ORIGINAL ARTICLE

Randomised controlled trial evaluating effects of morphine on plasma adrenaline/noradrenaline concentrations in newborns

S H P Simons, M van Dijk, R A van Lingen, D Roofthoof, F Boomsma, J N van den Anker, D Tibboel

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Objectives: To determine the effects of continuous morphine infusion in ventilated newborns on plasma concentrations of adrenaline (epinephrine) and noradrenaline (norepinephrine) and their relation to clinical outcome.

Design: Blinded, randomised, placebo controlled trial.

Setting: Level III neonatal intensive care units in two centres.

Patients: A total of 126 ventilated neonates (inclusion criteria: postnatal age <3 days, duration of ventilation <8 hours, indwelling arterial catheter for clinical purposes; exclusion criteria: severe asphyxia, severe intraventricular haemorrhage, major congenital anomalies, neuromuscular blockers).

Interventions: Plasma adrenaline and noradrenaline concentrations were determined in patients during blinded morphine (n = 60) and placebo (n = 66) infusion (100 µg/kg plus 10 µg/kg/h).

Results: Plasma concentrations at baseline (nmol/l with interquartile range in parentheses) were comparable in infants treated with morphine (adrenaline, 0.22 (0.31); noradrenaline, 2.52 (2.99)) or placebo (adrenaline, 0.29 (0.46); noradrenaline, 2.44 (3.14)). During infusion, median adrenaline concentrations were 0.12 (0.28) and 0.18 (0.35) and median noradrenaline concentrations were 2.8 (3.7) and 3.8 (4.0) for the morphine and placebo treated infants respectively. Multivariate analyses showed that noradrenaline (p = 0.029), but not adrenaline (p = 0.18), concentrations were significantly lower in the morphine group than the placebo group. Furthermore, noradrenaline concentrations were related to the length of stay in the neonatal intensive care unit.

Conclusions: Continuous morphine infusion significantly decreased plasma noradrenaline concentrations in ventilated newborns compared with placebo treatment. The results of this study support the idea that routine morphine administration decreases stress responses in ventilated neonates.

See end of article for authors' affiliations

Correspondence to:
Dr Tibboel, Department of
Paediatric Surgery,
Erasmus MC-Sophia, Dr
Molewaterplein 60, 3015
GJ Rotterdam, the
Netherlands; j.tibboel@
erasmusmc.nl

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Preterm newborn infants suffer pain and stress during intensive care.^{1–5} Although even the most premature neonates react to painful stimuli and are probably able to feel pain, adequate analgesia by continuous opioid treatment is still limited.^{2–5, 6} This may be explained by the fact that there is disagreement as to whether the currently available evidence is sufficient to justify prolonged exposure to opioids in this vulnerable group of patients. As it is still a major challenge to quantify the degree of pain relief in neonates, there is still lack of evidence about the analgesic effect of opioids. Observational pain scales, using both physiological and behavioural indicators, have been validated for premature and term neonates,^{7–9} but failed to identify the analgesic effects of opioids.¹⁰

Preterm neonates are, however, capable of mounting hormonal responses to stress related to birth, illness, intensive care treatment, surgical procedures, and mechanical ventilation, as manifested by high plasma catecholamine concentrations.^{11–17} These plasma catecholamine concentrations were reduced by analgesic treatment in newborns,^{18, 19} and therefore may also represent the stress relieving effect of continuous morphine infusion in neonates.

Therefore we hypothesised that routine morphine administration would reduce stress responses of ventilated newborns. To test our hypothesis, plasma concentrations of adrenaline (epinephrine) and noradrenaline (norepinephrine) were analysed in ventilated newborns who participated in a blinded, randomised, placebo controlled trial evaluating the analgesic effect of routine morphine administration in

preterm ventilated newborns.¹⁰ As lower stress responses are associated with improved outcome^{15, 20} and decreased post-operative mortality in neonates,^{13, 21} we also aimed to determine if the possible decrease in neonatal stress response produced by continuous morphine treatment would be related to beneficial effects on neonatal outcome.

METHODS

Patients

Neonatal patients were included from December 2000 to October 2002 in two centres that were level III neonatal intensive care units (centre I, Erasmus MC-Sophia Rotterdam, a university hospital; centre II, The Isala Clinics Zwolle, a non-university hospital). Neonates of gestational age 25–42 weeks requiring mechanical ventilation, with postnatal age <3 days, endotracheal intubation <8 hours before the start, and with an indwelling arterial catheter were eligible for inclusion. Exclusion criteria were severe asphyxia (Apgar score after five minutes <4 or cord blood pH <7.0),²² severe intraventricular haemorrhage (IVH) (grade 3 or IVH + apparent periventricular haemorrhagic infarction),²³ major congenital anomalies and facial malformations such as cleft lip and palate, neurological disorders, and continuous or intermittent neuromuscular blockers.

