Plasma catecholamines after endotracheal administration of adrenaline during postnatal resuscitation

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Abstract

To analyse the degradation of adrenaline after cardiopulmonary resuscitation of preterm neonates, free and sulphoconjugated adrenaline, noradrenaline, and dopamine were determined in 31 preterm neonates by a radioenzymatic method. Nine of the neonates received a high dose (250 µg/kg) of endotracheally administered adrenaline (1:1000); three of them had more than one dose of adrenaline. With the exception of sulphoconjugated dopamine, the free and sulphoconjugated catecholamine concentrations in preterm infants treated with adrenaline initially exceeded those in the untreated group. The concentrations decreased to the same range about two hours after birth. Free and sulphoconjugated adrenaline concentrations remained significantly increased in the adrenaline treated group, however, indicating a plateau effect. The correlation between free adrenaline and noradrenaline concentrations with their respective sulphoconjugated concentrations was highly significant. It is concluded that free catecholamines are rapidly degraded by sulphoconjugation in preterm neonates.

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Compared with the synthesis and secretion of catecholamines, little is known about their inactivation. Large amounts of free catecholamines are inactivated by the enzymatic degradation of catechol-O-methyltransferase and monoamine oxidase, especially during the passage of blood through the liver and kidney.^{1 2} Catecholamines are also taken up from plasma by different active processes of transportation. Erythrocytes and platelets are reported to be storage sites of free catecholamines.^{3 4} Finally, growing evidence suggests a role for the sulpho-

conjugation of free catecholamines by the enzyme phenolsulphotransferase in regulating haemodynamic and metabolic effects.^{3 5 6} This enzyme is ubiquitously located, with the highest concentrations in the intestines, liver, kidneys, brain, skeletal muscles, platelets, and erythrocytes.^{4 6 7} Sulphoconjugated catecholamines account for approximately 70–80% of total circulating adrenaline and noradrenaline and for 98% of total dopamine in resting adults.^{5 6} In children similar values are observed.⁸

Few data exist on sulphoconjugated catecholamines in term and preterm newborns.^{9 10} There is no experimental data on sulphoconjugated catecholamines after postnatal cardiopulmonary resuscitation of preterm infants. The endotracheal administration of adrenaline is usually recommended in postnatal cardiopulmonary resuscitation, however.^{11 12} In our experience the use of 250 µg/kg adrenaline as an endotracheal high dose administration of adrenaline is successful and not dangerous.

The aim of our study was to measure free and sulphoconjugated catecholamines in plasma after cardiopulmonary resuscitation in preterm neonates and to test the ability of preterm neonates to inactivate free catecholamines by sulphoconjugation when high dose diluted adrenaline was used endotracheally.

Patients and methods

PATIENTS

Thirty one preterm neonates were examined after postnatal cardiopulmonary resuscitation. Entry criteria for the study were the need for intubation and artificial ventilation of preterm neonates because of postnatal depression. All preterm neonates received external cardiac massage. Nine (29%) received an endotracheal dose of 250 µg/kg adrenaline (Suprarenin, Hoechst, 1:1000) diluted 1:1 with sodium chloride solution (0.9%) because they did not improve after intubation, ventilation, and chest compression; all had heart rates less than 100 beats/minute. Two of the nine preterm infants received a second adrenaline dose because of sepsis and the need for further circulatory support. After initial resuscitation by outside colleagues one preterm neonate received a third adrenaline dose because of an initially unrecognised right sided pressure pneumothorax with severe asphyxia and bradycardia. Table 1 gives the patients' data. There was no difference in gestational age, birth weight, or birth length between the adrenaline treated and the untreated group of preterm neonates. The initial capillary pH and Apgar scores

Table 1 Characteristics of the patients. Values are median (range)

Patient characteristic	Without adrenaline (n=22)	With adrenaline (n=9)
Girls/boys	11/11	5/4
Birth weight (g)	1473 (845–3465)	1225 (900–2745)
Birth length (cm)	41.7 (33–52)	37 (35–44)
Gestational age (weeks)	30.5 (28–36)	28.9 (28–35)
Apgar score		
1 min	6 (1–9)	2 (0–8)
5 min	9 (5–10)	7 (1–10)
10 min	10 (7–10)	8 (1–10)
No of deaths	3	3
Age and cause of death	(1) 1 day; intractable sepsis (2) 13 days; lung hypoplasia due to diaphragmatocele (3) 3 months; bronchopulmonary dysplasia and right heart failure	(1) 1 day; lung hypoplasia (2) 1 month; bronchopulmonary dysplasia and right heart failure (3) 9 months; bronchopulmonary dysplasia and right heart failure

