Catecholamine Inotrope Resuscitation of Antibiotic-Damaged Staphylococci and Its Blockade by Specific Receptor Antagonists

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The increasing use of antibiotic-coated catheters, such as those containing rifampin or minocycline, has led to a decrease in catheter colonization by staphylococci but not to a decrease in the incidence of catheter-related bloodstream infection (BSI). Because catheters are used for the administration of catecholamine inotropes to maintain cardiac function, we examined whether 2 commonly employed inotropes, dopamine and norepinephrine, could affect bacterial viability after exposure to rifampin and minocycline. Rifampin inhibition and minocycline inhibition of staphylococcal growth could be reversed by exposure to dopamine or norepinephrine as a result, in part, of catecholamine-mediated increased provision of host-sequestered iron. The simultaneous addition of inotropes with an antibiotic did not affect antibiotic susceptibility. Inotrope-induced growth in bacteria previously exposed to antibiotics was blocked by the inclusion in culture media of specific catecholamine-receptor antagonists. Considered collectively, these results provide a mechanistic basis for understanding how host-related factors, such as inotrope-based therapeutics, may influence the recovery of antibiotic-stressed bacteria in clinical settings.

The ability of staphylococci to colonize indwelling medical devices, such as central venous catheters (CVCs), is recognized as the most common source of infection encountered in the intensive care setting [1]. The incidence of nosocomial infections has been estimated at approximately 2 million cases per year, and approximately half of those cases are associated with indwelling medical devices, such as CVCs [2]. The majority of catheter-associated nosocomial infections are caused by coagulase-negative staphylococci, and the normal skin commensal Staphylococcus epidermidis is responsible for 50%–70% of reported cases [3]. Coagulase-positive staphylococci, such as S. aureus, are involved to a lesser extent, but CVC-related infections due to these organisms still present a significant comorbidity risk with no effective antibiotic treatment [4]. In an effort to combat CVC-related infection and the possible subsequent progression to catheter-related bloodstream infection (BSI) [5], the use of antimicrobial-impregnated catheters, most prominently those incorporating rifampin and minocycline antimicrobial formulations, has been proposed and evaluated in a number of clinical studies [6–10]. However, concern about the efficacy of antimicrobial-impregnated CVCs in the prevention of catheter-related BSI persists [10, 11], as does their possible contribution to the emergence of drug-resistant clones [12, 13].

Previous work has demonstrated that medications administered through CVCs, such as the catecholamine inotropes used to maintain cardiac function, can directly contribute to the ability of very low cell numbers of S. epidermidis to increase their planktonic growth level [14] and their ability to form biofilms on intravenous catheter material [15] in the presence of host tissue fluids such as plasma. The ability of the catecholamine inotropes, most notably norepinephrine, dopamine, and dobutamine (an analogue of dopamine), to increase both bacterial growth and the production of virulence-associated factors has been noted for both gram-negative [15–21] and gram-positive [14, 15, 22] patho-
obtained from Amersham Life Science. SAPI medium, solved in DMSO and minocycline in distilled water; antibiotic prepared as described elsewhere [17, 26]. Rifampin was dissolved in DMSO with or without 100 µmol/L of catecholamine (norepinephrine or dopamine) [15]. Cultures were then incubated for 24 hours at 37°C in a 5% CO₂ humidified incubator, and enumerated by use of pour-plate analysis on Luria broth agar [25].

To ensure maximal antibiotic sensitivity for the second experimental approach, overnight staphyloccocal cultures grown in serum-SAPI medium were cultured twice in fresh serum-SAPI medium to mid-exponential phase (OD₆₀₀ ~0.4; ~10⁸ cfu/mL) before being supplemented with rifampin or minocycline at approximately 100 times the MIC (5 µg and 2 µg/mL, respectively); controls consisted of bacteria similarly prepared and cultured, but without the antibiotic, and diluted to give an equivalent OD₆₀₀ to that of the less well-grown, antibiotic-treated cultures. Antibiotic-treated cultures and control cultures were then incubated for an additional 4 h, and the degree of antibiotic damage was determined with viable counts of serially diluted cultures plated onto Luria agar (this method also produced a precise enumeration of the bacterial inoculum size for each analysis). Antibiotic-treated cultures and untreated controls were then serially diluted into fresh serum-SAPI medium that contained 100 µmol/L catecholamine. Unless otherwise stated, cultures were incubated at 37°C in a 5% CO₂ humidified incubator for up to 3 days, and viable growth levels were determined at the times indicated [25].