Abbreviations: NICU, neonatal intensive care unit; IVH, intraventricular haemorrhage; IQR, interquartile range; CI, confidence interval

Figure 1 Flow diagram showing administration of morphine or placebo.

Procedure

The local ethics committees of the participating centres approved the study protocol. If possible, the parents were informed about the study before the birth of their child. Written informed parental consent was obtained for all included patients. Masked study medication consisted of either morphine hydrochloride or placebo (sodium chloride), both dissolved in 5% glucose. After enrolment, patients were randomly allocated to receive a masked loading dose (100 µg/kg) followed by masked continuous infusion (10 µg/kg/h). To prevent possible overdosing, the loading dose was not given if, before intubation, a morphine loading dose had been given less than three hours before the start of the study. Study medication was continued for a maximum of seven days; if the patient's clinical condition required it, it was discontinued earlier. After seven days, the study medication was weaned and replaced by real morphine infusion if necessary.

All patients judged to be in pain or distress were given additional morphine during the study on guidance of the attending physician (independent of the study) with allowed doses of 50 µg/kg followed by 5–10 µg/kg/h continuous "open label" morphine. Blood samples for catecholamine analyses were taken at baseline—that is, before the start of study medication—and at 24, 48, and 96 hours after the start of study medication at rest in centre I, and at days 2, 3, and 5 within five minutes of endotracheal suctioning in centre II. Blood samples of 0.6 ml were drawn from the arterial catheter into a heparin microcontainer and taken to the laboratory in ice water. The samples were centrifuged (4°C, 10 minutes, 3000 g), and plasma was separated and stored at –80°C. Plasma concentrations of adrenaline and noradrenaline were determined using high performance liquid chromatography with fluorimetric detection.²⁴

Table 1 Background characteristics of study participants

	Morphine (n = 60)	Placebo (n = 66)
Sex (boys/girls)	38/22	37/29
In/outborn	45/15	44/22
Gestational age (weeks)	30.3 (27.5 to 32.1)	29.6 (28.4 to 32.1)
Birth weight (g)	1380 (1004 to 1840)	1340 (1024 to 1674)
Postnatal age (hours)	8.5 (5.0 to 13.0)	8.0 (5.0 to 12.0)
Apgar score at 1 min	6 (4 to 8)	6 (4 to 8)
Apgar score at 5 min	8 (7 to 9)	8 (7 to 9)
Dopamine infusions (%)	23	23
Prenatal corticosteroids (%)	57	55
CRIB score	2 (1 to 5)	3 (1 to 6)

Unless otherwise indicated, data are median (25th to 75th centile). In/outborn, Born inside or outside of the participating hospitals; CRIB, clinical risk index for babies.

Outcome

Primary outcome measures were the concentrations of adrenaline and noradrenaline measured in arterial blood plasma.

To determine the association between these concentrations and the clinical outcome, secondary outcome measures (development of IVH, poor neurological outcome (severe IVH (grade 3 or IVH + apparent periventricular haemorrhagic infarction), periventricular leucomalacia, or death within 28 days), total duration of artificial ventilation, and total duration of NICU stay) were evaluated.

Randomisation and blinding

A power analysis showed that 60 patients per group were needed to achieve a medium effect size (Cohen's *d* = 0.59), with α error of 0.05 (two tailed) and power of 90%.

Neonates had an equal probability of being assigned to either condition, using a randomisation code and stratification into five groups of gestational age ranges (<27, 27–30.6, 31–33.6, 34–36.6, and ≥37 weeks) to obtain a balanced number of morphine and placebo participants within each stratum.

Independent pharmacists, using the computer generated randomisation list, placed ampoules of either 1 ml morphine/HCl or 1 ml placebo into boxes numbered with the study numbers. If a new patient was enrolled, the next box in line for the relevant age group was taken. All research and clinical staff, as well as the parents of the participants, were blinded to the treatment.

