Stability of norepinephrine infusions prepared in dextrose and normal saline solutions

[La stabilité des perfusions de norépinéphrine préparées dans des solutions dextrosées ou salées]

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Purpose: Norepinephrine (NE) infusions are commonly used in the intensive care unit and in the operating room. Data on long term stability of NE solutions are lacking. This prospective study was designed to evaluate the stability of NE, in dextrose (5%) in water (D5W) and in normal saline (NS) solutions, for a period up to seven days.

Methods: We prepared norepinephrine solutions in quadruplicate, by aseptically diluting 1 mg NE in 250 mL of D5W or NS and 4 mg NE in 250 mL of D5W or NS (final concentrations, 4 $\mu g \cdot mL^{-1}$ and 16 $\mu g \cdot mL^{-1}$, respectively) and stored the solutions at room temperature under ambient light. We sampled the solutions, in duplicate, at times 0, 24, 48, 72, 120, and 168 hr and stored them at -80°C for later assay. Norepinephrine concentrations were measured by high-performance liquid chromatography with electrochemical detection (coefficient of variation 4.6%). Statistical analysis was done by nonparametric, repeated measures ANOVA (Friedman test).

Results: There was no significant decrease in NE concentration for either, NE 4 $\mu g \cdot mL^{-1}$ in D5W or NS ($P = 0.09$ and 0.11, respectively) or for NE 16 $\mu g \cdot mL^{-1}$ in D5W or NS ($P = 0.18$ and 0.40, respectively). The ratios of NE concentration at 168 hr, compared to baseline, were 95.7% and 96.4%, for NE 4 $\mu g \cdot mL^{-1}$ in D5W and NS, respectively, and 104.5% and 96.4%, for NE 16 $\mu g \cdot mL^{-1}$ in D5W and NS, respectively.

Conclusion: Norepinephrine solutions, in concentrations commonly used in the clinical setting, are chemically stable for seven days, at room temperature and under ambient light, when diluted either in D5W or NS.

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Objectif *: Les perfusions de norépinéphrine (NE) sont fréquemment utilisées aux soins intensifs et en salle d'opération. Toutefois, les données concernant la stabilité à long terme des solutions de NE font défaut. Cette étude prospective a été conçue dans le but d'évaluer la stabilité de la NE préparée dans des solutions de dextrose (5 %) dans l'eau (D5W) et de chlorure de sodium 0,9 % (NS), pendant une durée maximale de sept jours.*

Méthode *: Nous avons préparé des solutions de norépinéphrine en quatre exemplaires en diluant en milieu stérile 1 mg de NE dans 250 mL de D5W ou de NS et 4 mg de NE dans 250 mL de D5W ou de NS (concentrations finales de NE de 4 $\mu g \cdot mL^{-1}$ et de 16 $\mu g \cdot mL^{-1}$, respectivement). Nous avons stocké ces solutions à température et lumière ambiantes. Nous avons échantillonné les solutions en deux exemplaires à 0, 24, 48, 72, 120 et 168 h et les avons stockées à une température de –80°C pour fin d'analyses ultérieures. Les concentrations de norépinéphrine ont été mesurées par chromatographie liquide à haute performance avec une détection électrochimique (coefficient de variation 4,6 %). L'analyse statistique a été effectuée en utilisant une ANOVA (test de Friedman) pour mesures non paramétriques répétées.*

Résultats *: Il n'y a eu de réduction significative dans la concentration de NE dans aucune des solutions, que ce soit NE 4 $\mu g \cdot mL^{-1}$ dans une solution de D5W ou de NS ($P = 0,09$ et 0,11, respectivement) ou pour la solution de NE à 16 $\mu g \cdot mL^{-1}$ dans du D5W ou du NS ($P = 0,18$ et 0,40, respectivement). Les ratios de concentration de norépinéphrine à 168 h, comparées aux données initiales, étaient de 95,7 % et 96,4 % pour la NE 4 $\mu g \cdot mL^{-1}$ dans les solutions de D5W et de NS, respectivement, et de 104,5 % et 96,4 % pour la NE 16 $\mu g \cdot mL^{-1}$ dans les solutions de D5W et de NS, respectivement.*

Conclusion *: Les solutions de norépinéphrine, aux concentrations fréquemment utilisées dans un contexte clinique, sont chimique-*

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ment stables pour sept jours si elles sont conservées à température et lumière ambiantes, qu'elles soient diluées dans des solutions de D5W ou de NS.

