Mechanisms of dopamine and dobutamine interference in biochemical tests that use peroxide and peroxidase to generate chromophore

Brad S. Karon, Thomas M. Daly, and Mitchell G. Scott

Dopamine and dobutamine have recently been shown to produce a negative interference in several biochemical tests that use peroxide and peroxidase to generate a chromophore. To define the chemical mechanism of this interference, we examined the effects of dopamine and dobutamine in various peroxidase-based biochemical tests. Dopamine interfered stoichiometrically with peroxidase-based tests that use 4-aminophenazone to form chromophore but interfered little in those that use other compounds to generate chromophore. Dopamine reacts with 4-aminophenazone in the presence of peroxide and peroxidase to form a novel quinone-imine dye, with a smaller absorptivity than the chromophore formed in the absence of dopamine. The smaller absorptivity of this novel chromophore results in negative interference by reducing the total absorbance at the wavelength used to measure analyte. In contrast, dobutamine interfered stoichiometrically with all peroxidase-based tests studied, regardless of whether 4-aminophenazone was used to form the chromophore. Dobutamine was rapidly oxidized by peroxide in the presence of peroxidase, thus depleting the peroxide necessary to generate chromophore. Dopamine and dobutamine demonstrate two distinct general mechanisms of interference in peroxidase-based biochemical tests.

Negative interference by the catecholamines dopamine and dobutamine has recently been reported in methods that use hydrogen peroxide and horseradish peroxidase to generate chromophores [1–3]. We demonstrated recently that dobutamine interfered extensively with enzymatic creatinine measurement on the Vitros analyzer, whereas dopamine interfered far less [1]. Another study reported interference by small amounts of dopamine in several enzymatic creatinine methods on the Eppendorf Epos Analyzer [2]. Furthermore, dopamine interferes with triglyceride, cholesterol, and uric acid measurement on the Hitachi analyzer [1, 3]. It has been speculated that the mechanism of this interference involves dobutamine and dopamine oxidation by peroxide/peroxidase, thus depleting the hydrogen peroxide necessary to produce an indicator chromophore [1, 3].

Such a mechanism has been described for interference by reducing agents such as ascorbic acid and acetaminophen in methods that use horseradish peroxidase to generate dyes, including glucose and uric acid measurement [4–6]. In these methods, the analyte of interest initiates a series of reactions that generates peroxide, and the amount generated is proportional to the concentration of the analyte. The resulting hydrogen peroxide (in the presence of peroxidase) oxidizes compounds that do not absorb visible light into compounds that do (i.e., generates a chromophore). Reducing agents are thought to interfere with peroxidase-based methods because they are oxidized more readily than the indicator dyes and deplete the peroxide necessary to generate the chromophore [5, 6]. Many of these interferences have been minimized by optimizing the indicator dye systems [4, 7].

Dopamine and dobutamine may also interfere with peroxidase-based tests by acting as reducing agents. Dopamine can be oxidized by peroxide in vitro to produce dopamine o-quinone (Fig. 1A) [8], whereas dobutamine oxidation by peroxide has not been studied in vitro or in vivo. In many clinical biochemical methods, peroxide oxidizes 4-aminophenazone, which combines with a phenolic compound to form a strongly absorbing quinone-imine dye, as described by Trinder (Fig. 1B) [9].

Dopamine and dobutamine may interfere with these tests by either: (a) depleting the peroxide necessary to oxidize chromophore (Fig. 1A); (b) interfering in the formation of chromophore, i.e., interfering with the Trinder reaction or similar reactions (Fig. 1B); (c) affecting

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the stability of formed chromophore, i.e., reducing the formed chromophore (Fig. 1C); or (d) some other mechanism not related to generation of chromophore by peroxide. Here, we determined the mechanisms responsible for dopamine and dobutamine interference in peroxidase-based tests by examining dopamine and dobutamine oxidation in vitro, and we correlated this with interference in clinical biochemical methods. The results define two distinct general mechanisms of interference in peroxidase-based biochemical tests.

