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Adrenomedullin increases in term asphyxiated newborns developing intraventricular hemorrhage

Romolo Di Iorio[a,*], Emanuela Marinoni[a], Mario Lituania[b], Giovanni Serra[b] Claudio Letizia[c], Ermelando V. Cosmi[a], Diego Gazzolo[b]

[a]*Laboratory of Perinatal Medicine and Molecular Biology, Institute of Gynecology, Perinatology and Child Health, University "La Sapienza," Rome, Italy*
[b]*Department of Neonatology, G. Gaslini Institute, Genoa, Italy*
[c]*Department of Clinical Sciences, University "La Sapienza," Rome, Italy*

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Abstract

Objectives: Adrenomedullin (AM) is a newly discovered vasodilator peptide that participates in the regulation of cerebral blood flow. The aim of this study was to investigate whether circulating AM was increased in infants with prenatal asphyxia who developed intraventricular hemorrhage (IVH).

Design and methods: A case-control study was performed on 40 full-term asphyxiated newborns: 20 developed IVH (group A) and 20 did not (group B). Forty term healthy newborns represented the control group. Biochemical laboratory parameters, neurological patterns, cerebral ultrasound scanning, and Doppler velocimetry were assessed at 12 and 72 h from birth. Plasma AM concentration was measured at 12 h from birth by means of a specific RIA.

Results: AM levels were significantly higher in group A (20.2 ± 5.2 fmol/ml) than in group B (8.4 ± 2.1 fmol/ml) or controls (9.3 ± 2.6 fmol/ml). In asphyxiated newborns, AM concentration was correlated with middle cerebral artery PI value only in group B.

Conclusions: Increased concentration of AM at 12 h from birth in asphyxiated newborns who later developed IVH suggests that this peptide may participate in the loss of cerebral vascular autoregulation in response to hypoxia and could be useful to discriminate, among newborns at risk, those with an adverse neurological outcome.

Keywords: Cerebral bleeding; Adrenomedullin; Intrapartum asphyxia; Cerebral circulation; Brain injury

Introduction

Major neurological deficits can complicate up to 20% of asphyxiated newborns [1]. Human and experimental studies on cerebral blood flow modifications have pointed out the importance of cerebrovascular autoregulation, which is under control of metabolic, myogenic, and neurological mechanisms, during the post-asphyxial phase. The main events causing brain injury are commonly identified in hypotension, cerebral ischemia, and reperfusion hyperemia with loss of vascular autoregulation [2].

Adrenomedullin (AM) is a 52-aminoacid peptide, first isolated from pheochromocytoma, that shares some homology with the calcitonin gene-related peptide (CGRP) [3]. It has been identified in a variety of tissues, including the central nervous system [4], and AM-like immunoreactivity has been described in human cerebrospinal fluid [5]. Cultured vascular endothelial cells and smooth muscle cells secrete AM [6], which elicits potent hypotension and vasodilatation in the vascular system through an increase in intracellular cAMP [7]. AM, as well as CGRP, exerts its vasodilator effect also in the cerebral circulation as

* Corresponding author. Laboratory of Perinatal Medicine and Molecular Biology, Institute of Gynecology, Perinatology and Child Health, University "La Sapienza," Viale Regina Elena, 324, I-00161 Rome, Italy. Fax: +39 06 5756635.

E-mail address: romolodi@hotmail.com (R. Di Iorio).

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demonstrated in vitro and in vivo [8,9], and there are evidences that this peptide is involved in the regulation of systemic and local blood flow during neonatal cardiovascular adaptation [10,11] and in response to birth stress [12].

The aim of this study was to investigate whether AM participates in the occurrence of intraventricular hemorrhage (IVH) in term-asphyxiated infants and whether its concentration in plasma may help to discriminate those infants who will develop cerebral hemorrhage.

