Mechanisms of disease

Stimulation of *Staphylococcus epidermidis* growth and biofilm formation by catecholamine inotropes

Mark Lyte, Primrose P E Freestone, Christopher P Neal, Barton A Olson, Richard D Haigh, Roger Bayston, Peter H Williams

Summary

Background Bacterial colonisation of indwelling medical devices by coagulase-negative staphylococci is a prevalent risk in intensive-care units. Factors determining biofilm formation and progression to catheter-related infection are incompletely understood. We postulated that administration of inotropic agents via indwelling intravenous catheters may stimulate growth and formation of biofilms by *Staphylococcus epidermidis*.

Methods Inocula representing physiologically relevant infecting doses of *S epidermidis* were incubated in a minimum medium supplemented with fresh human plasma in the presence or absence of pharmacological concentrations of norepinephrine or dobutamine. Biofilm formation on polystyrene and medical-grade silicone was examined. After incubation, cultures were assessed for growth and formation of biofilms by colony counting and scanning electronmicroscopy. The production of exopolysaccharide, a major constituent of *S epidermidis* biofilms, was also assessed by use of immunofluorescence microscopy.

Findings Incubation of *S epidermidis* with catecholamine inotropes in the presence of human plasma resulted in a significant increase in growth compared with control on both polystyrene and silicone surfaces, with pronounced increases in biofilm formation as visualised by scanning electronmicroscopy. Experiments with transferrin labelled with radioactive iron showed the ability of catecholamine inotropes to facilitate acquisition of iron by *S epidermidis*. Immunofluorescence microscopy revealed extensive exopolysaccharide production associated with *S epidermidis* biofilms.

Interpretation The ability of catecholamine inotropic drugs to stimulate bacterial proliferation and biofilm formation may be an aetiological factor in the development of intravascular catheter colonisation and catheter-related infection. The removal of iron from transferrin for subsequent use by *S epidermidis* is a possible mechanism by which catecholamine inotropes stimulate bacterial growth as biofilms.

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Introduction

Bacterial colonisation of indwelling medical devices such as catheters has been identified as the commonest source of infection among patients in intensive care.[1] An estimated 16 000 episodes of catheter-related bloodstream infection occur each year in the intensive-care setting in the USA alone,[2] and the cost of treating a single episode is calculated at between US$3700 and $29 000.[2,3] Most catheter-associated nosocomial infections are caused by coagulase-negative staphylococci, the normal skin commensal *Staphylococcus epidermidis* being the cause of 50–70% of reported cases.[4] In association with appropriate surfaces, bacteria such as *S epidermidis* adhere and proliferate to form BIOFILMS, highly complex structures that represent functional communities of microbes.[5] The formation of bacterial biofilms is an established process in many clinical contexts, including colonisation of urinary catheters[6] and prosthetic joints.[7] Phenotypic changes in the bacteria, including the production of POLYSACCHARIDE INTERCELLULAR ADHESIN (PIA),[8] are thought to confer resistance to both host defences and antimicrobial therapy.[9]

Environmental factors in the intensive-care setting that affect the ability of *S epidermidis* to adhere to biomaterials and form biofilms are largely unknown. One particular feature of catheter colonisation that has been given little attention is the direct effect on bacterial biofilm formation of therapeutic compounds infused through the catheter. Recent work in our laboratories has shown that the growth of free-floating, non-adherent, coagulase-negative staphylococci in liquid culture is increased by several orders of magnitude in the presence of catecholamine inotropes.[10] We therefore chose in this study to assess whether this group of drugs can also affect *S epidermidis* biofilm development. Particularly important features of the study design include use of low bacterial inocula, to mimic clinically relevant infecting doses of bacteria, and concentrations of inotropic drugs that are used pharmacologically in the intensive-care setting. Cumulative growth on surfaces such as polystyrene multiwell tissue culture plates is the commonly accepted standard indicator of bacterial biofilm formation.[11,12] However, because most indwelling devices are made of silicone, we also investigated the ability of this biomaterial to support biofilm formation.