**Catecholamine response and antagonism assays.** Catecholamine antagonism assays were performed in serum-SAPI medium supplemented with concentrations of the compounds shown in the text. Controls comprised equivalent volumes of the solvent used to dissolve the catecholamine or antagonist. To determine whether an antagonist was directly inhibitory to bacterial growth, all antagonism of catecholamine-growth induction assays were also performed in the presence of a concentration of iron which overcomes the iron-limitation of serum-SAPI medium (100 µmol/L Fe(NO₃)₃) and allows maximal bacterial growth levels [14]. Unless stated otherwise, bacteria were inoculated into serum-SAPI medium at ~50–100 cfu-mL. Cultures were incubated statically at 37°C in a 5% CO₂ humidified incubator for 24 h [26] and enumerated by pour-plate analysis [25].

**Transferrin-iron uptake analysis.** ⁵⁵Fe-labelled transferrin (⁵⁵Fe-Tf) was prepared as described elsewhere [25]. Exponentially growing staphyloccoci were inoculated at 10⁶ cfu/mL into serum-SAPI medium supplemented with 10⁵ cpm of ⁵⁵Fe-Tf with or without 100 µmol/L norepinephrine. Cultures were incubated at 37°C in a 5% CO₂ humidified incubator for 6 h, harvested by centrifugation at 5000 g for 5 min, washed in PBS, and assayed for cell numbers and ⁵⁵Fe incorporation by use of pour-plate analysis and scintillation counting as previously described [25].

**MATERIALS AND METHODS**

**Bacteria, chemicals, and growth medium.** S. epidermidis and S. haemolyticus (wound isolates) were obtained from Leicester Public Health Laboratory, Leicester Royal Infirmary (Leicester, United Kingdom) and S. aureus strains Newman and 832–4 were obtained from the Department of Genetics, University of Leicester. All chemicals were purchased from Sigma with the exception of ⁵⁵FeCl₃ (specific activity 5 mCi/mg Fe), which was obtained from Amersham Life Science. SAPI medium, L-(−)-norepinephrine bitartrate and dopamine hydrochloride were prepared as described elsewhere [17, 26]. Rifampin was dissolved in DMSO and minocycline in distilled water; antibiotic stocks were stored at ~20°C, and diluted into culture media immediately prior to use.

**Study design.** Two experimental approaches were employed: (1) determination of the antibiotic MIC for staphyloccoci in the presence or absence of inotropes when inotropes and antibiotics were added simultaneously (2) addition of inotropes after staphyloccoci had been exposed to levels of antibiotic at or above the MIC. For the first experimental approach, initial determinations of antibiotic MICs were performed in serum-SAPI medium, to more closely approximate in vivo conditions [27]. After growing overnight in serum-SAPI medium, staphyloccoci were inoculated into fresh serum-SAPI medium at 1:50 dilution and cultured for ~3 h until exponential growth was achieved (optical density at 600 nm [OD₆₀₀], ~0.4; ~10⁸ cfu/mL). Loss of viability in the antibiotic-treated cultures, compared with non-treated controls, was usually 3–4 logs. Replicate cultures were diluted to ~10⁶ cfu per mL into fresh serum-SAPI medium supplemented simultaneously with increasing concentrations of rifampin or minocycline with or without the addition of 100 µmol/L of catecholamine (norepinephrine or dopamine) [15].

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Statistical analysis. All growth assays were performed in triplicate and performed on at least 3 separate occasions. Where appropriate, statistical analysis was performed by use of an unpaired t test in which a 2-tailed P value was calculated (Instat; GraphPad). Statistical significance was defined as P < .05.