Materials and methods

Population

The study was carried out on full term neonates with a gestational age between 38 and 42 weeks who were admitted to the Neonatal Intensive Care Unit (NICU) at the Giannina Gaslini Institute of Genoa with a diagnosis of perinatal asphyxia in a 48-month period. This NICU serves as the referral unit for the regional hospitals. The number of deliveries that refers to this center is about 15,000 per year. Neonates were eligible for study entry if they were admitted at less than 12 h of age.

All asphyxiated newborns were delivered by emergency cesarean section due to acute fetal distress defined as non-reassuring fetal status according to 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) [13]. Asphyxia was defined according to the American College of Obstetricians and Gynecologists criteria: an Apgar score <3 at the 5th min, pH <7.0, BE ≤10 in cord blood or venous blood taken from newborns within 60 min from birth, or needing for positive pressure ventilation (>3 min). Neonates were eligible for study entry if they fulfilled three out of the above four criteria. Infants with any malformation, systemic infections, intrauterine growth retardation, and cardiac and hemolytic diseases were excluded from the study. Other exclusion criteria were multiple pregnancies, maternal hypertension, and diabetes.

During the study period we enrolled 20 asphyxiated term newborns who developed IVH (group A). The incidence of IVH in term infants in our study was about 0.4% per year. This group was matched with 20 asphyxiated term infants without IVH who were admitted consecutively to each case (asphyxiated controls; group B) and 40 healthy term newborns delivered by elective cesarean section (healthy controls).

In all newborns, clinical and laboratory parameters were recorded at birth and at 12 h from birth. Neonates in the asphyxiated group were mechanically ventilated and sedated using Fentanyl (Fentanest, Pharmacia and UpJohn, Milan, Italy) 0.5–2.5 μ/kg/h and Midazolam (Ipnovel, Roche, Milan, Italy) 50–400 μ/kg/h. In asphyxiated newborns, cerebral ultrasound scanning and cerebral Doppler velocimetry waveform patterns were recorded and neurological examination assessed at 12 and 72 h after birth. The diagnosis of IVH was performed at 72 h from birth on the bases of cerebral ultrasound patterns showing subependymal hemorrhage (n = 10), intraventricular hemorrhage alone (n = 8), and intraventricular hemorrhage with ventricular dilatation (n = 2). The two infants with intraventricular hemorrhage and ventricular dilatation died at 74 and 96 h from birth for cardiopulmonary failure.

The study protocol was approved by the Ethics Committee of the Giannina Gaslini Children's Hospital, Genoa University School of Medicine. Parents of the subjects gave informed consent and all procedures were performed according to the hospital guidelines.

Cerebral monitoring

Cerebral standard ultrasound was performed by a real-time ultrasound system (Acuson 128SP5, Mountain View, CA, USA) using a transducer frequency emission of 3.5 MHz. A pulsed Doppler apparatus with a transducer frequency emission of 2 MHz, high pass filter <100 mW/cm^2, was used for blood flow velocity measurements in the middle cerebral artery (MCA). In every record, three to five consecutive cardiac cycles were examined to obtain an average of maximal and minimal flow velocity. The waveform patterns were quantified by calculating the pulsatility index (PI) according to the formula: maximal systolic peak flow velocity − minimum diastolic peak flow velocity/mean peak flow velocity ($V_{max} - V_{min}/V_{mean}$).

Neurological examination

Neonatal neurological conditions were classified using a qualitative approach as described by Prechtl [14], assigning each infant to one of the three diagnostic groups: normal, suspect, abnormal. An infant was considered abnormal if one of the following neurological syndromes were present: hyper- or hypokinesia, hyper- or hypotonia, hemisyndrome, apathy syndrome, hyperexcitability syndrome. An infant was classified as suspect if only isolated symptoms were present, but no defined syndrome. All infants were tested by the same examiner, who did neither know the neurological condition at birth nor the sonogram outcomes of the infants.