INTRAVENOUS norepinephrine (NE) infusions are frequently used in anesthesia, and in intensive care medicine, to treat and to prevent hypotension.[1] The product monograph recommends that NE infusions should only be prepared in dextrose solutions and its maximal shelf time should be limited to no more than 24 hr. It is also recommended that NE solutions should be protected from light, although this recommendation is seldom followed in clinical practice. Many studies, on which these recommendations are based, were conducted before the emergence of modern, precise, assay techniques such as high-performance liquid chromatography (HPLC).[2–4] The few studies performed, using modern measurement technology, reported the stability of NE in dextrose (5%) in water (D5W) at room temperature for a period up to 48 hr.[5–8] Stability of NE solutions for longer periods has not been studied.

The use of normal saline (NS) for preparation of NE solutions, although allowed by the pharmacy department of some Canadian institutions, is currently not recommended.[9,10] This is based on the fact that pH is a major determinant of the degradation of NE: an increase of pH decreasing the stability of NE solutions.[5–10] Although, several decades ago, the pH of NS varied between 7.2 to 7.7, current formulations of NS have a much lower pH (approximately 5.7), closer to the pH of D5W (5.0).[2,10] This should slow the degradation of NE and should, theoretically, prolong the stability period of NE solutions. If NE were prepared in NS, it would avoid unwanted glucose infusion. This benefit would be appreciated, both in the operating room and in the intensive care unit (ICU), where a tight control of blood glucose levels has been recommended.[11,12]

The primary objective of this study was to evaluate the stability of NE in D5W and NS solutions for a period of up to seven days. The secondary objective was to measure the pH of NE solutions and to assess the impact of pH on the degradation of NE.

Materials and methods

The study was conducted at the biochemistry laboratory of the Centre hospitalier affilié universitaire de Québec (Hôpital de l'Enfant-Jésus) using an experimental, prospective design. In the first part of the study, a telephone survey of intensive care units and anesthesiology departments of university hospitals, in the provinces of Quebec and eastern Ontario, was undertaken to identify the most frequently used concentrations of NE solutions for infusion. The results showed that NE concentrations of 4 $\mu g \cdot mL^{-1}$ and 16 $\mu g \cdot mL^{-1}$ were the most frequently used concentrations in anesthesiology and in ICUs, respectively. We thus elected to study these two concentrations.

Experimental protocol

PREPARATION OF SOLUTIONS

We obtained norepinephrine D5W and NS solutions from the pharmacy department of the Hôpital de l'Enfant-Jésus (Quebec City) which were similar to those used in the operating room and in the ICU. Norepinephrine was manufactured by Sabex (Boucherville, QC, Canada); and all ampoules were taken from the same lot. Each ampoule contained 4 mg NE bitartrate, 8.2 mg NaCl, 0.46 mg sodium metabisulfate (as an antioxidant), 1.3 mg anhydric citric acid, 0.9 mg dihydrate sodium citrate, and sterile water to a volume of 4 mL. All solutions were prepared on the same day, by the same investigator (M.T.), using a sterile technique. Solutions were prepared by adding a precise volume of NE to 250-mL polyvinyl chloride bags containing either D5W or NS. Mixing of NE solutions was ensured by agitating bags for three seconds. Four different solutions were prepared in the following manner. One milligram of NE was drawn from the ampoule and added to a 250-mL D5W infusion bag, which yielded a concentration of 4 $\mu g \cdot mL^{-1}$ and was labelled D4. One milligram of NE was drawn from the NE ampoule and added to a 250-mL NS infusion bag, which yielded a concentration of 4 $\mu g \cdot mL^{-1}$ and was labelled S4. Four milligrams of NE were drawn from the NE ampoule and added to a 250-mL D5W infusion bag, which yielded a concentration of 16 $\mu g \cdot mL^{-1}$ and was labelled D16. Finally, 4 mg of NE were drawn from the NE ampoule and added to a 250-mL NS infusion bag, which yielded a concentration of 16 $\mu g \cdot mL^{-1}$ and was labelled S16. Each solution was prepared in quadruplicate. Once prepared, NE solutions were kept for seven days at a constant temperature of 20°C and exposed to ambient light. Storage room temperature was monitored throughout the experiment.