Table 2 Free and sulphoconjugated, adrenaline, noradrenaline, and dopamine concentrations (nmol/l) in 31 preterm neonates receiving endotracheal adrenaline (with adrenaline, *n*=9) during postnatal resuscitation or not (without adrenaline, *n*=22). I represents the time of blood sampling about 28 minutes after delivery in the delivery room, II about 78 minutes later at the intensive care unit, and III about 15 minutes later – that is, about two hours after birth

	I		II		III	
	With adrenaline (<i>n</i> =9)	Without adrenaline (<i>n</i> =22)	With adrenaline (<i>n</i> =9)	Without adrenaline (<i>n</i> =22)	With adrenaline (<i>n</i> =9)	Without adrenaline (<i>n</i> =22)
Median (range) free adrenaline	28.6 (7.4–1283)	0.95 (0.089–9.04)	5.07 (1.2–44.4)	0.35 (0.079–2.87)	4.77 (0.63–20.89)	0.44 (0.11–11.56)
Median (range) sulphoconjugated adrenaline	24.48 (4.46–271.89)	2.37 (0.24–18.63)	49.7 (5.65–108.1)	2.26 (0.18–14.35)	27.63 (3.46–141.5)	1.58 (0.065–13.23)
Sulphoconjugated adrenaline (median % of total)	31.5	65.6	83.5	77.1	86.6	76.4
Median (range) free noradrenaline	39.53 (3.32–309.8)	6.26 (1–85.9)	10.72 (0.56–75.9)	3.58 (0.53–12.9)	1.92 (0.46–9.9)	3.35 (0.44–13.35)
Median (range) sulphoconjugated noradrenaline	21.7 (5.16–173.5)	5.61 (1.62–27.46)	25.7 (4.78–86.9)	5.26 (1.78–22.18)	6.9 (3.72–54.9)	5.76 (1.67–22.9)
Sulphoconjugated noradrenaline (median % of total)	42.8	45.9	71.3	65.7	84.7	63.5
Median (range) free dopamine	1.35 (0.46–3.52)	0.63 (0.17–2.69)	0.81 (0.27–2.92)	0.39 (0.085–0.68)	0.41 (0.27–1.06)	0.43 (0.19–0.67)
Median (range) sulphoconjugated dopamine	17.8 (10–26)	27.8 (14.2–156.8)	21.2 (10.68–49.65)	26.4 (3.92–120.4)	12.6 (6.33–37.6)	28.2 (1.78–105.9)
Sulphoconjugated dopamine (median % of total)	91.6	98	95.7	99	97	99

differed significantly, however. Immediately after intubation an umbilical vein catheter was introduced for circulatory support and routine blood sampling. All catheters were examined for bacteriological contamination; none was found. At a median time of 28 minutes after birth a first blood sample of a maximum volume of 1 ml was taken through the umbilical catheter in the delivery room; a further blood sample at a median of 78 minutes later was taken in the intensive care unit. A third blood sample was taken 11–26 minutes later – that is, about two hours after birth – when the catheter was extracted. There was no significant difference in the time periods between blood sampling in the two groups. Whenever possible, the parents of our patients were informed about the procedure. The study was approved by the local ethics committee of the University of Wuerzburg.

METHODS

Free adrenaline, noradrenaline, and dopamine were determined with a modified radioenzymatic assay according to the principles of Peuler and Johnson.¹³ The most important modification includes deproteinisation of plasma with 9 N perchloric acid. This allowed us to determine catecholamines in plasma using a standard curve.^{10 14 15} The preparation of rat liver catechol-*O*-methyltransferase was modified according to the method of Axelrod and Tomchick.¹ The assay is characterised by small volumes of deproteinised plasma (25 µl/tube) and by a high sensitivity; the lowest limit of detection ranges from 0.5–2 pg. The interassay variance is 7–12% (10 samples); the intra-assay variance was lower than 5% (10 samples) for adrenaline, noradrenaline, and dopamine.

To determine the sulphoconjugated part of the total (equivalent to free plus sulphoconjugated) catecholamines, we determined total catecholamines by adding 60 µU arylsulphatase type VI per tube (Sigma) into the incubation solution, leading to the subtotal transformation of sulphoconjugated catecholamines into the free form. A second assay was performed to measure the respective free catecholamines. The sulphoconjugated catecholamines were

calculated by subtraction of the free from the total amount of the respective catecholamines. To decrease the interassay variance, total and free catecholamines were determined in one assay.¹⁴ The interassay variance of total catecholamines remained less than 7.3% and the intra-assay variance less than 4.8% for all catecholamines.