RESULTS

Effect of catecholamine inotropes on sensitivity of S. epidermidis and S. aureus to rifampin and minocycline (first experimental approach). Figure 1A shows the growth of S. epidermidis in response to increasing concentrations of rifampin in the presence or absence of 100 μmol/L norepinephrine or dopamine. After incubation for 24 h, plate counts showed that the growth level of S. epidermidis in the presence of concentrations of rifampin near the MIC (0.05 μg/mL) was increased when catecholamines were present (P < .001), although the organisms' overall antibiotic sensitivity was largely unchanged. Rifampin concentrations of 1 μg/mL and higher were markedly more bactericidal to S. epidermidis, with the result that norepinephrine and dopamine were no longer as effective at increasing cell numbers, compared with their effect on nonsupplemented cultures (data not shown). When analogous experiments were performed for S. aureus Newman (figure 1B), a similar pattern of catecholamine-induced growth enhancement in the presence of rifampin was observed (P < .001). Analyses of changes in staphylococcal growth rates in response to a wide range minocycline concentrations also revealed that the simultaneous addition of inotropes did not significantly change the MIC, though, as with rifampin, the level of bacterial growth was increased at minocycline concentrations around the MIC (0.02 μg/mL; data not shown).

Figure 1. Effect of adding increasing concentrations of rifampin simultaneously with catecholamine inotropes (experimental design 1). Analyses of Staphylococcus epidermidis and S. aureus isolates simultaneously exposed to antibiotics and catecholamine inotropes were performed in serum-SAPI medium, as described in Materials and Methods. The cultures (inoculated at 1 x 10⁶ cfu/ml) were incubated at 37°C for 24 h and counts of viable bacteria were performed by use of pour-plate analysis, as described elsewhere [17]. The results shown here are the mean of triplicate cultures. The SE of the mean was not shown. A, growth rate of S. epidermidis (inoculum size, 1.00 x 10⁶ cfu/ml); B, growth rate of S. aureus Newman (inoculum size, 0.92 x 10⁶ cfu/ml). White bar, no additions (control); black bar, 100 μmol/L norepinephrine; gray bar, 100 μmol/L dopamine.

Effect of catecholamine inotropes on growth of staphylococci previously exposed to rifampin and minocycline (second experimental approach). The ability of bacteria to survive exposure to antimicrobial-coated CVCs in a damaged state and then enter the systemic circulation may be a relevant pathway by which staphylococci cause catheter-related BSI. To examine whether the presence of catecholamines could affect the survival of bacteria after the bacteria had been exposed to antibiotics, we analyzed the response of staphylococci that had been exposed for 4 h to rifampin or minocycline at 100 times the MIC (5 μg and 2 μg/mL, respectively), and then serially diluted directly into fresh serum-SAPI medium with or without 100 μmol/L catecholamine. All bacteria were then incubated for an additional 24 h and viable cells were enumerated as described above.

Rifampin. Figure 2A shows that prior exposure of S. epidermidis and S. haemolyticus to rifampin resulted in ≥3 log decrease in the number of viable cells. However, both norepinephrine and dopamine were able to significantly increase the growth level of S. epidermidis previously exposed to rifampin, even when the antibiotic carryover was near to the MIC (the 10⁻² dilution) (P < .001). Figure 2B shows a comparative growth profile for the untreated control cultures. Figure 2A shows growth levels after 24 h; the growth levels of the bacteria after a total of 72 h incubation are shown in figure 2C. Because the viable cell count of the S. epidermidis inoculating culture in figure 2A was 5.40 x 10⁴ cfu/mL, the number of viable cells in the 10⁻³ and 10⁻⁶ dilutions were nominally ≤1 cell/mL. It can be seen that while there were only small increases in the numbers of bacteria in the cultures not supplemented with catecholamine, in the presence of norepinephrine or dopamine S. epidermidis reached the maximal level of growth supported by serum-SAPI medium, even though the carryover concentration of rifampin was still inhibitory (P < .001). Moreover, S. epidermidis isolates that had been previously exposed to 10 times the MIC of rifampin for as long as 6 days were still able to respond to norepinephrine and dopamine, as evidenced by the increased growth level when
compared with cultures not supplemented with catecholamine (figure 2D) \( (P < .001) \). Similar results to those shown for \textit{S. epidermidis} were also obtained for \textit{S. haemolyticus} (figure 2E and 2F).

