An Abstract of the Thesis of

Kenneth R. Dandurand for the degree of Master of Science in Pharmacy presented on April 29, 1983.

Title: The Effect of Phototherapy on Dopamine in Dextrose 5% Water Stability In Vitro

Abstract Approved. 

Douglass J. Stennett

The stability of dopamine in dextrose 5% water subjected phototherapy was assessed.

Solutions of dopamine (1000 mcg/ml) were prepared and triplicate samples were subjected to one of three light (normal, dark, phototherapy) and two flow (2ml/hr. and no flow) conditions at ambient room temperature. Photo-irradiance was maintained between 5.1 and 6.6 watts/cm² throughout the experiment. Samples were assayed at intervals from 0 to 36 hours by high performance liquid chromatography (HPLC).

There was no statistically significant decomposition of dopamine solutions exposed to phototherapy as compared to control samples over time.
Dopamine in dextrose 5% water exposed to phototherapy is stable for 36 hours. The results of this study indicate that the need for additional protection of dopamine from blue light irradiance emitted by phototherapy is not needed to maintain stability during administration to neonates.
The Effect of Phototherapy on Dopamine in Dextrose 5% Water Stability In Vitro.

by

Kenneth R. Dandurand

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Redacted for Privacy

Redacted for Privacy

Head of department of Pharmacy

Redacted for Privacy

Dean of Graduate School

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I. INTRODUCTION

Dopamine (3,4 dihydroxyphenylethylamine) HCL (Intropin®) has been used successfully as a vital ionotropic agent in adults (1,2) and more recently in neonates (3). It is not limited to beta adrenergic stimulating activity but exhibits alpha adrenergic stimulating activity as well as a unique dopaminergic receptor effect. This dose dependent, alpha, beta and dopaminergic activity results in large variations in its pharmacologic action. Low doses, two to five micrograms per kilogram per minute, stimulate dopaminergic receptors found in the renal and mesenteric vasculature causing vasodilation. This shunts blood from the periphery to vital organs in the gut and to the kidney. Beta receptors are stimulated with moderate doses, five to ten micrograms per kilogram per minute, initiating an increase in cardiac output because of increased ionotropic effect. Doses, greater than ten micrograms per kilogram per minute, such as those used in the treatment of shock, have almost entirely an alpha effect resulting in peripheral vasoconstriction.

Neonates, in particular premature infants, frequently require dopamine to maintain adequate kidney perfusion and
systemic arterial pressure.

Phototherapy is commonly used in the treatment of established neonatal hyperbilirubinemia as well as prophylactically in susceptible premature infants (4).

The purpose of this study is to determine the stability of dopamine in dextrose 5% water solutions when exposed to blue light (400-500nm) irradiance from phototherapy units.

Like all catecholamines, the oxidation of dopamine is catalyzed by oxygen, heat, heavy metal ions and light (5,6). The oxidation of dopamine (Figure 1) results in the formation of a quinone followed by cyclization to an adrenochrome and indoles. This reaction results in a pink-brown coloration of the solution. Polymerization of these products forms dark brown melanin-like compounds. These products of dopamine oxidation are therapeutically inactive (7). To enhance stability, dopamine (Intropin®) is packaged in light resistant containers with the addition of the antioxidant sodium bisulfite (5). Dopamine is formulated at a pH of 3.0-4.5 to maintain optimal stability as an alkaline environment enhances and promotes degradation (5). The manufacturer also recommends that dopamine be stored at room temperature but away from light to insure stability (8). Gardella et al. (5) have shown that dopamine in eight of nine intravenous solutions
Figure 1. Chemical degradation of dopamine
tested, including dextrose 5% water, is chemically and physically stable when subjected to heat (25-55°C) and light (natural and fluorescent) for forty-eight hours. This indicates that dopamine will not lose its potency under normal hospital conditions. However, the specific environment of a neonatal intensive care unit, with radiant warmers, incubators and phototherapy units, may subject this drug to more intense heat and light conditions which may lead to its degradation.

Within the visible spectrum, blue light has the shortest wavelength. According to Planck's theory, the shorter the wavelength of light the higher the energy per photon; thus, blue light (400-500nm) has a higher energy than other portions of the visible spectrum. This increased energy enhances the photodegradation of drugs (9).