Adrenomedullin measurement

Plasma AM concentration was measured after extraction and purification in samples routinely collected at 12 h from birth and stored at −80°C. Briefly, 2 ml of samples was applied to conditioned Sep-pak C18 cartridges (Millipore Corp., Waters Chromatography, Mildford MA, USA) and the column was sequentially washed with 5 ml of isotonic saline, 5 ml of 0.1% trifluoracetic acid, and 5 ml of 20% acetonitrile in 0.1% trifluoracetic acid. The absorbed material was eluted with 4 ml of 50% acetonitrile and the

elute was lyophilized. After lyophilization, samples were dissolved in 50 mM phosphate buffer (pH 7.4) and AM was measured in plasma by RIA using a commercial kit (Phoenix Pharmaceuticals Inc., Mountain View, CA, USA) with rabbit polyclonal antibody raised against human AM 1–52, as reported [15]. Each measurement was performed in duplicate and the averages were reported. The antibody cross reacts 100% with human AM and no cross reactivity was reported with rat AM, amylin, CGRP, endothelin-1, α-atrial natriuretic peptide, brain natriuretic peptide. The 2intra- and inter-assay coefficients of variance were 5.1% and 12.0%, respectively.

Statistical analysis

Data are expressed as mean values ± SD. Data were analyzed by Mann–Whitney *U* two-sided test and linear regression analysis. Comparisons between proportions were performed with Fisher's exact test. Multiple logistic regression analysis was performed with the AM levels as the dependent variables to analyze the influence of various clinical parameters (MCA PI, Apgar score, incidence of respiratory distress syndrome, blood pH, oxygen and carbon dioxide partial pressures, base excess, mean blood pressure, type of treatment, occurrence of IVH) on AM levels. A value of $P < 0.05$ was considered significant.

Results

In Table 1, clinical characteristics and biochemical findings recorded at birth in the three studied groups are shown. As expected, in asphyxiated newborns, independently on the occurrence of IVH, Apgar score at the 1st and 5th min, pH, PvCO2, and base excess were significantly different from healthy controls ($P < 0.001$, for all). After clinical stabilization, at 12 h from birth, neonatal outcome and all laboratory parameters, except for base excess, were similar in the three groups (Table 2). At this time point, in all but two infants, cerebral ultrasound examination showed any pathological finding. At the neurological examination, 19 out of the 40 asphyxiated infants were classified as suspect (group A, $n = 7$; group B, $n = 12$; ns; healthy controls = 0). Hypertonia–hypotonia was present in nine asphyxiated infants (Group A: $n = 3$; Group B: $n = 6$), hyperexcitability in six infants (Group A: $n = 3$; Group B: $n = 5$), and seizures

Table 1
Neonatal monitoring parameters at birth in asphyxiated newborns developing (group A) and not developing IVH (group B) and in healthy controls

	Group A ($n = 20$)	Group B ($n = 20$)	Controls ($n = 40$)
Gestational age (week)	39.5 ± 1.2	40.0 ± 1.4	40.6 ± 1.0
Birth weight (g)	3453 ± 237	3335 ± 387	3542 ± 198
Apgar 1st min <3 (N°)	18*	17*	0
RBC (10^{12}/L)	3.9 ± 0.2	3.8 ± 0.4	3.9 ± 0.3
Hb (g/L)	136 ± 4	135 ± 3	137 ± 1
Ht	0.40 ±0.20	0.40 ± 0.22	0.42 ± 0.21
PH	6.97 ± 0.6*	6.98 ± 0.8*	7.30 ± 0.1
$PvCO_2$ (mm Hg)	25.2 ± 5.0*	27.1 ± 7.3*	41.7 ± 4.1
PvO_2 (mm Hg)	30.6 ± 7.9	33.0 ± 9.0	40.7 ± 5.6
BE	−15.5 ± 1.1*	−14.2 ± 2.8*	0.2 ± 2.2
Na^+ (mmol/L)	138 ± 5	140 ± 3	140 ± 3
K^+ (mmol/L)	4.7 ± 0.3	4.1 ± 0.2	4.2 ± 0.2
Ca^{++} (mmol/L)	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.1
Plasma glucose (mmol/L)	4.2 ± 1.1	4.1 ± 1.2	4.2 ± 0.2

Values are expressed as mean ± SD.
* $P < 0.05$ vs. controls.