Materials and Methods

Instrumentation. Plasma concentrations of creatinine, cholesterol, and uric acid were measured on a Hitachi 747 analyzer (Boehringer Mannheim), according to the manufacturer’s procedures. Creatinine and uric acid were also measured on a Vitros 750 analyzer (Johnson and Johnson Clinical Diagnostics). Absorbance spectra were recorded on a Beckman DU 650 spectrophotometer (Beckman Instruments).

Reagents. Uric acid/PAP and cholesterol/HP reagents were obtained from Boehringer Mannheim. Dopamine HCl injection (80 g/L) was obtained from American Regent Labs. Dobutamine HCl injection (12.5 g/L) was obtained from Abbott Labs. Hydrogen peroxide (300 g/L) solution, horseradish peroxidase type II (200 kU/L, EC 1.11.1.7), HEPES, and 4-aminoantipyrine (4-amino-phenazone) were purchased from Sigma Chemical Co.

Plasma experiments. Plasma pools were prepared from randomly chosen nonicteric, nonhemolyzed excess samples received by the Barnes-Jewish Hospital Clinical Chemistry Laboratory for routine testing. Various concentrations of dopamine and dobutamine were added to pooled plasma and the above biochemical tests were performed. Dopamine was added to the plasma pool such that the total sample dilution was <1%, and dobutamine was added such that the total sample dilution was <2%. Final dopamine and dobutamine concentrations varied between 8 and 320 mg/L. Separate plasma pools were used for experiments involving dopamine and dobutamine. Duplicate samples were run under each condition, and the average of the two samples is reported.

Absorbance spectra of dopamine and dobutamine. Absorbance spectra were obtained by using a 1-cm quartz cuvette containing a total volume of 1 mL. Absorbance spectra of dopamine or dobutamine were determined in 75 mmol/L HEPES, pH 6.8, at 37 °C in the presence or absence of hydrogen peroxide and horseradish peroxidase. To measure dopamine and dobutamine oxidation, 40 mg/L (0.261 mmol/L) dopamine or 80 mg/L (0.265 mmol/L) dobutamine was added to HEPES buffer in the presence of various concentrations of horseradish peroxidase. After an initial absorbance measurement in the absence of peroxide, hydrogen peroxide was added to attain a final peroxide concentration of 9 mmol/L, and the absorbance spectra were recorded after a 5-min incubation.

Absorbance spectra of uric acid and cholesterol reagents. Absorbance spectra of uric acid/PAP and cholesterol/HP reagents were measured by preparing the reagents according to the manufacturers’ recommendations and using a 1-cm quartz cuvette with 1-mL total volume described above. The absorbance of uric acid and cholesterol reagents was measured after the addition of hydrogen peroxide and (or) dopamine or dobutamine. For experiments in which the absorbance of uric acid reagent was measured after the addition of plasma, plasma was added to uric acid reagent in the same proportion as outlined in the Boehringer Mannheim uric acid/PAP package insert, with a total volume of 1 mL rather than 368 μL.

Results

Effects of dopamine and dobutamine on peroxidase-based tests. Various amounts of dopamine and dobutamine were added to pooled plasma to determine their effects on several biochemical tests that use peroxide/peroxidase. Cholesterol, uric acid, and triglyceride tests on the Hitachi analyzer all use peroxide/peroxidase to generate qui-
none-imine dyes, based on the reaction originally described by Trinder (Fig. 1B) [9]. These test reagents all contain 4-aminophenazone and either phenol (cholesterol), 2,4,6-tribromo-3-hydroxybenzoic acid (uric acid), or 4-chlorophenol (triglyceride). The creatinine and uric acid reagents used on the Vitros analyzer do not contain 4-aminophenazone but use peroxide/peroxidase in a similar manner to generate a triarylimidazole leuco dye (creatinine) or an imidazole dye (uric acid). Creatinine measurement on the Hitachi analyzer, which is not peroxidase-based, was used as a control.