Methods

Experimental materials

The slime-producing *S epidermidis* strain RP62A (number 35984, American Type Culture Collection, Manassas, VA, USA) was donated by V L Thomas (University of Texas Health Science Center, San Antonio, TX, USA). Fresh *S epidermidis* clinical isolates F1423, F1553, and F1580 were obtained intraoperatively from patients who had prosthetic-valve endocarditis, infection of a total hip replacement, and infection of a ventriculoperitoneal shunt, respectively, at the City Hospital, Nottingham, UK. Intraoperatively obtained bacteria were identified in the clinical microbiology laboratory as *S epidermidis* by the APISTAPH IDENTIFICATION SYSTEM (BioMérieux,

GLOSSARY

APISTAPH IDENTIFICATION SYSTEM
Apistaph is a kit with freeze-dried substrates, indicators, and reagents, which generates a numerical profile that can be used with a computerised database to identify staphylococci.

BIOFILMS
A surface-attached community of microorganisms, which undergoes phenotypic change in response to nutritional deprivation and other stresses, resulting in infections that are mostly chronic, persistent, and recalcitrant to antibiotic treatment.

POLYSACCHARIDE INTERCELLULAR ADHESIN (PIA)
PIA is a β-1, 6-linked glycan produced by staphylococci, especially when growing as a biofilm. It serves to attach bacterial cells one to another thereby facilitating biofilm development. It is known colloquially as slime.

Basingstoke, UK). All bacterial strains were maintained by serial passage on trypticase soy agar plates.

Trypticase soy agar was obtained from Difco (Detroit, MI, USA). SAPI, a nutritionally poor minimum salts medium, was prepared as previously described,[13] except that the dextrose concentration was 1 g/L. Iron-55-labelled ferric chloride (specific activity $1{\cdot}85\times10^{11}$ Bq Fe) was obtained from Amersham Pharmacia Biotech (Amersham, UK). Human apotransferrin, norepinephrine bitartrate, dopamine hydrochloride, and dobutamine hydrochloride were obtained from Sigma-Aldrich (St Louis, MO, USA). Norepinephrine-3-*O*-sulphate was synthesised by Research Biochemicals, Natick, MA, USA, as part of the National Institute of Mental Health's Drug Chemical Synthesis and Drug Supply Program. All chemicals were dissolved in distilled deionised water on the day of the experiment and sterile filtered. Medical-grade silicone elastomer sheet (unfilled) of thickness 0·45 mm was donated by Codman and Shurtleff, Raynham, MA.

In previous studies on mechanisms of *S epidermidis* attachment to surfaces, less bacterial adherence was observed in the presence of platelet-poor plasma than in the presence of platelets.[14,15] In this study, we used both platelet-rich and platelet-free plasma preparations to establish the role of platelets in the adherence of *S epidermidis* to biomaterial surfaces. 60 mL heparinised blood was obtained between 0700 h and 0900 h from healthy volunteers (six male, four female; aged 30–56 years, median 37), who were not currently taking anticoagulant therapy, according to the protocol for blood donation and use approved by the Minneapolis Medical Research Foundation and Hennepin County Medical Center Institutional Review Board. Heparinised blood, which was used immediately in all experimental protocols, was centrifuged at 1500 *g* for 20 min to give platelet-rich plasma. Platelet counts in plasma preparations were measured with a Coulter Hema-Stks analyser. To prepare platelet-free plasma, platelet-rich preparations were diluted one in ten in SAPI medium and passed through a 0·8 μm/0·2 μm dual-stage sterile filter. The need for additional platelet-free plasma from the same donor in some experiments necessitated an additional step in which the blood remaining after removal of platelet-rich plasma was further centrifuged at 100 *g* for 20 min followed by sterile filtration. The results were similar whichever method of preparation was used. Media containing platelet-rich and platelet-free plasma were always prepared fresh and used immediately. The use of fresh-frozen plasma and plasma obtained from outdated donor blood from the blood bank was also assessed but gave results that were not consistently reproducible on multiple testing. Thus, only freshly obtained blood was used for the preparation of plasma used for experiments reported in this study.