Statistical analysis

Multiple regression analyses were used to simultaneously estimate the effect of treatment condition (morphine *v* placebo), the amount of additional open label morphine, gestational age, deviation from mean birth weight, clinical risk index for babies (CRIB),²⁵ sex, and participating centre on the plasma adrenaline and noradrenaline concentrations (means per patient during masked medication infusion) corrected for the baseline concentrations, prenatal corticosteroid use, and dopamine infusion. To achieve normal distributions of adrenaline and noradrenaline concentrations, outcome variables as well as baseline concentrations were logarithmically (ln) transformed. After the use of the enter method, non-significant covariates ($p_{in} < 0.05$; $p_{out} > 0.10$) were excluded from the analyses to minimise the number of covariates in both analyses.

In addition, multiple regression analyses were used with duration of artificial ventilation and duration of NICU stay as outcome variables, predicted by adrenaline and noradrenaline concentrations, corrected by the number of samples per infant. Logistic regression analyses were used with the incidence of IVH and poor neurological outcome as outcome variables, also predicted by adrenaline and noradrenaline concentrations, and corrected for the number of samples per infant. Data were analysed using SPSS statistical software version 10.1 (SPSS Inc, Chicago, Illinois, USA). Multicollinearity was tested by determining the variance inflation factors.

RESULTS

During the inclusion period, 210 patients were eligible; informed parental consent was obtained for 150 of them. No differences in background characteristics between the infants with and without informed parental consent were found.¹⁰ The 150 newborns were randomly allocated to receive morphine or placebo. For practical reasons—that is,

lack of venous/arterial access—and ethical reasons—that is, less than 3 ml/kg of blood sampling allowed for the duration of the study—plasma catecholamine concentrations could not be determined in 23 patients. One other patient was given intravenous noradrenaline because of persistent hypotension and was therefore excluded from analysis. Thus catecholamine concentrations could be determined and analysed for 126 patients (fig 1).

Sixty patients were allocated to receive continuous morphine infusion (31 in centre I, 29 in centre II), and 66 patients to receive placebo (38 in centre I, and 28 in centre II). Median duration of infusion was 47 hours (interquartile range (IQR) 19–92). Infusion was stopped for the following reasons: extubation ($n = 98$), seven days in study ($n = 15$), hypotension (defined using a normative data model for different birth weights; $n = 4$), continuous administration of neuromuscular blockers ($n = 4$), surgery ($n = 2$), deceased ($n = 1$), requiring too much additional morphine ($n = 1$), and overdosing ($n = 1$). Table 1 shows patient characteristics for both treatment groups. They were all similar in the two groups. Plasma adrenaline and noradrenaline concentrations (nmol/L; median (IQR)) were comparable at baseline: adrenaline, 0.22 (0.31) and 0.29 (0.46) in the morphine and placebo treated infants respectively; noradrenaline, 2.52 (2.99) and 2.44 (3.14) in the morphine and placebo treated infants respectively. During the infusion, median adrenaline concentrations were 0.12 (0.28) and 0.18 (0.35) and median noradrenaline concentrations were 2.8 (3.7) and 3.8 (4.0) for the morphine and placebo treated infants respectively.

Multiple regression analysis with the (mean per infant) adrenaline concentration (ln transformed) during masked study medication as outcome variable showed that adrenaline concentrations were not predicted by treatment condition ($B = -0.079$; 95% confidence interval (CI) -0.20 to

Table 3 Clinical outcome for the morphine and placebo treated infants

	Morphine (n = 60)	Placebo (n = 66)
NICU stay (hours)	312 (150 to 552)	288 (138 to 906)
Artificial ventilation (hours)	67 (28 to 126)	73 (28 to 158)
IVH (all grades) (%)	18	38
Poor neurological outcome (%)	5	15

Unless otherwise indicated, data are median (25th to 75th centile). NICU, Neonatal intensive care unit; IVH, intraventricular haemorrhage; poor neurological outcome, severe IVH (IVH grade 3 or IVH + apparent periventricular haemorrhagic infarction), periventricular leucomalacia, or death within 28 days.

Table 2 Multiple regression analysis with ln transformed plasma adrenaline and noradrenaline concentrations (means per patient) as outcome variables

	Adrenaline			Noradrenaline		
	B*	95% CI of B	p Value	B*	95% CI of B	p value
Condition (placebo/morphine)	-0.079	-0.20 to 0.037	0.18	-0.25	-0.48 to -0.027	0.029
Amount of extra morphine†	-0.0091	-0.028 to 0.010	0.34	-0.0081	-0.046 to 0.030	0.67
Deviation birth weight‡	-0.039	-0.077 to 0.001	0.046	-0.091	-0.17 to -0.015	0.019
Dopamine infusion	0.23	0.082 to 0.37	0.003	0.42	0.13 to 0.71	0.005
Prenatal corticosteroids				0.41	0.17 to 0.65	0.001
Participating centre	-0.11	-0.24 to 0.013	0.078	-0.23	-0.47 to 0.004	0.054
	$R = 0.50$, $R_{adj}^2 = 0.20$			$R = 0.64$, $R_{adj}^2 = 0.36$		