Phototherapy of hyperbilirubinemia involves the use of visible light in the 400-500 nanometer spectral range. It has been shown that bilirubin undergoes maximal photodecomposition in this blue region of the light spectrum (10,11). These studies have shown a direct dose/response relationship between blue light irradiance and decrease in bilirubin levels in jaundiced infants.

One of the most important factors in maximizing bilirubin degradation is the irradiance of the light source (11). Irradiance is a measure of light energy per
unit surface area (microwatts/cm²) while illuminance is the degree of light intensity (foot-candles). Normal fluorescent daylight sources have a high degree of illuminance; however, they do not have high energy per surface area in the blue region. This makes them less effective in bilirubin degradation than lights with a high irradiance in the blue region of the visible light spectrum. Lights with an irradiance between four and six microwatts per cm² are effective in decreasing serum bilirubin levels in neonates (12,13). At this level of radiant flux a photo-oxidation (14,15) and a more recently discovered photoisomerization (15) occur which significantly accelerates bilirubin turnover.

Excess unconjugated bilirubin can cross the blood brain barrier and is toxic to the central nervous system. Serum bilirubin levels greater than 20 mg per 100 ml are associated with an increased risk of kernicterus which can be fatal. Sufficient data have shown that phototherapy can prevent serum bilirubin (unconjugated) levels from rising to 15 mg per 100 ml; (16,17) therefore, the need for phototherapy is evident. Despite the life saving potential of phototherapy and its widespread use, little is known of its specific adverse effects. Phototherapy in vitro has been reported to cause alterations in DNA (18), riboflavin (19), and protein (20). These changes have not been confirmed and the clinical significance is
unclear. However, these and other studies (21) imply that phototherapy may cause in vitro alterations of light sensitive drugs in intravenous solutions.

Neonatal dopamine doses usually range from two to twenty-five micrograms per kilogram per minute. Many neonates are likely to weigh less than two kilograms; therefore, the total dose of dopamine administered per minute will be relatively small. A common admixture for dopamine in pediatric patients is one hundred milligrams in one hundred milliliters of dextrose 5% water. At this concentration the infusion rate may be as low as approximately two milliliters per hour. This slow infusion rate, additionally necessitated by the need to minimize fluid intake, may enhance the degradation of light sensitive drugs such as dopamine by increasing exposure time to light sources. A solution of dopamine at this rate may be subjected to the light from phototherapy units for as long as six hours in the intravenous tubing or longer in the intravenous bottle. Plastics of the type used in intravenous tubing and glass intravenous bottles will filter out light of wavelengths below three hundred nanometers; however, they allow light of greater wavelengths to pass through (9). Therefore, the wavelength of light emitted by phototherapy (400-500nm) may affect the drug in solution.
Dopamine in dextrose 5% water was analyzed over 36 hours to determine if a significant decrease in concentration occurred when exposed to phototherapy. Temperature, flow rate, light sources and initial dopamine concentration were controlled in order to assess the specific effects of blue light irradiation. The statistical null hypothesis: there is no significant decomposition of dopamine (greater than ten percent) in dextrose 5% water when exposed to phototherapy (blue lights 400-500 nm) as compared to solutions exposed to normal fluorescent light or dark control conditions over thirty-six hours. The alternative hypothesis: there is a significant decomposition of dopamine (greater than ten percent) in dextrose 5% water when exposed to phototherapy as compared to solutions exposed to normal fluorescent light or dark control conditions over thirty-six hours.
II. METHODOLOGY

REAGENTS AND MATERIALS

The reagents - citric acid, sodium phosphate dibasic and acetonitrile\(^a\), used in the preparation of the high performance liquid chromatography (HPLC) mobile phase were analytical grade or better. Dopamine hydrochloride powder\(^b\) was used in the generation of the standard curve. Dopamine hydrochloride injection\(^c\) in dextrose 5% water \(^d\), USP, was used throughout the experimental procedure. All assays were conducted using beta-hydroxyethyl theophylline \(^e\) as the internal standard. All water used was deionized by a Milli Q water purification system \(^f\). McGaw pediatric intravenous tubing \(^g\) was used with all intravenous admixtures of dopamine. The phototherapy unit consisted of four F4OB Sylvania blue bulbs\(^h\). All chemical reagents were weighed on a Mettler H18 analytical balance\(^i\).