Dopamine and dobutamine did not show negative interference with creatinine as measured on the Hitachi analyzer but did produce a negative interference with all the peroxidase-based tests studied (Table 1). Dopamine interference in the Hitachi cholesterol, uric acid, and triglyceride methods was consistent with a 1:1 stoichiometry (Fig. 2A). For instance, a dopamine:analyte molar ratio of 0.5:1 resulted in ≥50% negative interference in all three procedures. In contrast, dopamine interference with the Vitros creatinine and uric acid methods was not consistent with stoichiometric inhibition because a >5:1 ratio of dopamine:analyte was necessary to produce >50% negative interference (Fig. 2A). Interestingly, dobutamine interference was consistent with a 1:1 stoichiometry (dobutamine:analyte) on both the Hitachi and Vitros analyzers for all peroxidase-based tests examined (Fig. 2B).

**Effects of dopamine and dobutamine on the absorbance of uric acid reagent.** To directly examine the effects of dopamine and dobutamine on chromophore formation in the Boehringer Mannheim reagents, we measured the absorbance of the uric acid/PAP reagent in the presence and absence of peroxide, dopamine, and dobutamine. In the absence of peroxide, the uric acid reagents showed no absorbance in the visible range. When peroxide was added, an absorbance spectrum with a maximum at 510 nm appeared rapidly, with dye formation complete within 5 min at 25 °C. When dopamine was added before peroxide, it clearly interfered with dye formation at 5 min (Fig. 3A). Absorbance of the uric acid reagent at 510 nm decreased from 2.26 ± 0.02 to 1.50 ± 0.02 (34%) in the presence of a 0.3 molar ratio of dopamine:peroxide, to 0.79 ± 0.01 at a ratio of 0.59 (65%) (not shown), and to 0.30 ± 0.01 at a molar ratio of 1.18:1 dopamine:peroxide (Fig. 3A). Dobutamine, added before peroxide, interfered with chromophore formation in the uric acid reagent in a similar manner (Fig. 3B). However, addition of dopamine or dobutamine at a 1:1 molar ratio with peroxide after chromophore formation (after a 5-min incubation with peroxide) had no effect on chromophore absorbance (not shown). We also measured uric acid reagent absorbance after the addition of pooled plasma with a uric acid concentration of 64 mg/L. Addition of plasma to uric acid reagent also resulted in an absorbance spectrum with a maximum at 510 nm. When dobutamine was added

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**Table 1. Effects of dopamine and dobutamine on measurements made on Hitachi 747 and Vitros 750**

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* Separate plasma pools were used for dopamine and dobutamine experiments. Values shown are the average of duplicate determinations.

**Fig. 2.** Values from Table 1 for cholesterol (■), uric acid (●), and triglyceride (†) measured on a Hitachi analyzer and creatinine (■) and uric acid (●) measured on a Vitros analyzer, plotted to reflect the percentage of interference of dopamine and dobutamine.

The percentage of interference is defined as follows: [control (no dopamine or dobutamine) − observed]/control × 100. (A) Percentage of interference vs moles dopamine per mole analyte. (B) Percentage of interference vs moles dobutamine per mole analyte.
(before plasma) at a ratio of 0.44 mol of dobutamine per mole of uric acid, $A_{510}$ was reduced by $\sim 50\%$ ($0.27$ to $0.13$), whereas the addition of $0.87$ mol of dobutamine per mole of uric acid reduced $A_{510}$ even further (Fig. 3C).

Similar results were observed when dopamine and dobutamine were added to Boehringer Mannheim cholesterol/HP reagent either before or after addition of peroxidase (not shown). Thus, dopamine and dobutamine interfered with dye formation and had no effect on either the initial reactions necessary to produce peroxide or the stability of the formed chromophore. However, it is not possible to determine from these experiments whether dopamine and dobutamine were oxidized more rapidly than the chromophore, and thus depleted the peroxide generated (Fig. 1A), or directly interfered with the Trinder reaction (Fig. 1B).

