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 comorbid 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-
gens. Recently published work [23, 24] has also demonstrated that the mechanism(s) by which inotropes influence bacterial processes appear to be more complex than the simple provision of extracellular iron from iron-sequestering glycoproteins, such as transferrin and lactoferrin, as had originally been believed [22, 25].

The endogenous and exogenous factors that may influence the ability of bacteria exposed to antimicrobial-coated CVCs to go on to become the cause of catheter-related BSI are not fully understood. In the present study, we investigate whether therapeutic levels of dopamine and norepinephrine are able to influence the growth rate of staphylococci in the presence or absence of antibiotics or after staphylococci have been exposed to antimicrobial levels at or above the MIC. The latter experimental design mimics the clinical scenario in which the bacterial cell would first come into contact with an antimicrobial-impregnated CVC, incurring injury that would initially prevent it from attaching to the catheter. As a result of not being able to attach to the catheter, the antimicrobial-stressed bacterium could then enter the systemic circulation, where it would potentially be exposed to high concentrations of catecholamine inotropes, but not antimicrobials, resulting in catheter-related BSI.

**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 staphylococci in the presence or absence of inotropes when inotropes and antibiotics were added simultaneously (2) addition of inotropes after staphylococci 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, staphylococci 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; ~10⁷ 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 ~10⁵ cfu per mL into fresh serum-SAPI medium supplemented with increasing concentrations of rifampin or minocycline with or without the addition of 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 staphylococcal cultures grown in serum-SAPI medium were cultured twice in fresh serum-SAPI medium to mid-exponential phase (OD660 ≈ 0.4; ~10⁴ 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 OD660 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 staphylococci 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].

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presence or absence of 100 in response to increasing concentrations of rifampin in the midis (experimental approach).

**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).

**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 × 10⁶ cfu/mL, the number of viable cells in the 10⁻³ and 10⁻⁸ 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

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**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 × 10⁸ 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. Figure 1A shows a comparative growth profile for the untreated control cultures. Figure 1B shows growth rates of *S. epidermidis* inoculated at 1 × 10⁸ cfu/mL, the number of viable cells in the 10⁻³ and 10⁻⁸ 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

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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.

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compared with cultures not supplemented with catecholamine (figure 2D) \( (P < .001) \). Similar results to those shown for \( S. \) epidermidis were also obtained for \( S. \) haemolyticus (figure 2E and 2F).

Previous work has shown that \( S. \) 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 \( S. \) 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 only very low cell densities \(( \approx 10 \text{ cfu/mL}) \). However, antibiotic treatment of the \( S. \) 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 \( S. \) aureus culture dilutions is different from that obtained for \( S. \)
Newman and 832–4; of control cultures of growth levels after 24 h incubation of rifampin-treated corresponding nonsupplemented control culture (Asterisk, statistically significant increase in growth level over the corre-
cultures. The SE of the mean was not

Materials and Methods. Results shown represent the mean of triplicate

levels were enumerated by use of pour-plate analysis as described in

and control cultures were incubated at 37°C for 24 h, and viable cell

count,2.67

to a final concentration of 100

times the MIC), as described in Materials and Methods. Antibiotic-treated
cultures (Newman: viable cell count,2.21

or dopamine

bar)

to norepinephrine and dopamine. Table 2 shows that the

does not statistically change the growth of coagulase-

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 55Fe) from 55Fe-Tf in both the presence and absence of norepinephrine. As shown in table 1, significant incorporation of transferrin-derived 55Fe 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 55Fe 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 α-adrenergic, β-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 β-adrenergic receptor antagonist propranolol had no effect on the ability of norepinephrine or dopamine to induce growth in S. epidermidis. Other β-adrenergic antagonists, such
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 inoculated at 10⁸ cfu/mL into serum-SAPI supplemented with 10⁵ 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% 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. 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%.

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

<table>
<thead>
<tr>
<th>Organism</th>
<th>In medium without norepinephrine mean ± SD, cpm/mL</th>
<th>In medium with norepinephrine</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em> Newman</td>
<td>1398 ± 23</td>
<td>2990 ± 123</td>
</tr>
<tr>
<td><em>S. aureus</em> 843–2</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 10⁸ cfu/mL into serum-SAPI supplemented with 10⁵ 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% 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. 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%.

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 by interaction with specific dopamine receptors that are not targets for α- or β-adrenergic antagonists [28]. We therefore investigated whether dopaminergic antagonists had any effect on the response of *S. epidermidis* to dopamine. Inclusion of apomorphine and haloperidol (nonselective) and raclopride (D₄-specific) did not alter the ability of dopamine to induce growth in *S. epidermidis* (data not shown). However, the D₄ receptor antagonist chlorpromazine was able to block growth in response to dopamine by >3 log orders (< .001) (table 2). Chlorpromazine had no significant effect on norepinephrine-induced growth and by itself did not induce growth. The addition of Fe(NO₃)₃ induced growth in dopamine-supplemented *S. epidermidis* cultures that contained chlorprom-
antagonist chlorpromazine showed significant decreases in growth levels, compared with control cultures (\(P < .001\)). 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\%.