Table 2
Monitoring parameters at 12 h from birth in asphyxiated infants developing (group A) and not developing (group B) IVH and in control group

	Group A ($n = 20$)	Group B ($n = 20$)	Controls ($n = 40$)
RBC (10^{12}/L)	3.9 ± 0.2	4.0 ± 0.3	3.9 ± 0.3
Hb (g/L)	136 ± 2	135 ± 4	135 ± 4
Ht	0.40 ± 0.27	0.40 ± 0.25	0.40 ± 0.20
pH	7.28 ± 0.2	7.30 ± 0.1	7.31 ± 0.1
$PvCO_2$ (mm Hg)	44.2 ± 12.0	44.1 ± 6.3	42.4 ± 4.1
PvO_2 (mm Hg)	41.6 ± 6.3	45.0 ± 5.2	42.1 ± 7.2
BE	−5.2 ± 6.3*	−4.0 ± 3.7*	−0.9 ± 2.1
Plasma glucose (mmol/L)	4.4 ± 0.7	4.2 ± 1.1	4.1 ± 0.3
MCA PI	1.71 ± 0.25*	1.48 ± 0.22	1.47 ± 0.29
Cerebral ultrasound (normal/abnormal)	20/2	20/0	Not recorded
Neurological examination (normal/suspect/abnormal)	13/7/0	8/12/0	Not assessed

Values are expressed as mean ± SD.
* $P < 0.05$ vs. controls.

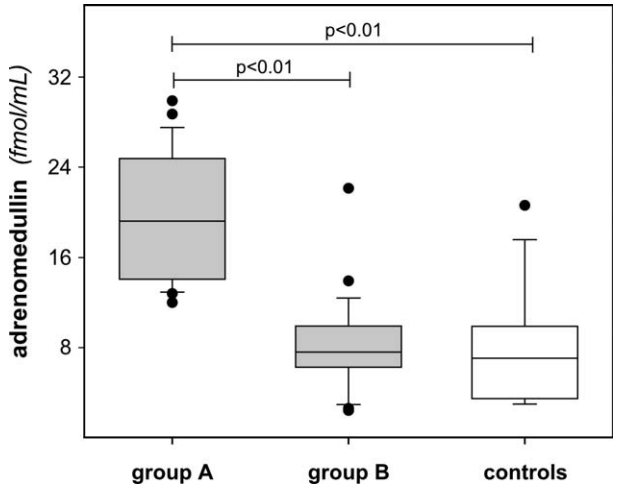

Fig. 1. Adrenomedullin concentrations 12 h from birth in asphyxiated infants developing (group A) and not developing (group B) IVH and in healthy controls. The lower and upper bars represent the 10th and 90th percentiles, respectively, and the interquartile range is indicated by the box, the median value being the horizontal line in the box. Adrenomedullin was significantly higher ($P < 0.01$) in asphyxiated infants developing IVH than in the other two groups.

in two infants (Group A: $n = 1$; Group B; $n = 1$). Multiorgan failure has been shown in the two asphyxiated infants developing IVH who died in the first 96 h after birth. Overall MCA PI value in total asphyxiated infants was higher than in healthy controls (1.59 ± 0.29 vs. 1.47 ± 0.29); however, this difference did not reach the level of significance ($P = 0.14$). In contrast, when asphyxiated infants were subgrouped according to the occurrence of IVH, mean MCA PI value at 12 h from birth was significantly higher in infants developing IVH than in group B or controls (Table 2; $P < 0.05$, for all). No difference was found between asphyxiated infants who did not develop IVH and controls ($P > 0.05$).

