apgar score, 5 min	8.2 ± 0.6	8.3 ± 0.7

Values are expressed as mean ± SD. There were no statistical differences between the two groups.

Cerebral ultrasound and Doppler velocimetry. Standard cerebral ultrasound was performed by a real-time ultrasound machine and a pulsed Doppler apparatus (Acuson 128SP5, Acuson, Mountain View, CA, U.S.A.) was used for blood flow velocity measurements of the pulsatility index (PI) in the middle cerebral artery (MCA), performed at the same time of blood sampling.

Adrenomedullin measurement. Plasma adrenomedullin concentration was measured after extraction and purification, as reported (18), in 500 µL of samples collected at admission to the NICU for clinical evaluation, and stored at –20°C. A specific RIA kit (Phoenix Pharmaceuticals, Mountain View CA, U.S.A.) with rabbit polyclonal antibody raised against human adrenomedullin 1–52 was used. Each measurement was performed in duplicate and the averages were reported. The sensitivity of the assay was 0.34 fmol/mL. The antibody cross-reacts 100% with human adrenomedullin and no cross-reactivity was reported with rat adrenomedullin, amylin, CGRP, endothelin-1, α-atrial natriuretic peptide, or brain natriuretic peptide. The intra- and interassay coefficients of variance were 5.1% and 12.0%, respectively.

Statistical analysis. Data are expressed as mean ± SD. Adrenomedullin blood concentrations and neonatal monitoring parameters between the groups were analyzed by means of Mann-Whitney U two-sided test. Comparison between proportions was performed with Fisher’s exact test; correlation between adrenomedullin levels and MCA PI was analyzed by linear regression analysis. Multiple linear regression analysis was performed with adrenomedullin as the dependent variable to analyze the influence of various clinical parameters (occurrence of IVH, MCA PI, mode of delivery, indication for cesarean section, gestational age, birth weight, Apgar score, incidence of respiratory distress syndrome [RDS], corticosteroids and tocolytic therapy) on adrenomedullin values. A value of $p < 0.05$ was considered as significant.

RESULTS

At blood sampling performed within 6 h after birth, no significant differences were observed in laboratory parameters between the two study groups (Table 2). Although no cerebral ultrasound features suggestive of IVH were observed during the first 48 h of age, MCA PI recorded at the time of blood sampling was significantly higher ($p < 0.01$) in infants developing IVH. In this group, mean adrenomedullin concentration was significantly higher ($p < 0.01$) than in controls, being 20.1 ± 4.5 fmol/mL and 7.5 ± 3.0 fmol/mL, respectively (Fig. 1). As shown in Figure 2, there was a negative correlation ($r = -0.75$; $p < 0.01$) between adrenomedullin concentrations and MCA PI values in IVH group, whereas no correlation was found in the control group ($r = 0.20$). In IVH group, higher adrenomedullin concentrations were found in infants who developed a more severe IVH (grades II and III) when compared with IVH grade I (23.2 ± 2.3 fmol/mL versus 14.9 ± 0.9 fmol/mL; $p < 0.05$). There was a significant correlation between adrenomedullin levels and degree of brain lesion ($r = 0.83$; $p < 0.01$), whereas no correlation was present between MCA PI assessed within 6 h after birth and severity of IVH (r

Table 2. Neonatal monitoring parameters at blood sampling performed within 6 hr from birth in infants developing IVH and controls