Culture conditions

S epidermidis strains were grown on trypticase soy agar plates at 37°C in 95% air with 5% carbon dioxide. After overnight culture, one colony was selected from a plate, suspended in phosphate-buffered saline, and serially diluted in phosphate-buffered saline to obtain a suspension of about 10^5 colony-forming units (CFU) per mL. Plasma was diluted in SAPI to a final concentration of 10% (by volume), which for platelet-rich plasma represented 221 000–528 000 platelets per μL of culture. Diluted plasma (980 μL), inotrope solution (10 μL of the desired concentration; 0·1 mmol/L for norepinephrine and 1·0 mmol/L for dobutamine unless otherwise stated), and bacterial suspension (10 μL containing about 10^2–10^3 CFU) were added sequentially to 24-well flat-bottomed polystyrene tissue-culture plates (BD Biosciences, Franklin Lakes, NJ, USA) that either did or did not contain silicone squares. A low bacterial inoculum was chosen to reflect more accurately the in-vivo conditions that would exist around the entry point for indwelling medical devices after preparation of the area for insertion of the device and its continued maintenance. Previous in-vitro work had shown that such low bacterial inocula grow poorly in serum-based medium in the absence of catecholamine inotropes.[10,16] Well contents were thoroughly mixed by orbital agitation of the plates for 10 s. The plates were placed on a rocking platform in a 37°C incubator with an atmosphere of 95% air and 5% carbon dioxide and rocked at seven strokes per min through a plane of 16°.

Bacterial adherence to polystyrene and silicone

After incubation, wells that did not contain silicone squares were washed three times in phosphate-buffered saline at room temperature to remove planktonic and lightly adherent growth, then 1 mL phosphate-buffered saline was added to each well and the bottom surfaces were vigorously scraped with a sterile bacteriological loop. For wells containing silicone squares, the squares were removed with sterile forceps and rinsed by immersion ten times in 3 mL volumes of fresh phosphate-buffered saline at room temperature. Each square was placed in a well of a new 24-well plate containing 1 mL phosphate-buffered saline, scraped with a sterile bacteriological loop to remove adherent bacteria, and then discarded.

Well contents from the scraped wells and scraped silicone squares were harvested, vortexed for 30 s to dissociate adherent bacterial clumps, and serially diluted in phosphate-buffered saline. 50 μL of each serial dilution was pour-plated on trypticase soy agar, and bacterial colonies were counted after overnight incubation at 37°C. Results are expressed as CFU per mL of well contents.

Scanning electronmicroscopy

Wells of duplicate polystyrene plates were washed three times with phosphate-buffered saline at room temperature and fixed with either cold 2·5% buffered glutaraldehyde or 100% acetone followed by dehydration in a graded series of ethanol (10 min per grade). Individual polystyrene well bottoms were cut out of the plates with a Dremmel cutting tool, and the resulting plastic discs were stored in fresh 100% ethanol overnight at 4°C. Films of adherent bacteria were dried by immersion in tetramethylsilane (Sigma) and rapid evaporation in a fume hood. After sputter coating with gold palladium, samples were examined in a Hitachi S-3500N variable-pressure scanning electronmicroscope with electronic image capture. Analysis was done at the Electron Optical Facility, CBS Imaging Center of the University of Minnesota under the direction of Janet L Parkin.