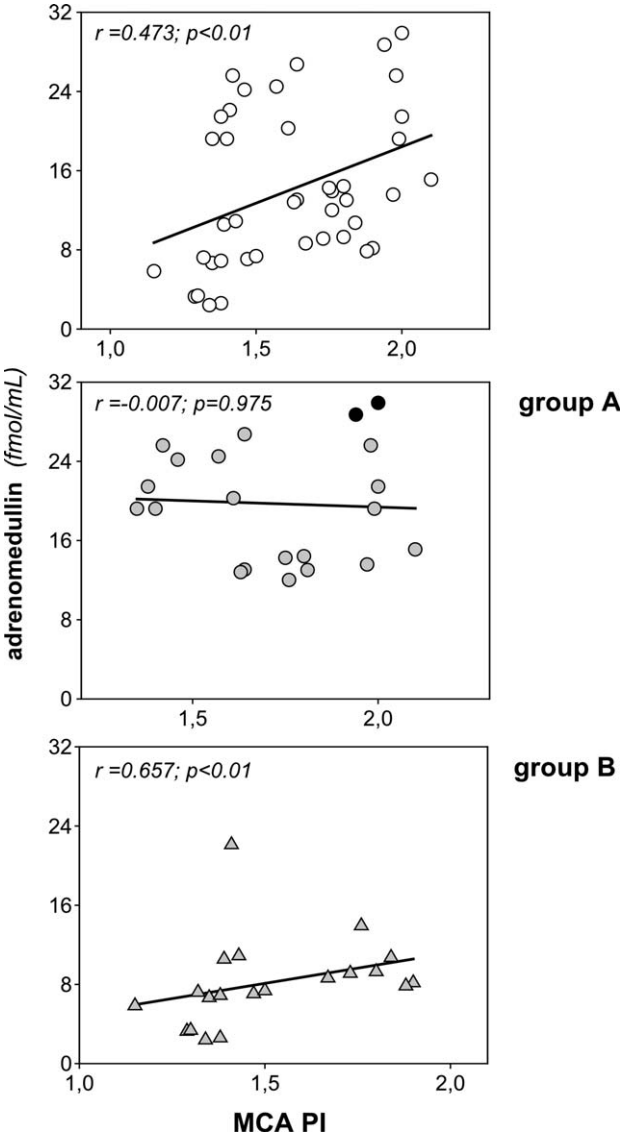

Fig. 2. Correlation between adrenomedullin concentrations and middle cerebral artery (MCA) PI 12 h from birth in all asphyxiated infants (upper panel) in those developing (group A) and not developing (group B) IVH. There was a positive correlation in total asphyxiated infants and in those who did not develop IVH, whereas no correlation was found in group A. The two filled circles indicate adrenomedullin levels in infants with severe IVH who died at 72 and 96 h after birth.

Mean AM concentration was significantly higher ($P <$ 001) in group A (20.2 ± 5.2 fmol/mL) than in group B (8.4 ± 2.1 fmol/mL) or healthy controls (9.3 ± 2.6 fmol/mL; Fig. 1). Since only 2 out of 20 infants of group A had a severe IVH with intraventricular hemorrhage and ventricular dilatation, we were unable to assess a statistical correlation between clinical feature (grade of IVH) and AM concentration. However, the highest levels of AM were found in the two infants with intraventricular hemorrhage and ventricular dilatation (29.9 and 28.7 fmol/ml, respectively). A significant correlation was present between circulating AM and MCA PI in asphyxiated newborns; however, this correlation was lost when group A alone was analyzed (Fig. 2).

Multiple logistic regression analysis with AM levels as the dependent variable showed that AM concentrations correlated exclusively with the occurrence of IVH ($P <$ 0.001), whereas all the other variables were not correlated.