Previous work has shown that \textit{S. aureus} and other coagulase-positive staphylococcal strains evidence little increase in growth in response to catecholamines in serum-SAPI medium [17]. This result was confirmed by the responses to norepinephrine and dopamine observed for \textit{S. aureus} strains Newman and 832–4 cultures not treated with an antibiotic (figure 3B and 3D), which showed that the enhancement of growth was evident at very low cell densities \( (\leq 10 \text{ cfu/mL}) \). However, antibiotic treatment of the \textit{S. aureus} cultures (figure 3A and 3C) caused the bacteria to become more responsive to the catecholamine \( (P < .001) \). This being so, the growth profile observed over the \textit{S. aureus} culture dilutions is different from that obtained for \textit{S. epidermidis}.}

\textbf{Figure 2.} Effect of catecholamine inotropes on growth of coagulase-negative staphylococcal strains previously exposed to rifampin (second experimental approach). Panels A–D, Replicate cultures of exponentially growing \textit{Staphylococcus epidermidis} cultures grown in serum-SAPI medium were incubated for 4 h at 37°C with 5 \( \mu \text{g/mL} \) rifampin (100 times the MIC), as described in Materials and Methods. Antibiotic-treated cultures (inoculum size, \( 5.40 \times 10^4 \text{ cfu/mL} \)) and untreated control cultures (inoculum size, \( 1.05 \times 10^8 \text{ cfu/mL} \)) were each serially diluted in 10-fold dilution steps into fresh serum-SAPI medium with no additions \( (\text{white bar}) \) or supplemented with norepinephrine \( (\text{black bar}) \) or dopamine \( (\text{gray bar}) \) to a final concentration of 100 \( \mu \text{mol/L} \). Test and control cultures were incubated at 37°C for 24 h \( (\text{panel A, B, and D}) \) and 72 h \( (\text{panel C}) \), and viable cells levels were enumerated by use of pour-plate analysis as described in Materials and Methods. Panels A and C, growth levels of \textit{S. epidermidis} 24 and 72 h after rifampin treatment; panel B, growth levels of untreated \textit{S. epidermidis} control culture; panel D, growth levels of \textit{S. epidermidis} after 6 days’ exposure to 0.5 \( \mu \text{g/mL} \) rifampin \( (\text{inoculum size, } 2.63 \times 10^4 \text{ cfu/mL}) \); panels E and F, replicates of exponentially growing \textit{S. haemolyticus} cultures grown in serum-SAPI medium were treated with 5 \( \mu \text{g/mL} \) rifampin (100 times the MIC) and further processed as described above for \textit{S. epidermidis}. Antibiotic-treated cultures \( (\text{inoculum size, } 7.35 \times 10^4 \text{ cfu/mL}) \) \( (\text{panel E}) \) and control cultures \( (\text{inoculum size, } 2.07 \times 10^8 \text{ cfu/mL}) \) \( (\text{panel F}) \) were incubated at 37°C for 24 hours and then viable cell levels were enumerated for as described for \textit{S. epidermidis}. Results shown in panels A–F represent the mean of duplicate cultures; the SE of the mean was not \( > 7\% \) for all of the cultures shown. Asterisk, statistically significant increase in growth level compared with the corresponding nonsupplemented control culture \( (P < .001) \).
Acquisition of transferrin-bound iron by staphylococci. Earlier reports [14, 15, 17] have demonstrated that the growth of coagulase-negative staphylococci in inotrope-supplemented serum-SAPI medium is the result of inotrope-mediated provision of iron from the serum iron-binding protein transferrin. We therefore investigated whether the ability of \( S. \) aureus strains to grow effectively in the absence of inotrope support (figures 3B and 3D) was caused by possession of host-iron acquisition systems that were more effective than those possessed by coagulase-negative staphylococci. Iron-uptake assays were used to test the ability of each of the staphylococcal strains to acquire iron (in the form of \( ^{55} \text{Fe} \)) from \( ^{55} \text{Fe}-\text{Tf} \) in both the presence and absence of norepinephrine. As shown in table 1, significant incorporation of transferrin-derived \( ^{55} \text{Fe} \) in coagulase-negative staphylococci only occurred in the presence of norepinephrine, whereas in the absence of norepinephrine, both \( S. \) aureus cultures were able to acquire up to 10 times more iron, compared with the coagulase-negative organisms. However, norepinephrine supplementation still increased \( ^{55} \text{Fe} \) uptake from transferrin for \( S. \) aureus, as described elsewhere [14].