	IVH (n = 24)	Controls (n = 48)
RBC (10 ⁶ /L)	4.2 ± 0.2	4.2 ± 0.3
Hb (g/L)	13.7 ± 3	13.9 ± 2
Ht (%)	41.4 ± 2.4	41.7 ± 3.2
pH	7.30 ± 0.4	7.32 ± 0.1
PvCO ₂ (kPa)	5.84 ± 1.5	5.83 ± 1.2
PvO ₂ (kPa)	4.16 ± 0.9	4.19 ± 1.1
BE (mmol/L)	-2.1 ± 1.9	-1.7 ± 2
Na ⁺ (mmol/L)	138 ± 5	140 ± 3
K ⁺ (mmol/L)	4.1 ± 0.3	4.1 ± 0.2
Ca ⁺⁺ (mmol/L)	1.14 ± 0.08	1.15 ± 0.1
Plasma glucose (mmol/L)	4.1 ± 1.1	4.3 ± 1.1
Systolic arterial pressure (kPa)	44.2 ± 13.1	45.3 ± 12.3
Diastolic arterial pressure (kPa)	24.7 ± 11.1	25.3 ± 10.2
MCA PI	1.71 ± 0.21*	1.49 ± 0.19

Values are expressed as mean ± SD. RBC, red blood cells; BE, base excess; PvO₂, venous oxygen partial pressure; PvCO₂, venous carbon dioxide partial pressure.

* $p < 0.01$.

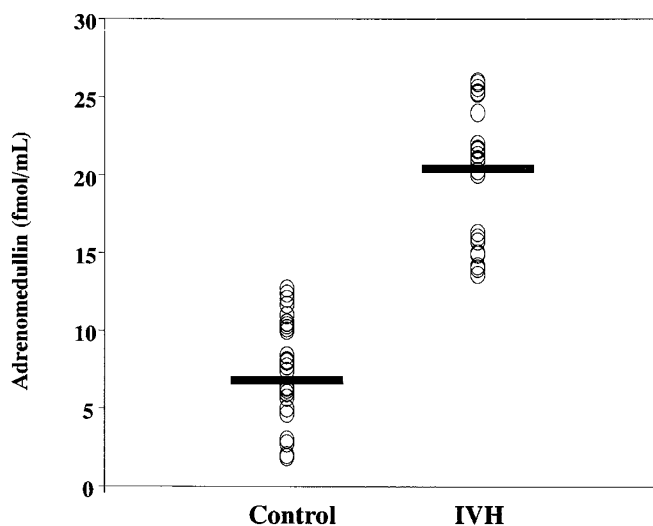
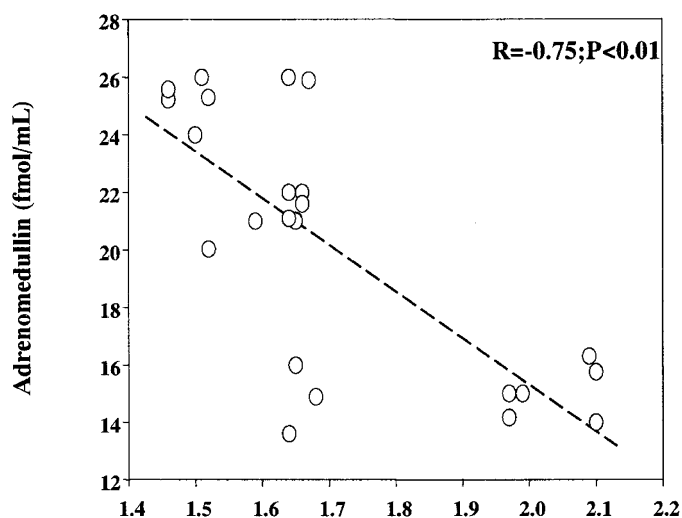


Figure 1. Individual values of adrenomedullin concentrations in plasma of newborns who did not develop IVH (controls) and in those that developed IVH (IVH). Horizontal bars represent the mean values. Values for IVH were higher than those for controls ($p < 0.01$, Mann-Whitney U two-sided test).

= 0.29; $p > 0.05$). The incidence of RDS was not significantly different in the two groups (IVH group: $n = 12$; controls: $n = 10$) and was not significantly associated with adrenomedullin levels (odds ratio = 0.38). All the other clinical and laboratory parameters were not correlated with adrenomedullin concentrations and multiple linear regression analysis confirmed a significant correlation between adrenomedullin and MCA PI ($p < 0.001$) and showed that the occurrence of IVH represented an independent variable associated with adrenomedullin values ($p < 0.001$) (Table 3).