**Oxidation of dopamine.** To determine the mechanism of dopamine and dobutamine interference in chromophore formation, we studied oxidation of dopamine and dobutamine by peroxidase in vitro. The Boehringer Mannheim reagents for cholesterol, uric acid, and triglyceride all contain $<1000$ U/L horseradish peroxidase in buffers between pH 6.8 and 7.8, and absorbance is measured after a 5-min incubation at $37\,^\circ C$. To determine whether dopamine and dobutamine could be oxidized under these conditions, the absorbance spectra of dopamine and dobutamine were determined in the presence and absence of peroxidase in a HEPES buffer, pH 6.8, with 500 U/L horseradish peroxidase, after a 5-min incubation at $37\,^\circ C$. Dopamine and peroxidase in HEPES buffer alone (pH 6.8) showed no visible absorbance. When excess peroxide was added and allowed to incubate at $37\,^\circ C$ for 5 min, the solution slowly turned brown. A small, broad absorbance was present with a maximum at 465 nm (Fig. 4A), which is consistent with dopamine $\alpha$-quinone ($\epsilon_{465} = 2455 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$) [10] (Fig. 1A). However, only $30\%$ of the added dopamine was converted to dopamine $\alpha$-quinone within 5 min at $37\,^\circ C$ (moles of dopamine $\alpha$-quinone = $A_{465}/2455$). This is consistent with a previous report.

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**Fig. 3. Absorbance spectra.**

(A) Absorbance spectra of Boehringer Mannheim uric acid/PAP reagent after a 5-min incubation at $25\,^\circ C$ in the presence of (a) 44.1 $\mu$mol/L peroxide, (b) peroxide plus dopamine at a molar ratio of 0.3:1 (dopamine:peroxide), and (c) peroxide plus dopamine at a molar ratio of 1.18 (dopamine:peroxide). One experiment is shown; values in the text represent the average of three experiments. (B) Absorbance spectra of Boehringer Mannheim uric acid reagent after a 5-min incubation at $25\,^\circ C$ in the presence of (a) 44.1 $\mu$mol/L peroxide, (b) peroxide plus dobutamine at a molar ratio of 0.25:1 (dobutamine:peroxide), and (c) peroxide plus dobutamine at a molar ratio of 1.17 (dobutamine:peroxide). (C) Absorbance of Boehringer Mannheim reagent after a 5-min incubation at $25\,^\circ C$ in the presence of (a) plasma with a uric acid concentration of 64 mg/L, (b) plasma plus dobutamine at a molar ratio of 0.44:1 (dobutamine:uric acid), and (c) plasma plus dobutamine at a molar ratio of 0.87:1 (dobutamine:uric acid).

**Fig. 4. Absorbance spectra.**

(A) Absorbance spectra of dopamine in HEPES buffer, pH 6.8, as described in Materials and Methods. Absorbance spectra shown are (a) dopamine and peroxidase in the absence of peroxide, (b) dopamine, 500 U/L peroxidase, and peroxide after a 5-min incubation at $37\,^\circ C$, (c) dopamine, 20 kU/L peroxidase, and peroxide after a 5-min incubation at $37\,^\circ C$, and (d) dopamine, 4-amino-phenazone, 500 U/L peroxidase, and peroxide after a 5-min incubation at $37\,^\circ C$. (B) Absorbance spectra of dobutamine in HEPES buffer, pH 6.8, as described in Materials and Methods. Absorbance spectra shown are (a) dobutamine and peroxidase in the absence of peroxide, (b) dobutamine, 500 U/L peroxidase, and peroxide after a 5-min incubation at $37\,^\circ C$, and (c) dobutamine, 4-amino-phenazone, 500 U/L peroxidase, and peroxide after a 5-min incubation at $37\,^\circ C$. 
demonstrating that dopamine oxidation to dopamine o-quinone is slow at neutral pH [8]. Horseradish peroxidase at 20 kU/L was necessary to achieve oxidation of 87% of added dopamine at 5 min (Fig. 4A).