SAMPLING TECHNIQUE AND MEASUREMENT OF NE CONCENTRATION

At times 0, 24, 48, 72, 120, and 168 hr, the first three bags of each solution were aseptically sampled in duplicate (2 mL). Samples were transferred into

test tubes and were stored at -80°C until further assay. Concentrations of free catecholamines were measured by HPLC with amperometric detection. Norepinephrine, in glucose or saline, was diluted 1:40 with dihydroxybenzylamine (as internal standard) in HCl 0.1N. Twenty microlitres were injected into the HPLC system. The catecholamines were then separated by HPLC on a reversed-phase column (Nova-Pak C18; 3.9 × 150 mm; 4-µm bead size; Waters), with a mobile phase of water containing 50 mM anhydrous sodium acetate, 20 mM citric acid, 4 mM sodium 1-ocanesulfonate (as an ion-pairing agent), 0.61 mM triethylamine, 0.5 mM d-n-butylamine, and 0.133 mM Na2EDTA (pH 4.3). The flow rate was 1 $mL \cdot min^{-1}$. The electrochemical detector (Model 464; Waters) was set at +0.6 volt. The following order of elution was observed: norepinephrine and dihydroxybenzylamine. At concentrations of 37.5 and 187.5 $\mu g \cdot L^{-1}$, the inter assay coefficients of variation were 4.6% and 7.4%, respectively.

PH MEASUREMENT

Ten millilitres of the fourth bag of each solution were drawn aseptically using a 10-mL syringe and a 20G needle, before the addition of NE to the solution bag, and then, at times 1, 24, 48, 72, 120, and 168 hr. The pH was measured immediately with the pHmeter PHM64 (Radiometer Inc. Copenhagen, Denmark) using a two-point standardization with buffer solutions. Standardization was repeated before each measurement.

Data analysis

Norepinephrine concentrations were analyzed with nonparametric, repeated measures ANOVA (Friedman test). Temperature is presented as mean ± SD. Norepinephrine concentrations are presented as mean ± SD. Stability of drug concentration was defined as a concentration larger than 90% of the initial concentration.[5,8,13]

Results

The average storage temperature, throughout the seven days of the experiment, was 20.4 ± 1.2°C. Measurements of NE concentrations were made on 144 samples. Norepinephrine concentrations are presented in the Table and in Figures 1 and 2. There was no significant decrease in NE concentration in any of the solutions tested over the seven day study period (D4: $P = 0.09$, S4: $P = 0.11$ D16: $P = 0.18$, S16: $P = 0.40$). The ratios of day seven NE concentrations on baseline concentration were consistently above 90% (Table). The pH values of D5W and of NS, before the addition of NE, were 4.6 and 6.0, respectively.

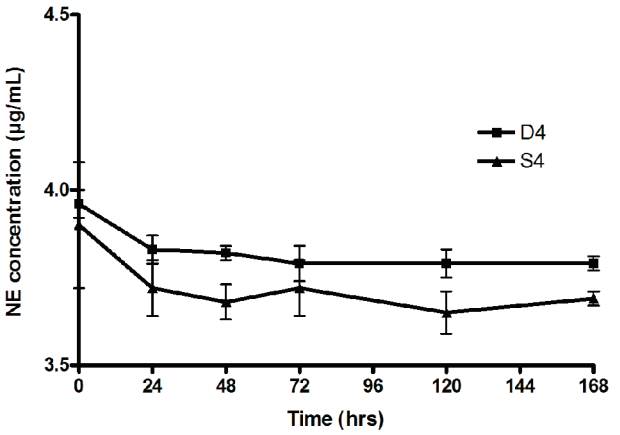

FIGURE 1 Concentrations of norepinephrine (NE) 4 $\mu g \cdot mL^{-1}$ prepared in dextrose 5% in water or normal saline, as measured by high-performance liquid chromatography at baseline, 24, 48, 72, 120, and 168 hr. Concentrations did not change significantly ($p = 0.09$ and 0.11, respectively). D4 = NE 4 $\mu g \cdot mL^{-1}$ in dextrose 5% in water; S4 = NE 4 $\mu g \cdot mL^{-1}$ in normal saline. Data are presented as mean ± SD.