The assay methods have been validated for use on neonatal plasma in several investigations.^{9 10 14 17}

STATISTICS

All statistical analyses were performed at the data centre of the University of Wuerzburg. The Spearman rank correlation coefficient was used as well as Wilcoxon's test and the Mann-Whitney U test.

Results

A wide range of free and sulphoconjugated adrenaline, noradrenaline, and dopamine concentrations was found (table 2).

About two hours after delivery, free noradrenaline and free dopamine concentrations of preterm neonates treated with adrenaline are within the equivalent range of untreated neonates (table 2). Free adrenaline concentrations remain significantly increased in the adrenaline treated group compared with untreated neonates (I, II *p*<0.00003; III *p*<0.003; see table 2 for definition of I–III). Free adrenaline concentrations decrease considerably in the adrenaline treated group of preterm neonates (*p*<0.015) and in the untreated group (*p*<0.002) from the first to the second blood sample (table 2). This also is true for free noradrenaline concentrations (*p*<0.01 and *p*<0.0001 respectively) and free dopamine concentrations (*p*<0.01 and *p*<0.001 respectively).

In the adrenaline treated group all sulphoconjugated catecholamine concentrations non-significantly increased between the first and the second blood sample (table 2). Concentrations of sulphoconjugated adrenaline (*p*<0.0003) and noradrenaline (*p*<0.005) are significantly higher than in the untreated

Figure 1: Relation of free plasma adrenaline concentration to their respective sulphoconjugated concentrations in 31 preterm neonates after intubation and resuscitation about 106 minutes after birth. The broken inner lines represent the 95% confidence interval of the mean values, whereas the outer lines are the 95% confidence interval of the individual values.

group 28 minutes after birth. In contrast, sulphoconjugated dopamine concentrations are significantly lower ($p<0.002$). Two hours after delivery concentrations of sulphoconjugated noradrenaline and dopamine are within the same range (table 2), with the exception of sulphoconjugated adrenaline concentrations which remain significantly ($p<0.0003$) increased over the whole period of observation. Sulphoconjugated adrenaline ($p<0.01$) and dopamine ($p<0.001$) in per cent of total was lower in adrenaline treated than in untreated neonates at the time of the first blood sample, whereas sulphoconjugated noradrenaline in per cent of total was equal at the first and significantly higher ($p<0.01$) at the third blood sample (table 2). In the adrenaline treated group of preterm neonates, sulphoconjugated

adrenaline ($p<0.01$), noradrenaline ($p<0.01$), and dopamine ($p<0.02$) in per cent of total increased significantly from the first to the second blood sample. The same is true for untreated neonates ($p<0.03$, $p<0.001$, and $p<0.02$ respectively).

Correlation between the free and the respective sulphoconjugated catecholamines is significantly positive with respect to adrenaline at the first ($r=0.6$, $p=0.0003$; 31 samples), second (fig 1), and third ($r=0.56$, $p=0.0041$; 24 samples) blood sampling, and to noradrenaline at the first ($r=0.567$, $p=0.0009$) and second ($r=0.66$, $p=0.0001$; 29 samples) sampling. In contrast with this, free dopamine concentrations are not significantly correlated with their respective sulphoconjugated dopamine concentrations.

Of the nine adrenaline treated preterm neonates (fig 2), the three receiving adrenaline endotracheally on two or three occasions had the highest concentrations of free and sulphoconjugated adrenaline. All concentrations of free adrenaline decreased significantly. Concentrations of sulphoconjugated adrenaline in preterm neonates receiving more than one adrenaline dose decreased, however, whereas all other concentrations of sulphoconjugated adrenaline increased.

Discussion

The endotracheal route of adrenaline administration is rapid and not dangerous^{11 12} and is proposed for use in neonates without intravenous access who did not respond to a standard dose of adrenaline.¹⁶ The aim of this paper is to show that preterm neonates are able to inactivate large amounts of free catecholamines by sulphoconjugation in the course of postnatal cardiopulmonary resuscitation, especially after endotracheal administration of adrenaline.