A much different result was seen when a twofold molar excess of 4-aminophenazone (to dopamine) was incubated with dopamine and 500 U/L peroxidase in HEPES buffer, pH 6.8. This ratio of 4-aminophenazone (to dopamine) was used because 4-aminophenazone was present in at least twofold molar excess of dopamine in all experiments performed on the Hitachi analyzer (Table 1). In the absence of peroxide, no visible absorbance was observed (not shown). After the addition of excess peroxide, this solution rapidly turned pink, and a large absorbance spectrum with a maximum at 490 nm (rather than 465 nm) was observed after 5 min at 37 °C (Fig. 4A). This reaction (between dopamine and 4-aminophenazone) was more rapid than the formation of dopamine o-quinone and was complete within 5 min, there being no further change in absorbance after an additional 10-min incubation at 37 °C (not shown). Thus, dopamine and 4-aminophenazone most likely react to form a novel chromophore, and this may account for the negative interference in tests using 4-aminophenazone. In the tests performed on the Vitros analyzer (which do not use 4-aminophenazone), slow oxidation of dopamine to dopamine o-quinone (i.e., depletion of peroxide) likely accounts for the much lesser interference seen.

Oxidation of dobutamine. Dobutamine can be oxidized to adrenochrome, which produces a bright pink color (Eli Lilly, technical information on file); oxidation of dobutamine by peroxide/peroxidase, however, has not been described. When dobutamine was incubated in HEPES buffer, pH 6.8, at 37 °C in the presence of 500 U/L horseradish peroxidase, no absorbance in the visible range was observed. After the addition of peroxide, the solution rapidly turned pink. An absorbance spectrum with a maximum at 485 nm was observed, which is consistent with adrenochrome (ε$_{485}$ = 3800 L · mol$^{-1}$ · cm$^{-1}$) [11]. Oxidation of dobutamine to adrenochrome was >95% complete within 5 min (moles of adrenochrome = A$_{485}$/3800) (Fig. 4B). In the presence of a twofold molar excess of 4-aminophenazone (to dobutamine), a slightly larger absorbance at 485 nm was observed (Fig. 4B).

Dobutamine oxidation, in the absence and presence of 4-aminophenazone, produced an absorbance spectrum with a maximum at 485 nm. Thus, it was not possible to determine whether a specific reaction took place between dobutamine and 4-aminophenazone. However, because oxidation of dobutamine to adrenochrome is rapid (complete within 5 min) under the Boehringer Mannheim reagent conditions, it is clear that reaction with 4-aminophenazone is not required for dobutamine to interfere in peroxidase-based tests. Thus, dobutamine most likely interferes with peroxidase-based biochemical tests by depleting the peroxide necessary to oxidize chromophore.

Discussion

Dopamine and dobutamine interference with biochemical tests has been described only recently. The first report of dopamine interference was a study that found that low amounts of dopamine (20–100 mg/L) interfere with four different enzymatic methods for creatinine determination. All four methods used 4-aminophenazone to generate chromophores [2]. Our previous study found that similar amounts of dopamine did not greatly interfere with the enzymatic measurement of creatinine on a Vitros analyzer (which does not use 4-aminophenazone), whereas small amounts of dobutamine produced a large negative interference [1]. A recent report found that dopamine (10–100 mg/L) interfered with the measurement of triglyceride, cholesterol, and uric acid on a Hitachi 917 analyzer [3]. The present study explains why dopamine interferes in some, but not all, peroxidase-based tests and provides information about the mechanism of dopamine and dobutamine interference in peroxidase-based reactions.