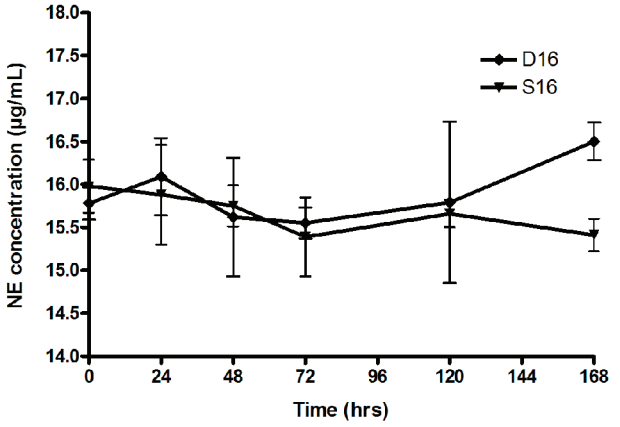

FIGURE 2 Concentrations of norepinephrine (NE) 16 $\mu g \cdot mL^{-1}$ prepared in dextrose 5% in water or normal saline, as measured by high-performance, liquid chromatography at baseline, 24, 48, 72, 120, and 168 hr. Concentrations did not change significantly ($p = 0.18$ and 0.40, respectively). D16 = NE 16 $\mu g \cdot mL^{-1}$ in dextrose 5% in water; S16 = NE 16 $\mu g \cdot mL^{-1}$ in normal saline. Data are presented as mean ± SD.

The pH values of D4 and D16 remained stable over the 168-hr study period. The pH values of S4 and S16 showed an initial decrease to 5.0 and to 4.5, respectively (at one hour), and remained stable for the remainder of the study period (Figure 3).

TABLE Concentration of norepinephrine in the 4 μg·mL^{-1} and in the 16 μg·mL^{-1} solutions, prepared in dextrose 5% in water or in normal saline

Solution	*Baseline*	*168 hr*	*Ratio*
D4	3.96 ± 0.04	3.79 ± 0.02	95.7 (94.9 – 96.5)
S4	3.71 ± 0.08	3.69 ± 0.02	94.6 (90.5 – 97.1)
D16	16.09 ± 0.45	16.50 ± 0.94	104.5 (103.0 – 107.5)
S16	15.88 ± 0.58	15.41 ± 0.19	96.4 (93.4 – 98.5)

Concentration of norepinephrine (NE) measured by high-performance liquid chromatography at baseline and at 168 hr. There was no significant decrease in NE concentration in any of the solutions tested over the seven-day study period (see also Figures 1 and 2). The ratio of NE concentration at 168 hr to baseline concentration was above 90% for all solutions tested. D4 = NE 4 μg·mL^{-1} in dextrose (5%) in water; S4 = NE 4 μg·mL^{-1} in normal saline; D16 = NE 16 μg·mL^{-1} in dextrose (5%) in water; S16 = NE 16 μg·mL^{-1} in normal saline. Data are presented as mean ± SD or as percentages (range).

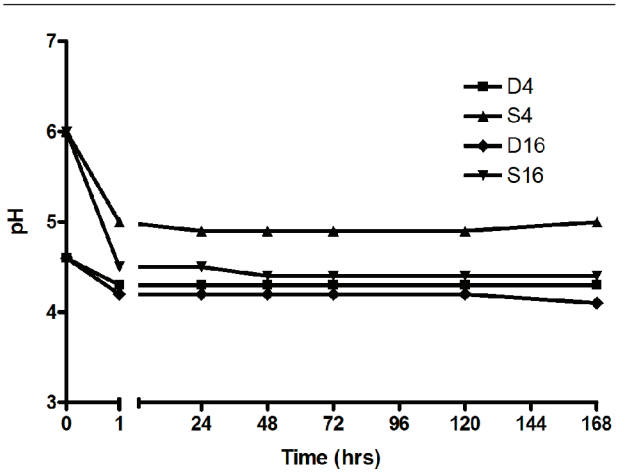

FIGURE 3 The pH of the four norepinephrine (NE) solutions as measured at baseline (before the addition of NE to solution bag), then at 1, 24, 48, 72, 120, and 168 hr. The pH of D4 and D16 remained stable over the 168-hr study period. Initially, the pH of S4 and S16 decreased from 6.0 to 5.0 and to 4.5, respectively (at one hour) and remained stable thereafter.