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\(^a\) J.T. Baker Chemical Co. Philipsburg NJ.
\(^b\) American Critical Care, McGaw Park, IL. Lot #420-50A
\(^c\) American Critical Care, McGaw Park, IL. Lot #A2008
\(^d\) McGaw Laboratories, Santa Anna CA. Lot #J2B028A
\(^e\) Pierce Chemical Co., Rockford IL. lot #31590
\(^f\) Millipore Corporation, Bedford, MA.
\(^g\) McGaw Laboratories, Santa Anna CA. Lot #P2D006
\(^h\) Sylvania GTE, Danvers, MA.
\(^i\) Mettler Instrument Corp., Hightstown, NJ.
INSTRUMENTATION

Chromatographic analysis was performed using a Waters model U-6000 high performance liquid chromatograph (HPLC) pump with a model 440 variable wavelength detector. All injections were made with a Wisp 710B automatic injector. A Corning model 7 pH meter was used for all pH readings. Irradiance measurements of the light source were made with an Olympic Mark II bilimeter spectroradiometer with a type B sensor.

CHROMATOGRAPHIC CONDITION

A Waters C-18 Bondapak column (3.9mm i.d. x30cm) consisting of organosilane bonded to polar porous silica with a 10 micron particle size was used for the HPLC separation. The mobile phase was made using an aqueous portion containing citric acid 0.1M, disodium phosphate buffer 0.2M at a pH of 4 and an organic portion of 7% acetonitrile. The mobile phase was deaerated by water bath ultrasound under vacuum for 20 seconds. The fluid rate for HPLC separation was 2.0ml/min with a column back pressure of 2000 PSI. Retention time was approximately four minutes for dopamine and eleven minutes for beta-hydroxyethyl theophylline.

i. Mettler Instrument Corp., Hightstown, NJ.
j. Waters Associates, Milford, MA.
k. Corning Glass Works, Medfield, MA.
l. Olympic Medical Co., Seattle, WA.
m. Branson Instrument Co., Shelton, CN
PROCEDURES

The reverse phase HPLC assay used in this experiment for detecting dopamine concentrations was developed by modification of the methods of Flood(22) and Mell(23). Each of these methods was concerned with the isolation of urinary catecholamines. The specificity of the assay used in this study was determined by degrading the dopamine in solution with sodium bicarbonate until the solution was a pink-brown color due to the breakdown products of dopamine. The assay procedure, with identical chromatographic conditions as used in the experiment, was then performed. Peak heights for the dopamine were reduced accordingly and the retention time was identical to the standard calibration curve. No new peaks indicating degradation products of dopamine appeared on the chromatograph. This is consistent with both the Mell and Flood findings.

Dopamine solutions at 100mg/100ml were compounded by adding the appropriate amount of dopamine injection (Intropin® 200mg/5ml) to 100ml of dextrose 5% water under aseptic conditions. Three bottles with attached intravenous tubing were exposed to normal fluorescent, dark or phototherapy light conditions. Identical groups
subjected to these same conditions with the addition of a flow rate of two ml/hour controlled by a Gilson Minipulse 2 model HP pump$^2$ were also assayed (Table 1).

The intravenous admixture set, glass bottle and poly-vinylchloride tubing, were exposed to the phototherapy unit at a distance of 45 cm. Irradiance levels were maintained between 5.1 and 6.6 microwatts per cm$^2$ throughout the experimental conditions (Table 2). The flow rate was based on a theoretical infant weighing 2 kg and receiving 15-20 micrograms/kilogram/minute. All solutions were maintained at ambient room temperature.

Fifty microliter samples were taken at 0, 2, 4, 6, 8, 16, 24 and 36 hours from the tubing. An equal volume of the internal standard (beta-hydroxyethyl theophylline) was pipetted into vials with the samples. These were then sealed, vortexed for 30 seconds and stored in a refrigerator for less than twelve hours until HPLC analysis was conducted. Vials were then placed into the automatic injector. Injection volume was set at one microliter for all sample solutions. The run time for all samples was 11 minutes with a detector sensitivity of 0.05.

A four point standard curve was generated using concentrations of dopamine in dextrose 5% water from 900 to 1050 micrograms per milliliter in increments of 50 micrograms per milliliter (Figure 2).
Table 1.

Experimental and control conditions.