Stoichiometric interference by dopamine is observed only in tests that use 4-aminophenazone to form chromophore. Whereas oxidation of dopamine to dopamine o-quinone is slow under the Boehringer Mannheim reagent conditions, the reaction between 4-aminophenazone and dopamine is rapid under these same conditions (Fig. 4A). The oxidation product formed in the presence of dopamine and 4-aminophenazone has an absorbance maximum at 490 nm, different from the absorbance maximum of dopamine o-quinone (465 nm) and closer to the absorbance maximum of the quinone-imine dye formed by the uric acid reagent (510 nm, Fig. 3A). Thus, dopamine directly interferes with the Trinder reaction by reacting with 4-aminophenazone to form a novel quinone-imine dye, which accounts for dopamine negative interference (i.e., dopamine acts as the phenolic compound in the Trinder reaction). The slower oxidation of dopamine to dopamine o-quinone may account for the lesser extent of interference observed in peroxidase-based tests that do not use 4-aminophenazone.

To interfere with the biochemical tests, the absorbance of the novel quinone-imine dye must be less than the absorbance of the chromophore formed in the absence of dopamine. The absorbivity of the novel quinone-imine dye must be similar to that of dopamine o-quinone (~2455 L · mol$^{-1}$ · cm$^{-1}$), because the total absorbance of dopamine reacted with 4-aminophenazone was similar to the absorbance of the same amount of dopamine oxidized to dopamine o-quinone (Fig. 4A). In contrast, the absorbivity of the quinone-imine dye usually formed in the uric acid reagent (in the absence of dopamine) must be much greater. When 44.1 μmol/L hydrogen peroxide was added to uric acid reagent, an absorbance of 2.26 was observed at 510 nm (Fig. 3A). Given the stoichiometry of 2 mol of peroxide per mole of dye produced (see the
Boehringer Mannheim procedure for uric acid/PAP), the absorbivity of the quinone-imine dye usually formed in the uric acid reagent must be \(>100\,000\,\text{L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}\). Thus, dopamine reaction with 4-aminophenazone interferes with peroxidase-based tests by preventing the formation of the proper quinone-imine dye, thereby reducing the total absorbance at the detection wavelength (505 nm).

In contrast to dopamine, stoichiometric interference by dobutamine was observed in all peroxidase-based tests studied, regardless of whether 4-aminophenazone was used. Because it was not possible to determine the peroxidase concentration on the Vitros slides, we were unable to duplicate in solution dopamine or dobutamine oxidation on the Vitros slides. Dobutamine oxidation under the Boehringer Mannheim reagent conditions was rapid, with complete oxidation to adrenochrome occurring in <5 min. The absorbance of dobutamine in the presence of 4-aminophenazone was also slightly greater than in the absence of 4-aminophenazone (Fig. 4A). Because the absorbance maxima were identical (485 nm), it was not possible to determine whether dobutamine reacted with 4-aminophenazone to form a novel chromophore. Even if this reaction occurred, it is not necessary for dobutamine interference, as evidenced by the stoichiometric interference with peroxidase-based reactions that do not use 4-aminophenazone. Thus, dobutamine most likely interferes with peroxidase-based tests by depleting peroxide.

Taken together, it is clear that dopamine and dobutamine interference in peroxidase-based tests are the result of two distinct mechanisms. Dopamine reacts with 4-aminophenazone to form a novel chromophore, whereas dobutamine depletes the peroxide necessary to oxidize chromophore. Steady-state plasma concentrations of dopamine and dobutamine in vivo are \(<1\,\text{mg/L}\), even when multiple catecholamine agents are administered simultaneously [12–14]. Thus, only intravenous fluid contamination of plasma samples is expected to produce interference by dopamine and dobutamine. In a previous study, the concentration of dopamine and dobutamine resulting from a 50 mL/L intravenous contamination of plasma was 415 mg/L for dobutamine and 160 mg/L for dopamine [11]. Thus, a small (1–5%) intravenous contamination from a dopamine solution may produce interference with any peroxidase-based test that uses 4-aminophenazone to form chromophore. A small intravenous contamination from a dobutamine solution may interfere with any peroxidase-based test. The extent of dopamine or dobutamine interference can be predicted from the molar ratio of dopamine/dobutamine to analyte. By understanding the mechanisms of dopamine and dobutamine interference in peroxidase-based tests, the extent of interference from intravenous contamination by either catecholamine can be predicted.

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References