High concentrations of free and sulphoconjugated catecholamines were found in the umbilical vessels of term neonates after delivery.⁹ Moreover, in term neonates correlation between free and sulphoconjugated catecholamines was significantly positive, suggesting sufficient sulphoconjugation.^{10 14} Similar results were reported by studies in sheep investigating the effects of asphyxia on free and sulphoconjugated catecholamines.^{17 18} In human preterm neonates, however, an absence of correlation between free and sulphoconjugated catecholamines was observed.¹⁰ In spite of high free catecholamine concentrations some of the preterm infants were unable to adequately increase sulphoconjugated catecholamines, probably due to an immaturity of the enzyme phenylethylamine-*N*-methyltransferase.¹⁰ In the present study, however, free catecholamine concentrations decreased rapidly after postnatal cardiopulmonary resuscitation, whereas the percentages of all sulphoconjugated catecholamines of the respective total catecholamines increased significantly from the first to the second blood sample. The significant correlation of free adrenaline and noradrenaline with their

Figure 2: Free and sulphoconjugated adrenaline concentration at the first (I) and second (II) time of blood sampling in the nine preterm neonates who received a high dose of adrenaline endotracheally. All preterm neonates had a single adrenaline dose, but two also had a second and one a third adrenaline dose. Preterm neonates with two or three adrenaline administrations are characterised by dotted lines. (NS=non-significant, **= $p<0.015$.)

respective sulphoconjugated levels suggests a rapid inactivation of free adrenaline and noradrenaline in human preterm neonates by sulphoconjugation.

After endotracheal administration of adrenaline free adrenaline concentrations significantly exceeded those of the untreated group, indicating that the administered adrenaline dose reaches the circulation. As the result of asphyxia, however, endogenous stimulation of catecholamines may contribute to the significant increase of adrenaline concentrations in the adrenaline treated group of preterm neonates. With regard to preterm neonates receiving more than one administration of adrenaline a further increase in adrenaline concentration dependent on the dose was observed. By the administration of high doses of adrenaline endotracheally, a plateau of sustained increased adrenaline concentrations can be shown,^{19 20} whereas the other catecholamines decreased into the range of untreated preterm neonates. This plateau may be the result of delayed adrenaline absorption in the lungs.

With regard to the nine preterm neonates receiving endotracheal administration of adrenaline, those receiving more than one adrenaline administration had the highest sulphoconjugated noradrenaline and adrenaline concentrations, supporting the effective inactivation of free catecholamines by sulphoconjugation. With the exception of the three preterm neonates with high adrenaline concentrations, all showed an increase in sulphoconjugated adrenaline after postnatal cardiopulmonary resuscitation. In the case of high adrenaline concentrations of about 100 ng/ml, however, the inactivation of free and sulphoconjugated catecholamines by enzymatic degradation as well as tissue and platelet deposition seems to predominate, leading to decreasing sulphoconjugated adrenaline concentrations, whereas in lower concentrations of free adrenaline the inactivation by sulphoconjugation seems to dominate, leading to increasing sulphoconjugated adrenaline concentrations. Similarly, the percentages of all sulphoconjugated derivatives of total catecholamines increased considerably during about 100 minutes after delivery. Thus preterm neonates are able to metabolise large amounts of free adrenaline and noradrenaline, effectively protecting the body against an overflow of free catecholamines after postnatal cardiopulmonary resuscitation.

Whereas sulphoconjugated noradrenaline and adrenaline are initially increased in the adrenaline treated compared with the untreated group of preterm neonates, sulphoconjugated dopamine concentrations are significantly decreased in spite of significantly increased free dopamine concentrations. On the one hand this suggests a conversion of sulphoconjugated dopamine into the free form of catecholamines. By using animal studies an in vivo conversion of labelled sulphoconjugated dopamine into free catecholamines was demonstrated.²¹ On the other hand, the initially low sulphoconjugated dopamine concentrations might be caused by a competitive inhibition of dopamine sulpho-

conjugation by the high free adrenaline and noradrenaline concentrations in plasma. Moreover, the non-significant correlation of free and sulphoconjugated dopamine may be caused by the small concentrations of free dopamine compared with the large amounts of sulphoconjugated dopamine. Thus free dopamine concentrations might be in the range of the interassay variance of sulphoconjugated dopamine, leading to a poor correlation.

To summarise, preterm neonates are able to inactivate rapidly high concentrations of free adrenaline and noradrenaline after postnatal cardiopulmonary resuscitation with or without endotracheal adrenaline administration. This may contribute to the fact that we did not observe undesirable cardiovascular or metabolic side effects, even after high doses of adrenaline in preterm neonates (K O Schwab, H B von Stockhausen, H Müller, L Schrod, unpublished data).