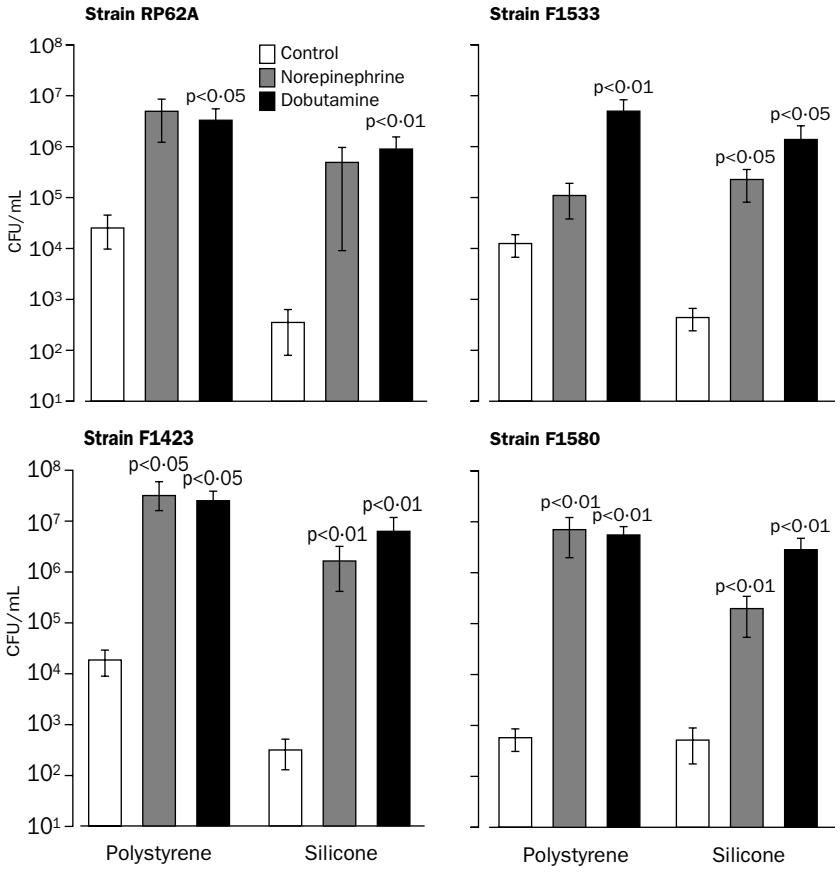

Figure 1: **Adherent growth of *S epidermidis* on polystyrene and silicone in the presence or absence of catecholamine inotropes**
Cultures were harvested at 48 h. Results obtained from four or five separate experiments done on separate days are presented on a logarithmic scale with each bar representing the mean calculated from the raw CFU counts; error bars=SE. p values are for comparison with control.

Anti-exopolysaccharide immunofluorescence
Wells of duplicate polystyrene plates were washed three times with 0·5% bovine serum albumin in phosphate-buffered saline, preblocked with the same medium, and incubated statically at 25°C for 30 min. Polyclonal rabbit antiserum to *S epidermidis* PIA[8] was added at a dilution of 1 in 200 in phosphate-buffered saline with 0·5% bovine serum albumin, and incubated statically at 25°C for a further 30 min; controls consisted of an equivalent volume of medium only. Anti-PIA serum was raised in rabbits and absorbed by use of PIA-positive and PIA-negative mutants (gift from Dietrich Mack, Hamburg, Germany). Wells were then washed three times with phosphate-buffered saline with 0·5% bovine serum albumin, then goat antibody to rabbit IgG conjugated with fluorescein isothiocyanate was added (Sigma; 1 in 200 dilution in phosphate-buffered saline with 0·5% bovine serum albumin) and the mixture incubated for 30 min. After three more buffer washes, test and control biofilms were viewed by reflected-light fluorescence, and images were recorded by a Kodak DC290 photodocumentation system. Controls consisting of biofilms incubated with secondary fluorescein isothiocyanate conjugate only did not show any significant staining, confirming the specificity of the anti-PIA serum (data not shown).

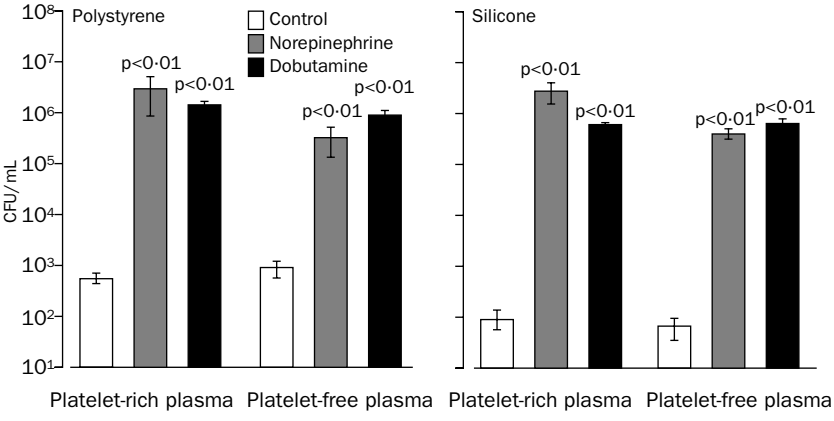

Figure 2: ***S epidermidis* biofilm formation (strain F1553) in the presence or absence of platelets**
Cultures were harvested at 48 h. Results are presented on a logarithmic scale with each bar representing the mean calculated from the raw CFU counts obtained from quadruplicate cultures in platelet-free and platelet-rich plasma prepared from the blood of the same individual; error bars=SE. Polystyrene and silicone cultures were done on separate days on blood donated by the same individual. p values are for comparison with control. Essentially the same results were obtained for strains RP62A, F1423, and F1580.