Blocking responsiveness to catecholamine in \( S. \) epidermidis by use of catecholamine-receptor antagonists. Recent studies from our laboratories have demonstrated that catecholamine-induced growth in enteric bacteria can be blocked by use of drugs employed therapeutically as catecholamine receptor antagonists [23]. A range of \( \alpha \)-adrenergic, \( \beta \)-adrenergic, and dopaminergic antagonists were therefore used to determine whether they could similarly block the responses of \( S. \) epidermidis to norepinephrine and dopamine. Table 2 shows that the nonselective \( \beta \)-adrenergic receptor antagonist propranolol had no effect on the ability of norepinephrine or dopamine to induce growth in \( S. \) epidermidis. Other \( \beta \)-adrenergic antagonists, such
Methods. Viable counts of the antibiotic treated cultures after treatment were enumerated by use of pour-plate analysis, as described in Materials and Methods. Viable counts of the antibiotic treated cultures after treatment were incubated at 37°C for 24 h and viable cell levels were normalized for differences in optical density, compared to untreated control cultures, and then the test cultures were each serially diluted in 10-fold dilution steps into fresh serum-SAPI medium with or without supplements. Variation within individual assay sets was <5%, and between experiments, it was <10%.

### Table 1. Uptake of transferrin-complexed iron from serum-SAPI medium by *Staphylococcus*.

<table>
<thead>
<tr>
<th>Organism</th>
<th>In medium without norepinephrine</th>
<th>In medium with norepinephrine</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus Newman</em></td>
<td>1398 ± 23</td>
<td>2990 ± 123</td>
</tr>
<tr>
<td><em>S. aureus 843–2</em></td>
<td>1366 ± 78</td>
<td>3099 ± 56</td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>123 ± 20</td>
<td>3330 ± 11</td>
</tr>
<tr>
<td><em>S. haemolyticus</em></td>
<td>163 ± 4</td>
<td>4800 ± 34</td>
</tr>
</tbody>
</table>

NOTE. Exponentially growing *staphylococci* were inoculated at 10^6 cfu/mL into serum-SAPI supplemented with 10^6 cpm of 55Fe-labelled transferrin (55Fe-Tf) in the absence and presence of 100 μmol/L norepinephrine. Cultures were incubated at 37°C in a 5% CO_2 humidified incubator for 6 h, harvested by centrifugation at 5000 g for 5 min, washed in PBS, and assayed for cell numbers and 55Fe incorporation by use of pour-plate analysis and scintillation counting. Assays were performed in triplicate on at least 2 occasions. There were no significant difference in cell numbers between control cultures and norepinephrine-supplemented cultures. Variation within individual assay sets was <5%, and between experiments, it was <10%.