Discussion

Experimental studies [16,17] have demonstrated that alteration of cerebral blood flow is one of the signs of impaired cerebral autoregulation in which vasoactive molecules and calcium-mediated effects might play an important role. Although in the clinical setting MCA PI is currently used for monitoring cerebrovascular resistances, in asphyxiated infant its reliability is reduced since it can be influenced by mechanical ventilation, patent ductus arteriosus, and sedation [18]. Recently, it has been reported that cerebral blood flow and cerebrovascular resistances in the first 24 h of life might not change in asphyxiated infants developing ischemic encephalopaty [19], thus suggesting the need for additional parameters to detect earlier the occurrence of IVH. The neurological examination is also of limited value because of pharmacological sedation.

In the present investigation, we confirmed that neither the neurological or the ultrasound examination, nor the MCA PI assessment performed 12 h from birth was able to discriminate infants who developed IVH from those that did not. In contrast, at this time, plasma AM levels were significantly higher in asphyxiated infants in whom IVH occurred.

In vitro and in vivo studies have shown that AM is secreted by endothelial cells and participates in the regulation of cerebral blood flow, and its ability to exacerbate ischemic brain damage [20,21]. Correlation between circulating AM and cerebrovascular resistance patters in asphyxiated infants suggests that AM may increase in response to acute hypoxia and play a compensatory role in the post-asphyxial period. This especially holds for the prevention of hypoxic–ischemic insult and ischemia reperfusion injury. Another explanation lies in the possibility that this correlation could be expression of safe brain–blood barrier permeability after

asphyxia insult since cerebrovascular autoregulation system is aimed to maintain cerebral blood flow at constant levels. The increased AM plasma levels shown in infants with increased cerebrovascular resistance might be suggestive of an altered release of AM in the systemic circulation at a stage when protective cerebrovascular autoregulation system is still active. Therefore, the loss of correlation between AM and MCAPI values in asphyxiated infants developing IVH can be ascribable to an excessive AM secretion causing the loss of cerebral vascular autoregulation, which, in turn, leads to the occurrence of cerebral bleeding due to the massive reactive vasodilatation, as demonstrated for other vasoactive factors. It should be noted, in this respect, that hypotension, cerebral ischemia, and reperfusion are the main events affecting cerebral vascular autoregulation, and severe and prolonged hypoxia or ischemia results in cell death and tissue damage.

Since asphyxial insult may be directly responsible for increased circulating AM levels, plasma protein concentration was measured 12 h from birth, after clinical and biochemical stabilization of the infants, to infer an association between AM and IVH, independently from the original event, being the protein half-life of about 2 h. In fact, it has been shown that birth stress itself increases circulating AM that correlates with pH values in the newborn [12], and that hypoxia induces AM mRNA expression and peptide production in different type of cells [22]. Lack of difference in AM levels at 12 h from birth between asphyxiated infants who did not develop IVH and healthy controls suggests that hypoxic insult per sé is not responsible for elevated concentration detected at 12 h of life in infants developing IVH.

AM infusion in dogs increases cerebral blood flow in a dose-dependent manner without affecting systemic blood pressure and other cardiovascular parameters [23]. Recently, we have shown that AM contributes to the hemodynamic modification that determines the redistribution of cardiac output (brain-sparing effect) in growth-retarded fetuses [20], confirming an important role of this peptide in the regulation of cerebral blood flow also in humans. Moreover, an experimental study in rats demonstrated the ability of AM to exacerbate ischemic brain damage [21].

In conclusion, our study indicates that AM is increased in the post-asphyxial period, very likely as a compensatory mechanism in response to acute hypoxia, suggesting that it may participate in the loss of cerebral vascular autoregulation that leads to IVH. It also suggests that the use of this peptide in the post-asphyxial period may help in the early detection of IVH when clinical examination and cerebral ultrasound are still silent.

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