<table>
<thead>
<tr>
<th>Group</th>
<th>Light</th>
<th>Flow</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>No Flow</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>Normal</td>
<td>Flow</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>Dark</td>
<td>No Flow</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>Dark</td>
<td>Flow</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>Phototherapy</td>
<td>No Flow</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>Phototherapy</td>
<td>Flow</td>
<td>3</td>
</tr>
</tbody>
</table>

Total Number of Samples 18
Table 2.

Irradiance measurements of blue light.a

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Readings (microwatts/cm²)</th>
<th>30cm</th>
<th>60cm</th>
<th>90cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td></td>
<td>5.5</td>
<td>6.6</td>
<td>6.1</td>
</tr>
<tr>
<td>24</td>
<td></td>
<td>5.3</td>
<td>6.2</td>
<td>6.1</td>
</tr>
<tr>
<td>36</td>
<td></td>
<td>5.1</td>
<td>6.1</td>
<td>6.3</td>
</tr>
</tbody>
</table>

aMeasurements were taken 45 cm from a bank of four blue lights in the center of light exposure at 30 cm intervals.
STATISTICAL METHODS

Analysis of variance (ANOVA) was used to determine main and interactive effects of light and flow conditions on the dependent variable (concentration) over the thirty-six hour study period. Mean square for main and interactive effects were compared to the mean square error (within treatment error) to obtain an F statistic. Values of p less than 0.05 were considered significant which would result in rejection of the null hypothesis.
Figure 2. Assay standard curve

$F(x) = 0.01657 \cdot \text{CONC} - 8.719 \quad R = 0.9940$
III. RESULTS

Eighteen samples at 1000 micrograms/milliliter of dopamine in dextrose 5% water were analyzed after exposure to light and flow conditions (Table 1). Drug concentrations (dependent variable) were determined using the linear regression equation for the standard curve (Figure 3).

The standard curve for the 1000 microgram/milliliter sample had a correlation coefficient of 0.9940 and a mean square error of 4.6904.

Mean concentration-time data for each category (Table 3.) indicate that dopamine admixtures have a coefficient of variation less than 7.0% for all samples.

It is clear that there is no dopamine degradation as indicated by the analysis of variance (Table 4) for main and interactive effects. The main and interactive effects over the thirty-six hour study period (Table 5) show similar results. The F-statistic for the main and interactive effects was consistent with not rejecting the null hypothesis (p<0.05) in all cases. There was a statistically significant change in concentration with respect to time as a main effect (p= 0.0002). This did not
Table 3.

Mean concentration-time data (with time interaction)

<table>
<thead>
<tr>
<th>Time</th>
<th>Normal</th>
<th>Normal</th>
<th>Dark</th>
<th>Dark</th>
<th>Photo</th>
<th>Photo</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>941±25</td>
<td>945±22</td>
<td>934±12</td>
<td>939±20</td>
<td>964±9</td>
<td>950±16</td>
</tr>
<tr>
<td>2</td>
<td>945±4</td>
<td>949±10</td>
<td>947±14</td>
<td>955±12</td>
<td>950±20</td>
<td>952±24</td>
</tr>
<tr>
<td>4</td>
<td>936±10</td>
<td>935±12</td>
<td>935±17</td>
<td>921±10</td>
<td>941±31</td>
<td>934±18</td>
</tr>
<tr>
<td>6</td>
<td>921±13</td>
<td>926±50</td>
<td>950±5</td>
<td>944±18</td>
<td>952±14</td>
<td>919±24</td>
</tr>
<tr>
<td>8</td>
<td>919±13</td>
<td>936±4</td>
<td>953±39</td>
<td>931±12</td>
<td>927±17</td>
<td>927±17</td>
</tr>
<tr>
<td>16</td>
<td>951±42</td>
<td>928±6</td>
<td>896±43</td>
<td>919±46</td>
<td>924±13</td>
<td>936±11</td>
</tr>
<tr>
<td>24</td>
<td>951±23</td>
<td>965±66</td>
<td>929±27</td>
<td>929±28</td>
<td>929±18</td>
<td>941±50</td>
</tr>
<tr>
<td>36</td>
<td>953±5</td>
<td>972±21</td>
<td>983±55</td>
<td>930±28</td>
<td>974±49</td>
<td>973±17</td>
</tr>
<tr>
<td>C.V.</td>
<td>4.5</td>
<td>6.9</td>
<td>5.9</td>
<td>4.9</td>
<td>5.1</td>
<td>5.3</td>
</tr>
</tbody>
</table>

*aExpressed as mean concentration + standard deviation.