Figure 4. Effect of catecholamine inotropes on growth of coagulase-negative staphylococcal strains previously exposed to minocycline. Replicates of exponentially growing *Staphylococcus epidermidis*, *S. haemolyticus*, and *S. aureus* (strains Newman and 832–4) cultured in serum-SAPI medium were incubated for 4 h at 37°C with 2 μg/mL minocycline (100 times the MIC), as described in Materials and Methods. Viable counts of the antibiotic treated cultures after treatment were as follows: *S. epidermidis*, 5.40 × 10^8 cfu/ml; *S. haemolyticus*, 5.0 × 10^4 cfu/ml; *S. aureus* strain Newman, 8.90 × 10^4 cfu/ml; and *S. aureus* strain 832–4, 5.10 × 10^4 cfu/ml. Cultures were normalized for differences in optical density, compared to untreated control cultures, and then the test cultures were each serially diluted in 10-fold dilution steps into fresh serum-SAPI medium with no additions (white bar) or supplemented with norepinephrine (black bar) or dopamine (gray bar) to a final concentration of 100 μmol/L. Test cultures were incubated at 37°C for 24 h and viable cell levels were enumerated by use of pour-plate analysis, as described in Materials and Methods. Results shown represent the mean of triplicate cultures. The SE of the mean was not >7% for all cultures shown. Asterisk, statistically significant increase in growth level, compared with the corresponding nonsupplemented control culture (P < .001). Panels A–D, growth levels after 24 h incubation of minocycline-treated *S. epidermidis* (A), *S. haemolyticus* (B), *S. aureus* strain Newman (C), and *S. aureus* strain 832–4 (D).

as labetalol, atenolol, and yohimbine, were similarly without effect (data not shown). In marked contrast, the α-adrenergic antagonists phenotamine (table 2) and prazosin (data not shown) were able to inhibit norepinephrine-induced growth by >3 log orders, in a concentration-dependent manner, compared with control cultures not supplemented with an antagonist (P < .001). None of the α- or β-antagonists induced growth in *S. epidermidis* when tested alone, even at concentrations of 500 μmol/L. Furthermore, addition of Fe(NO_3)_3 overcame antagonist blockage of growth induction (table 2), which indicated that growth inhibition by the α-adrenergic receptor antagonists did not result from any cellular toxicity due to the antagonist, but instead represented a specific antagonism of the bacterial response to the catecholamines.

Interestingly, the results presented in table 2 also show that the α- and β-adrenergic antagonists caused little inhibition of the *S. epidermidis* response to dopamine. From the perspective of eukaryotic receptors this should not be surprising, given that dopamine does not operate through either α- or β-adrenergic receptors, but instead represented a specific antagonism of the bacterial response to the catecholamines. Inclusion of apomorphine and haloperidol (nonselective) and raclopride (D_2-specific) did not alter the ability of dopamine to induce growth in *S. epidermidis* (data not shown). However, the D_2 receptor antagonist chlorpromazine was able to block growth in response to dopamine by >3 log orders (P < .001) (table 2). Chlorpromazine had no significant effect on norepinephrine-induced growth and by itself did not induce growth. The addition of Fe(NO_3)_3-induced growth in dopamine-supplemented *S. epidermidis* cultures that contained chlorprom-
Table 2. Antagonism of catecholamine-induced growth in Staphylococcus epidermidis.

<table>
<thead>
<tr>
<th>Antagonist, catecholamine</th>
<th>Growth at antagonist concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolamine</td>
<td>0 µmol/L</td>
</tr>
<tr>
<td>Norepinephrine, 50 µmol/L</td>
<td>8.01</td>
</tr>
<tr>
<td>Norepinephrine, 50 µmol/L + Fe(NO₃)₃, 100µmol/L</td>
<td>8.21</td>
</tr>
<tr>
<td>Dopamine, 50 µmol/L</td>
<td>8.01</td>
</tr>
<tr>
<td>Propranolol</td>
<td>8.02</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>8.01</td>
</tr>
<tr>
<td>Dopamine, 50 µmol/L</td>
<td>8.00</td>
</tr>
<tr>
<td>Dopamine, 50 µmol/L + Fe(NO₃)₃, 100µmol/L</td>
<td>8.06</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>8.03</td>
</tr>
</tbody>
</table>