Discussion

Norepinephrine is frequently used as a vasopressor, both in the operating room and in the ICU.[1] It is identical to the endogenous catecholamines synthesized in the adrenal medulla and in the sympathetic nervous tissue. Both the endogenous molecule and the drug are the levorotatory isomer, which is several times more active than the dextrorotatory isomer. Like other catecholamines, NE in water solutions is subject to degradation by a number of mechanisms, such as oxidation, cyclization, and polymerisation.[13] Degradation is caused by oxygen, heat, light, alkalinity, and the presence of trace elements such as copper and iron.[5,6,14] Synthetic norepinephrine is prepared as the bitartrate salt of levo-norepinephrine. Each 4mL ampoule contains 4 mg NE, with sodium metabisulfate as an antioxidant, and water, with pH adjusted between 3 and 4.5. It is recommended that NE solutions should only be prepared with glucose containing solutions, and should be discarded after 24 hr.[14]

The main finding of this study is that NE can be diluted in NS and that the stability of this solution is comparable to a solution prepared in D5W. This finding has important implications for the clinician, since the infusion of a large volume of D5W can sometimes result in a sizable glucose load in patients for whom hyperglycemia may be detrimental (e.g., brain injury, trauma).[11] Furthermore, it is now recognized that tight control of blood glucose levels may improve outcome in critically ill patients; thus critical care physicians are trying to avoid infusing glucose when deemed unnecessary.[11,15] Although this has important clinical implications, only one study has investigated the degradation of NE in NS using HPLC. Studying concentrations of 4 and 8 μg·mL^{-1}, Baumgartner *et al.*,[9] found that 98% of NE was still present after 24 hr, which is in agreement with the results of our study. However, the authors did not make any further measurements. No previous study has investigated the stability of NE in NS over prolonged periods.

The second important finding is that NE solutions are stable for a considerably longer period than the standard 24-hr recommendation. Such recommendations are probably based on older studies which were conducted before the availability of precise assay techniques. In this study, the NE concentration of all tested solutions remained greater than 90% of the baseline concentration, which is the accepted threshold of concentration stability.[13] Peddicord *et al.*[7] also reported that NE is stable for 24 hr, at a concentration of 64 μg·mL^{-1} in D5W, stored at ambient temperature but protected from light. Stewart *et al.*[8] have reported that NE, at concentrations of 4 and 8 μg·mL^{-1} in D5W, was stable for 48 hr, when exposed to room temperature and ambient light. Newton *et al.*[5] reported the stability of NE concentration in the same conditions up to 36 hr and estimated, by a mathematical extrapolation, that it should be stable up to 100 days. However, this estimation has never been confirmed by direct measurements. Finally, none of those studies fulfilled the requirements for the study of stability of injectable drugs.[13] Our study was able to confirm,

by direct measurement, the stability of NE in D5W and NS, for a period longer that 48 hr. Moreover, we found that NE concentrations were stable in temperature and light conditions that reproduce those normally encountered in the operating room and in the ICU. This has favourable implications from both human resource and economic perspectives.

Although light and temperature have an impact on the degradation of catecholamines, the major determinant is believed to be pH.[4,5,14] Consequently, manufacturers prepare NE ampoules at a pH between 3.0 and 4.5; and only dextrose solutions are recommended for the preparation of catecholamine infusions. According to the manufacturer, the pH of D5W is 5.0, which compares with our measurement (4.6). The pH of NS is now around 5.7, which was confirmed by our measurement (6.0). More interestingly, when either 1 or 4 mg of NE was added to the 250-mL NS bags, pH showed an immediate decrease to 5.0 and to 4.5, respectively. Such pH levels are similar to the pH of D5W, with or without NE. This may explain how the degradation of NE is similar, whether prepared in D5W or in NS, and how the concentrations of NE remained stable over seven days, both in D5W and in NS.

These findings suggest that NE solutions can be used for a much longer period than the 24 hr currently recommended by manufacturers. However, the risk of bacterial contamination of solutions must also be taken into account, even more so when NE is prepared in D5W, which is more likely to sustain bacterial growth. However, in hospitals, where infusions are prepared by the pharmacy department under sterile conditions with a laminar flow hood, NE infusions could be safely kept, either in D5W or NS, for a period much longer than the presently recommended 24 hr.[16] In conclusion, we have shown that NE, in concentrations commonly used in the clinical setting, can be prepared either in D5W or NS; and that such solutions are chemically stable for seven days, at room temperature and under ambient light.

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