DISCUSSION

The present study shows that, within a few hours after birth, circulating adrenomedullin was increased in infants who later



MCA PI

Figure 2. Correlation between plasma adrenomedullin concentrations and MCA PI in the 24 infants developing IVH. Regression line is shown.

Table 3. Relationship of various independent variables with adrenomedullin levels analyzed by multiple linear regression

Variable	Regression coefficient	Standard Error	p
Constant	11.180	11.10	0.318
Gestational age	-0.079	0.210	0.701
Birth wt	0.003	0.001	0.200
Cesarean section	-0.566	0.846	0.513
Apgar score, 1 min	-0.089	0.249	0.722
Apgar score, 5 min	1.029	0.759	0.180
Corticosteroid therapy	-1.226	0.894	0.176
Respiratory distress syndrome	-0.357	0.938	0.705
Tocolytic therapy	-1.590	1.067	0.141
MCA PI	-7.889	2.152	<0.001
IVH	13.73	0.966	<0.001

developed IVH, suggesting that adrenomedullin might play a role in the modifications of cerebral circulation.

Human and experimental studies on cerebral blood flow have demonstrated the importance of the cerebrovascular autoregulation, which is under control of metabolic, myogenic, and neurologic mechanisms. The main events causing loss of vascular autoregulation, which can lead to IVH, are commonly identified in hypotension, cerebral ischemia, and reperfusion hyperemia (3-5). *In vitro* and *in vivo* studies have shown that adrenomedullin is secreted by endothelial cells (7) and participates in the regulation of cerebral blood flow (10, 11); adrenomedullin infusion in dogs increases cerebral blood flow in a dose-dependent manner without affecting systemic blood pressure and other cardiovascular parameters (19). Recently, we have shown that adrenomedullin contributes to the hemodynamic modifications that determine the occurrence of the brain-sparing effect in growth retarded fetuses, confirming an important role of this peptide in the regulation of fetal cerebral blood flow in humans (15). In the present study, higher levels of adrenomedullin were found in infants with increased resis-

tance to cerebral blood flow as detected by Doppler ultrasound. Increased plasma levels of adrenomedullin could be the result of an enhanced release of the peptide by vascular endothelial cells in an attempt to compensate reduced blood flow. Because it has been shown that reperfusion injury resulting from reactive excessive vasodilatation is a primary event in the occurrence of IVH, adrenomedullin released in response to vasoconstriction might have a role in the loss of cerebral vascular autoregulation that leads to brain injury. In this light, it has been demonstrated that adrenomedullin exacerbates ischemic brain damage in rats (20).

On the other hand, it is also possible that the same pathologic events leading to IVH are also responsible for stimulation of adrenomedullin release. Cerebral blood flow autoregulation in preterm infants is very sensitive to perinatal hypoxic-ischemic insult (5), which represents the major cause of IVH. It has been demonstrated that hypoxia stimulates adrenomedullin mRNA expression and peptide production in different type of cells (21, 22) and circulating adrenomedullin is increased in human subjects maintained in hypoxic condition (23). Moreover, in preterm infants it has been demonstrated that ischemia and reperfusion induce an inflammatory response with enhanced cytokines expression (5), which are known to stimulate adrenomedullin release (24). Thus, elevated adrenomedullin concentrations found in infants developing IVH may derive from direct stimulation of peptide release from endothelial or neuronal cells induced by hypoxia or inflammatory mediators. Last, we cannot exclude that other factors may independently affect adrenomedullin levels and occurrence of IVH, however, multivariate analysis failed to show any significant correlation between adrenomedullin and other clinical variables, such as mode of delivery, gestational age, birth weight, indication for cesarean section, incidence of RDS, or maternal therapy.

In conclusion, adrenomedullin plasma levels in preterm infants soon after birth might be a promising tool for the early detection of infants at risk for IVH. The mechanism that gives rise to this increase and its relevance to the pathophysiology of IVH remain to be established.

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