Gestational age ($p = 0.81$ and 0.67), baseline adrenaline and noradrenaline concentrations (ln transformed) ($p = 0.21$ and 0.13), and prenatal corticosteroid use (only in adrenaline analyses; $p = 0.42$) were excluded from the analysis ($p > 0.1$) by using the backward method. p Values in bold are significant.

*Unstandardised regression coefficients.

†The mean amount of extra morphine used for each infant in $\mu\text{g/kg}$ per hour during study.

‡Birth weight was compared with normal mean birth weight for each patient, as a measure of small for gestational age infants.

0.037; $p = 0.18$) or by the amounts of additional open label morphine used ($B = -0.0091$; 95% CI -0.028 to 0.010 ; $p = 0.34$). Table 2 shows the details of the regression analysis. Using the mean noradrenaline concentration (ln transformed) during masked study medication as outcome variable, multiple regression analysis showed that plasma noradrenaline concentrations were significantly lower in the morphine group ($B = -0.25$; 95% CI -0.48 to -0.027 ; $p = 0.029$) than in the placebo group. The amount of additional open label morphine used did not significantly influence noradrenaline concentrations (table 2). Infants who received dopamine infusions and those who had low birth weights for their gestational age had higher concentrations of both adrenaline and noradrenaline. Furthermore infants who had received prenatal corticosteroids had significantly higher plasma concentrations of noradrenaline. The adrenaline and noradrenaline concentrations tended to be higher in centre II than centre I.

Table 3 shows clinical outcome measures for the morphine and placebo treated infants. The duration of NICU stay was significantly related to plasma concentration of noradrenaline ($B = 50.2$; 95% CI 20.0 to 80.4 ; $p = 0.001$) but not adrenaline ($B = -137.5$; 95% CI -300.0 to 25.0 ; $p = 0.10$). Neither adrenaline nor noradrenaline concentration predicted the duration of artificial ventilation, incidence of IVH, or poor neurological outcome.

DISCUSSION

In this study we evaluated whether continuous morphine infusion in newborn ventilated infants would reduce stress responses as reflected by plasma concentrations of adrenaline and noradrenaline. Routine morphine infusions were shown to reduce plasma concentrations of noradrenaline ($p = 0.029$), but not adrenaline ($p = 0.18$). The use of additional open label morphine did not influence adrenaline or noradrenaline concentrations. Previous studies have shown lowering of plasma concentrations of adrenaline and noradrenaline by the use of opioids after surgery.^{26, 27} Quinn *et al*¹⁸ showed in a placebo controlled trial that high morphine doses ($100 \mu\text{g/kg}$ for two hours + $25 \mu\text{g/kg/h}$) had effectively decreased adrenaline, but not noradrenaline, concentrations in ventilated neonates after 24 hours compared with placebo treatment. Our and previous studies also show that increased concentrations are not only associated with stress but are also influenced by other variables such as the use of dopamine infusion²⁸ and prenatal corticosteroids, asphyxia, or being born small for gestational age.¹⁷ Our study design (a randomised, placebo controlled trial with multiple regression analyses taking the effects of other variables into account) evaluates the effect of morphine on the concentrations of adrenaline and noradrenaline in the best available and structured way. After the different methods of statistical analysis and the amounts of morphine used, the discrepancy in results between the different studies might also be explained by variability in patient characteristics and treatment of neonates.

Decreased concentrations of noradrenaline were related to a shorter NICU stay, but this was only detected using multiple regression analyses, as median duration of NICU stay was more or less the same for the morphine and placebo treated infants. No other relations between adrenaline/noradrenaline concentrations and clinical outcome were found. Plasma concentrations of adrenaline and noradrenaline have previously been associated with poor outcome in newborns.^{16, 20} Anand *et al*²¹ showed that high concentrations of adrenaline and noradrenaline in preterm neonates after patent ductus arteriosus ligation in the absence of analgesia were associated with high mortality. In another study the same authors showed that a decrease in neonatal stress

response was related to improved clinical stability during and after surgical operations.¹³ Although these findings suggest that outcome in premature neonates might be related to catecholamine concentrations, we were not able to show a relation between adrenaline/noradrenaline concentration and neurological outcome. Different circumstances (surgery *v* no surgery) and different patient criteria—for example, inclusion or exclusion of asphyxiated patients—in previous studies compared with ours may explain this disparity.