*bCoefficient of variation for largest standard deviation divided by mean. Values in percent.
Table 4.

Analysis of variance for main and interactive effects
(1000 mcg/ml)

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>Degrees of Freedom</th>
<th>Mean Square</th>
<th>F</th>
<th>Tail Prob.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light</td>
<td>1049.50</td>
<td>2</td>
<td>524.751</td>
<td>.31</td>
<td>.73</td>
</tr>
<tr>
<td>Flow</td>
<td>169.86</td>
<td>1</td>
<td>169.86</td>
<td>.10</td>
<td>.76</td>
</tr>
<tr>
<td>LxF</td>
<td>865.08</td>
<td>2</td>
<td>432.54</td>
<td>.26</td>
<td>.78</td>
</tr>
<tr>
<td>Error</td>
<td>20093.02</td>
<td>12</td>
<td>1674.42</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5.

Analysis of variance for main and interactive effects over time (1000 mcg/ml)

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>Degrees of Freedom</th>
<th>Mean Square</th>
<th>F</th>
<th>Tail Prob.</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>18486.96</td>
<td>7</td>
<td>2640.99</td>
<td>4.65</td>
<td>.0002</td>
</tr>
<tr>
<td>RxL</td>
<td>10255.78</td>
<td>14</td>
<td>732.561</td>
<td>1.29</td>
<td>.23</td>
</tr>
<tr>
<td>RxF</td>
<td>1833.81</td>
<td>7</td>
<td>261.97</td>
<td>.46</td>
<td>.86</td>
</tr>
<tr>
<td>RxLxF</td>
<td>7773.55</td>
<td>14</td>
<td>555.28</td>
<td>.98</td>
<td>.48</td>
</tr>
<tr>
<td>Error</td>
<td>47674.56</td>
<td>84</td>
<td>567.55</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*aTime variable = R, Light variable = L, Flow variable = F*
hold true for the interactive effects of time with light and flow (p=0.48).

Therefore, there was no statistically significant decomposition in dopamine solutions when subjected to experimental conditions of phototherapy as compared to control situations.
IV. DISCUSSION

Dopamine, as previously reported by Gardella et al. (4), is physically and chemically stable for at least 48 hours when exposed to natural and fluorescent light at room temperature. This present study concerns itself with the more specific effect of blue light irradiance on dopamine stability.

The test conditions to which dopamine in dextrose 5% water were subjected were designed to simulate the hospital pediatric intensive care setting with respect to phototherapy. Under clinical conditions, administration of dopamine to neonates at slow infusion rates is common due to its potency and the size and nature of fluid restriction in premature infants. This subjects dopamine solutions to blue light irradiance for up to 24 hours.

Recent evidence has shown that light sensitive chemicals may be altered by the presence of phototherapy (18-21). The nature and length of exposure may subject dopamine to conditions which may alter its stability and its resultant effectiveness. Due to its light sensitive characteristics, protection from blue light irradiance may be warranted to assure adequate dose and response in the neonate.
Results of this study indicate that there is no statistically significant difference in dopamine stability among samples exposed to the various light (p>0.73) and flow conditions (p>0.75). Also with respect to the interaction between flow and light there was no significant difference (p>0.77).

Similar results can be seen by analyzing these effects over time. The effect seen over time with the various light conditions (p>0.23), flow (p>0.85) and interaction (p>0.48) indicate no significant difference as compared to control samples.

Although there is statistically significant differences in concentrations seen over time alone, there was no significance when test conditions were included as interactive effects. This statistical significance with respect to time is not clinically significant as dopamine is maintained within ten percent of initial concentration (Table 3) in compliance with accepted guidelines (24).
V. CONCLUSION

Concern over the stability of light sensitive dopamine exposed to blue light irradiance (400-500 nm) prompted the present study. The data presented herein suggest that there is no significant degradation of dopamine at 1000 micrograms per milliliter when exposed to phototherapy as compared to control samples over thirty-six hours. Despite the fact that statistically there were changes seen over time, the light conditions had no effect on these changes. Therefore, no additional protection of dopamine solutions (1000 micrograms per milliliter) from blue light irradiance is warranted to insure stability when exposed to phototherapy.