**NOTE.** All data are log⁻¹⁰ cfu/mL. *S. epidermidis* was inoculated at approximately 10⁹ cfu/mL into duplicate 1-mL aliquots of serum-SAPI medium containing catecholamines plus the concentrations of antagonists shown in the table, incubated for 24 h, and enumerated for growth. Norepinephrine supplemented cultures containing an α-adrenergic (phenolamine) antagonist, but not those with a β-adrenergic (propranolol) antagonist, showed significant decreases in growth levels, compared with control cultures supplemented only with catecholamines (P < .001). Similarly, dopamine-supplemented cultures containing the dopaminergic antagonist chlorpromazine showed significant decreases in growth levels, compared with control cultures (P < .001). Further, the adrenergic antagonist did not affect dopamine-induced growth; similarly the dopaminergic antagonist did not inhibit norepinephrine-induced growth. Results shown are representative data from at least 3 separate experiments; all data points showed variation of <5%.

a Growth level for culture with phenolamine and no catecholamine supplement, 3.90 log⁻¹⁰ cfu/mL.
b Note that catecholamine antagonist assays containing Fe(NO₃)₃ were also performed, and it was confirmed, by the absence of an effect on bacterial growth induction by iron, that the antagonists were not toxic.
c Growth level for culture with propranolol and no catecholamine supplement, 4.00 log⁻¹⁰ cfu/mL.
d Growth level for culture with chlorpromazine and no catecholamine supplement, 4.03 log⁻¹⁰ cfu/mL.

**Discussion**

To our knowledge, the results presented in this report demonstrate for the first time that the catecholamine inotropes that are used in the clinical setting are capable of facilitating the recovery and growth of antibiotic-damaged staphylococci responsible for catheter-related BSIs. Of critical importance in the present study has been the use of very low bacterial inocula in an iron-restrictive, serum-based medium that more closely approximates the in vivo milieu. This extends previous work demonstrating that catecholamine inotropic drugs were capable of increasing both *S. epidermidis* planktonic growth levels [14] and biofilm growth on catheter materials [15] from very low initial bacterial inocula in an iron-restrictive, plasma- or serum-based medium. Taken together these reports echo the concerns recently expressed in a *Lancet* commentary by Singer [30] that addressed the use of inotropes in the treatment of sepsis [30].

Antimicrobial coatings for indwelling medical devices, such as those that incorporate rifampin (usually used in combination with minocycline, as for CVCs), are increasingly being used in the clinical setting to prevent colonization with opportunistic skin flora, of which *S. epidermidis* is one of the most pervasive [6–10]. However, controversy surrounds claims for the superiority of antimicrobial-impregnated CVCs, compared with CVCs not impregnated with antimicrobials, in preventing and reduc-
Materials and Methods. Viable counts of the antibiotic-treated cultures were enumerated by use of pour-plate analysis as described in Materials and Methods. Prior to inoculation into catecholamine-containing serum-SAPI medium, preincubated with 200 μmol/L phentolamine and dopamine supplemented with 200 μmol/L chlorpromazine were also included. Test and control cultures were incubated at 37°C for 24–72 h, and viable cell levels were enumeracted by use of pour-plate analysis as described in Materials and Methods. Viable counts of the antibiotic-treated S. epidermidis, S. haemolyticus, and S. aureus cultures were determined prior to inoculation into catecholamine-containing serum-SAPI medium and were determined to be 2.2 × 10^5, 3.8 × 10^3, and 7.8 × 10^2 cfu/mL, respectively. The values shown represent the means of triplicate plate counts; SE of the mean was not >7% for all cultures shown. White bar, no additions (control); black bar, 100 μmol/L norepinephrine; right diagonal hatch, 100 μmol/L norepinephrine plus 200 μmol/L phenolamine; gray bar, 100 μmol/L dopamine; left diagonal hatch, 100 μmol/L dopamine plus 200 μmol/L chlorpromazine. Panel A, growth levels after 24 h incubation of rifampin-treated S. epidermidis; panel B, growth levels after 24 h incubation of rifampin-treated S. haemolyticus; panel C, growth levels after 24 h incubation of rifampin-treated S. aureus Newman.