Increased neonatal stress has been suggested to change stress responses at older ages.²⁹ Studies in animals have suggested that acute fetal or neonatal stress can alter the trigger concentration of the hypothalamic-pituitary-adrenal axis for life, resulting in changed stress responses at older ages.³⁰ In concordance with this observation, human adrenomedullary and adrenocortical activity were still increased in 12 year old children born small for gestational age compared with full term appropriate for gestational age matched controls.³¹ This mechanism may also partly explain the protective role of analgesics against negative consequences of early pain experience.³² Extrapolation of these data to the patients in our study may lead to the suggestion that those treated with continuous morphine infusion from the first postnatal day onwards might show decreased stress responses at older ages. Peters *et al*³³ showed no difference in cortisol concentrations and pain response to immunisation between toddlers who received pre-emptive morphine after major surgery within the first 3 months of life compared with controls, and Evans *et al*¹⁶ showed no correlation between neonatal catecholamine concentrations and cognitive or motor impairment at 5–6 years. For our specific study population, no further data are yet available. Therefore this highly speculative suggestion is now systematically being evaluated in a follow up study of our cohort of patients at 3 years of age.

Unfortunately we were not able to collect plasma from all patients in our study. Although the amount of blood needed for analysis each time was only 0.6 ml, collection proved to be a problem, particularly in the smallest patients. Our finding that gestational age does not significantly influence catecholamine concentration may counteract this shortcoming. Although it was previously suggested that catecholamine concentrations are higher in preterm than near term infants,^{14, 17, 34} the data of our study suggest that catecholamine concentrations are not related to gestational age but are increased in small for gestational age neonates. Further research is necessary to confirm this. However, the results of our study probably reflect stress responses in neonates within a wide range of gestational ages. Although newborns with severe asphyxia or otherwise high catecholamine concentrations¹⁷ were excluded from our study, we were still able to measure the effects of low morphine doses on plasma noradrenaline concentrations. As neonates in centre II, sampled after suctioning, tended to show higher plasma adrenaline/noradrenaline concentrations than those in centre I, sampled at rest, this probably suggests that adrenaline/noradrenaline concentrations are probably increased by acute stress in neonates. A previous study also showed increased noradrenaline concentrations after suctioning.¹²

As a fast method of analysis is not yet available, determining plasma catecholamine concentrations has only limited usefulness for individual neonatal pain management in daily clinical practice. Evaluation of noradrenaline concentrations is, however, an objective method for detecting evidence of the stress relieving effects of pharmacological agents. Therefore we believe that stress hormone concentrations will be important variables for future studies evaluating pain and the effects of analgesics in particular age groups.

What is already known on this topic

As it is very difficult to measure the short and long term analgesic effects of morphine in neonates, the routine use of continuous morphine for neonatal pain during intensive care treatment is still under debate.

What this study adds

This study shows that plasma noradrenaline, but not adrenaline, concentrations are sensitive markers of neonatal stress and are decreased by the use of continuous morphine infusions. This decreased stress response supports the idea that continuous morphine treatment in ventilated neonates should be part of standard care.

CONCLUSIONS

In this blinded, randomised, placebo controlled trial, we show that routine administration of morphine in ventilated newborns reduces plasma noradrenaline concentrations, suggesting a beneficial effect of routine morphine administration in the neonate. In agreement with our previous report¹⁰ showing no decrease in pain scores by the use of routine morphine infusions in newborns who had received ventilatory support, we also found no decrease in plasma adrenaline concentrations. Follow up of our patients is required to evaluate long term stress responses and outcome.

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Authors' affiliations

S H P Simons, M van Dijk, D Tibboel, Department of Paediatric Surgery, Erasmus MC-Sophia, Rotterdam, the Netherlands

J N van den Anker, Department of Paediatrics, Erasmus MC-Sophia

S H P Simons, D Roofthoof, Division of Neonatology, Erasmus MC-Sophia

F Boomsma, Department of Internal Medicine, Erasmus MC

R A van Lingen, Department of Paediatrics, Division of Neonatology, Isala Clinics, Zwolle, the Netherlands

J N van den Anker, Division of Paediatric Clinical Pharmacology,

Children's National Medical Center, Washington DC, USA

J N van den Anker, Department of Paediatrics and Pharmacology, George Washington University MC, Washington DC, USA

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