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- 1 Axelrod J, Tomchick R. Enzymatic O-methylation of epinephrine and other catechols. *J Biol Chem* 1958; 233: 702-5.
- 2 Tipton KF. Biochemical aspects of monoamine oxidase. *Br Med Bull* 1973; 29: 116-9.
- 3 Kuchel O, Buu NT, Hamet P, Laroche P, Bourque M, Genest J. Essential hypertension with low conjugated catecholamines imitates pheochromocytoma. *Hypertension* 1981; 3: 347-53.
- 4 Radge D, Kohse KP, Steegmüller U, Wisser H. Distribution of free and conjugated catecholamines between plasma, platelets and erythrocytes: different effects of intravenous and oral catecholamine administrations. *J Pharmacol Exp Ther* 1991; 257: 232-8.
- 5 Johnson GA, Baker CA, Smith R. Radioenzymatic assay of sulfate conjugates of catecholamines and dopa in plasma. *Life Sci* 1980; 26: 1591-8.
- 6 Joyce DA, Beilin LJ, Vandongen R, Davidson L. Plasma free and sulfate conjugated catecholamine levels during acute physiological stimulation in man. *Life Sci* 1982; 30: 447-52.
- 7 Vandongen R. The significance of sulfate-conjugated catecholamines in man. *Neth J Med* 1984; 27: 129-35.
- 8 Eichler I, Eichler HG, Rotter M, Kyrle PA, Gasic S, Korn A. Plasma concentrations of free and sulfoconjugated dopamine, epinephrine and norepinephrine in healthy infants and children. *Klin Wochenschr* 1989; 67: 672-5.
- 9 Schwab KO, Wernze H, Paulick R, Kastendieck E. Circulating free and sulfoconjugated catecholamines in the umbilical vessels as related to fetal stress. *Acta Endocrinol* 1985; 267 (suppl): 60.
- 10 Schwab KO, Kruse K, Breitung B, Paulick R. Freie und sulfatkonjugierte Plasma-Katecholamine bei Frühgeborenen und reifen Neugeborenen nach der Geburt. *Monatsschr Kinderheilkd* 1989; 137: 28-32.
- 11 Benitz WE, Frankel LR, Stevenson DK. The pharmacology of neonatal resuscitation and cardiopulmonary intensive care. Part I - Immediate resuscitation. *West J Med* 1986; 144: 704-9.
- 12 Lindemann R. Resuscitation of the newborn. Endotracheal administration of epinephrine. *Acta Paediatr Scand* 1984; 73: 210-2.
- 13 Peuler J, Johnson GA. Simultaneous single isotope radioenzymatic assay of plasma norepinephrine, epinephrine and dopamine. *Life Sci* 1977; 21: 625-36.
- 14 Schwab KO, Kastendieck E, Paulick R, Wernze H. Production and inactivation of catecholamines during hypoxia and recovery. In: Künzel W, Jensen A, eds. *The endocrine control of the fetus*. Berlin-Heidelberg: Springer Verlag, 1988: S80-7.
- 15 Schwab KO, Heubel G, Bartels H. Free epinephrine, norepinephrine and dopamine in saliva and plasma of healthy adults. *European Journal of Clinical Chemistry and Clinical Biochemistry* 1992; 30: 541-4.
- 16 American Heart Association. Standard and guidelines for cardiopulmonary resuscitation and emergency cardiac care. Part VII: neonatal resuscitation. *JAMA* 1992; 268: 2276-81.
- 17 Paulick R, Schwab KO, Kastendieck E, Wernze H. Plasma free and sulfoconjugated catecholamines during acute asphyxia in the sheep fetus - relation to cardiovascular parameters. *J Perinat Med* 1988; 16: 113-21.
- 18 Padbury JF, Martinez AM, Thio SL, Burnell EE, Humme JA. Free and sulfoconjugated catecholamine responses to hypoxia in fetal sheep. *Am J Physiol* 1989; 257: E198-202.

- 19 Roberts JR, Greenberg MI, Knaub M, Baskin SI. Comparison of the pharmacological effects of epinephrine administered by the intravenous and endotracheal routes. *Journal of the American College of Emergency Physicians* 1978; **7**: 260-4.
- 20 Roberts JR, Greenberg MI, Knaub M, Kendrick ZV, Baskin SI. Blood levels following intravenous and endotracheal epinephrine administration *Journal of the American College of Emergency Physicians* 1979; **8**: 53-6.
- 21 Buu NT, Nair G, Kuchel O, Genest J. The extra-adrenal synthesis of epinephrine in rats. Possible involvement of dopamine sulphate. *J Lab Clin Med* 1981; **98**: 527-35.