Figure 5. Effects of α-adrenergic and dopaminergic antagonists on the ability of catecholamines to rescue antibiotic-stressed staphylococci. Replicates of exponentially growing cultures of the bacteria shown were cultured in serum-SAPI medium, preincubated with 5 μg/mL rifampin, as described in the figure 2 legend, and then further processed as described in Materials and Methods, except that additional catecholamine assays containing norepinephrine supplemented with 200 μmol/L phentolamine and dopamine supplemented with 200 μmol/L chlorpromazine were also included. Test and control cultures were incubated at 37°C for 24–72 h, and viable cell levels were enumerated by use of pour-plate analysis as described in Materials and Methods. Viable counts of the antibiotic-treated S. epidermidis, S. haemolyticus, and S. aureus cultures were determined prior to inoculation into catecholamine-containing serum-SAPI medium and were determined to be 2.21 × 10^5, 3.80 × 10^5, and 7.8 × 10^2 cfu/mL, respectively. The values shown represent the means of triplicate plate counts; SE of the mean was not >7% for all cultures shown. White bar, no additions (control); black bar, 100 μmol/L norepinephrine; right diagonal hatch, 100 μmol/L norepinephrine plus 200 μmol/L phenolamine; gray bar, 100 μmol/L dopamine; left diagonal hatch, 100 μmol/L dopamine plus 200 μmol/L chlorpromazine. Panel A, growth levels after 24 h incubation of rifampin-treated S. epidermidis; panel B, growth levels after 24 h incubation of rifampin-treated S. haemolyticus; panel C, growth levels after 24 h incubation of rifampin-treated S. aureus Newman.

Of considerable interest is our observation that inotropes were able to induce the growth of antibiotic-damaged S. aureus. Previous work from our laboratories had shown that S. aureus strains showed little increase in growth in response to catecholamines in serum-based media [17]. This might be the result of the possession of acquisition systems for host sequestered iron that enable them to independently overcome the iron-limitation of tissue fluids [29], and indeed the iron uptake analyses (table 1) confirmed that S. aureus was more efficient at accessing transferrin-complexed iron than either of the coagulase-negative strains. However, in the presence of a catecholamine inotrope, S. epidermidis and S. haemolyticus were able to assimilate levels of transferrin–derived iron similar to the levels achieved by both S. aureus strains. Additionally, the current work demonstrates that S. aureus cultures that had been stressed by prior exposure to antibiotics became more dependent on catecholamines and showed marked growth inhibition in their absence (P < .001), thereby suggesting that the antibiotic may have damaged the ability of the S. aureus strains to access host-sequestered iron.

The data presented herein is also suggestive of potential new avenues for the prevention of staphylococcal responses to catecholamine inotropes (which can lead to biofilm formation [23]) based on the use of adrenergic and dopaminergic antagonists. As such, these observations unexpectedly extend the recent findings obtained from gram-negative enteric bacteria [23] to gram-positive species. They also provide an important perspective on the evolutionary development of responsiveness to catecholamine in staphylococcal species. Previous reports proposed that adrenergic and dopaminergic catecholamines induced staphylococcal growth via an apparently identical mechanism involving the provision of iron from the host iron-binding protein transferrin [14, 15]. Our latest work, however, has revealed this to be too simplistic an interpretation, given our demonstration that adrenergic and dopaminergic antagonists are selective in their ability to block staphylococcal responses to adrenergic and dopaminergic agonists, and that antagonist blocking of responsiveness to catecholamine is independent of catecholamine iron provision. These new findings indicate the presence of specific adrenergic- and dopaminergic-type receptors in the staphylococcal catecholamine-mediated growth pathways. The antagonist data shown in table 2 further suggests that these staphylococcal response...
systems resemble α-adrenergic receptors, but not β-adrenergic receptors, in the mechanism of norepinephrine growth induction and for a dopamine response system with D2-dopaminergic–like specificity in dopamine-mediated growth induction, similar to that observed in enteric species [23]. This pharmacological similarity enables us to speculate that the evolution of bacterial catecholamine response pathways may have developed before the temporal separation of gram-positive and gram-negative species. Finally, from the clinical perspective, the present report may carry important implications related to diagnosis, and suggests that incorporation of catecholamine inotropes into clinical microbiological diagnostic media may provide a useful means to rescue otherwise damaged bacteria from clinical specimens that currently prove incapable of growth in standard diagnostic laboratory media.

References