\(\text{Table 2. Antagonism of catecholamine-induced growth in } S. \text{ epidermidis.}\)

<table>
<thead>
<tr>
<th>Antagonist, catecholamine</th>
<th>Growth at antagonist concentration</th>
<th>0 (\mu\text{mol/L})</th>
<th>0.1 (\mu\text{mol/L})</th>
<th>1 (\mu\text{mol/L})</th>
<th>10 (\mu\text{mol/L})</th>
<th>20 (\mu\text{mol/L})</th>
<th>50 (\mu\text{mol/L})</th>
<th>75 (\mu\text{mol/L})</th>
<th>100 (\mu\text{mol/L})</th>
<th>200 (\mu\text{mol/L})</th>
<th>300 (\mu\text{mol/L})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolamine(^{a})</td>
<td>Norepinephrine, 50 (\mu\text{mol/L})</td>
<td>8.01</td>
<td>7.94</td>
<td>7.86</td>
<td>6.90</td>
<td>6.08</td>
<td>5.15</td>
<td>4.90</td>
<td>4.60</td>
<td>3.75</td>
<td>3.75</td>
</tr>
<tr>
<td></td>
<td>Norepinephrine, 50 (\mu\text{mol/L}) + Fe(NO(_3))(_3), 100(\mu\text{mol/L}) (^{b})</td>
<td>8.21</td>
<td>8.13</td>
<td>8.13</td>
<td>8.13</td>
<td>8.06</td>
<td>8.06</td>
<td>8.04</td>
<td>8.15</td>
<td>8.09</td>
<td>7.99</td>
</tr>
<tr>
<td></td>
<td>Dopamine, 50 (\mu\text{mol/L})</td>
<td>8.01</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>7.96</td>
</tr>
<tr>
<td>Propranolol(^{c})</td>
<td>Norepinephrine, 50 (\mu\text{mol/L})</td>
<td>8.02</td>
<td>8.02</td>
<td>7.99</td>
<td>8.03</td>
<td>7.68</td>
<td>7.76</td>
<td>7.91</td>
<td>7.90</td>
<td>7.81</td>
<td>7.60</td>
</tr>
<tr>
<td></td>
<td>Dopamine, 50 (\mu\text{mol/L})</td>
<td>8.01</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>7.95</td>
</tr>
<tr>
<td>Chlorpromazine(^{d})</td>
<td>Dopamine, 50 (\mu\text{mol/L})</td>
<td>8.00</td>
<td>7.98</td>
<td>7.98</td>
<td>7.81</td>
<td>7.34</td>
<td>6.92</td>
<td>6.66</td>
<td>6.35</td>
<td>5.27</td>
<td>4.81</td>
</tr>
<tr>
<td></td>
<td>Dopamine, 50 (\mu\text{mol/L}) + Fe(NO(_3))(_3), 100(\mu\text{mol/L}) (^{b})</td>
<td>8.06</td>
<td>8.08</td>
<td>8.08</td>
<td>8.16</td>
<td>8.11</td>
<td>8.08</td>
<td>8.08</td>
<td>8.08</td>
<td>8.11</td>
<td>8.06</td>
</tr>
<tr>
<td></td>
<td>Norepinephrine</td>
<td>8.03</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>7.81</td>
</tr>
</tbody>
</table>

\(^{a}\) Growth level for culture with phenolamine and no catecholamine supplement, 3.90 log\(^{-1}\) cfu/mL.

\(^{b}\) Growth level for culture with dopamine and no catecholamine supplement, 4.03 log\(^{-1}\) cfu/mL.

\(^{c}\) Growth level for culture with propranolol and no catecholamine supplement, 4.00 log\(^{-1}\) cfu/mL.

\(^{d}\) Growth level for culture with chlorpromazine and no catecholamine supplement, 4.03 log\(^{-1}\) cfu/mL.

NOTE. All data are log\(^{-1}\) cfu/mL. \(S. \text{ epidermidis}\) was inoculated at approximately 10\(^2\) 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 \(\alpha\)-adrenergic (phenolamine) antagonist, but not those with a \(\beta\)-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\%.

**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 \(S. \text{ epidermidis}\) 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 results 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 \(S. \text{ epidermidis}\) 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. Control cultures were incubated at 37°C for 24–72 h, and viable cell numbers and were determined to be 2.21 × 10^5, 3.80 × 10^5, and 7.8 × 10^5 cfu/mL, respectively. The values shown represent the means 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 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