Bacterial incorporation of iron
Human transferrin was loaded with ^{55}Fe by incubation of apotransferrin (Sigma) for 5 h at 37°C with 0·952 MBq ^{55}Fe-ferric chloride (containing 1·5 μg iron) per mg of protein, with 2 mmol/L citrate as the iron donor.[17] Unincorporated ^{55}Fe was removed by two rounds of spin column chromatography (Micro Bio-spin 6 columns, Biorad Laboratories). ^{55}Fe-transferrin was added at a concentration of 1·5 mg/L (equivalent to 10^5 cpm) to triplicate 1 mL volumes of plasma-supplemented medium in polystyrene plates. Norepinephrine or dobutamine was added at the stated concentrations, bacterial suspension (10 μL containing about 10^3 CFU) was added to each well, and the cultures were incubated as described above. After 21 h incubation, well contents were harvested, washed, and enumerated for growth as described above; ^{55}Fe incorporation into bacterial cells was measured after mixing samples with 2 mL Emulsifier-safe scintillant (Canberra-Packard, Pangbourne, UK) by counting in the tritium channel of a Minaxi Tri-Carb 400 series scintillation counter (Canberra-Packard).[10,17]

Statistical analysis
Numbers of CFU obtained from pour plates were log transformed to ensure normalisation of values before statistical analysis. The process of log transformation in itself does not ensure that the resulting distribution of values will be normal. A one-way ANOVA with Dunnett's post-hoc test was done with the GraphPad InStat Statistical Program. Blood samples used in an ANOVA were from multiple donors, all of whom had undergone the same experimental conditions.

Role of the funding source
Funding sources, in both the USA and the UK, had no role in study design, collection, analysis, or interpretation of data, writing, or the decision to submit the paper for publication.

Results

Effect of catecholamine inotropes on S epidermidis *adherence*

Addition of catecholamine inotropes to *S epidermidis* cultures in medium supplemented with platelet-rich plasma resulted in a significant increase in biofilm growth on both polystyrene and silicone for all four strains examined (figure 1). Biofilm formation was observed as early as 16 h after inoculation and increased progressively with time (data not shown). The ability of the catecholamine inotropes to increase biofilm formation was dose-dependent (data not shown), which accords with a report that catecholamines increase the growth of a range of coagulase-negative staphylococci including *S epidermidis*.[10] Other catecholamine inotropes such as dopamine yielded similar results, but the primary circulating inactivated form of norepinephrine, norepinephrine-3-*O*-sulphate, had no effect on *S epidermidis* growth (data not shown). CFU values obtained by scraping are probably underestimates of the actual numbers of bacteria present, because biofilm clumps were extremely difficult to dissociate into single-cell suspensions for plate counting. Microscopic examination of bacterial suspensions before pour-plating showed the presence of bacterial aggregates primarily in the bacteria stimulated by catecholamine inotropes. Plating of these aggregates, which contain hundreds to thousands of bacteria, would result in the formation of a single pour-plate colony. The presence of the bacterial aggregates was probably the result of increased production of characteristic biofilm exopolysaccharide, which resulted in tight associations of bacteria that resisted separation into single cells.

Because the ability of bacteria to interact with platelets has been documented previously,[18] we also investigated the potential role of platelets in mediating biofilm formation. Biofilm growth occurred on both polystyrene and silicone in the presence of platelet-rich or platelet-free plasma (figure 2). Although the presence of platelets was not essential, supplementation of the medium with various concentrations of plasma did affect biofilm growth on both polystyrene and silicone (figure 3). 5% platelet-rich plasma or more was required for reliable formation of biofilms by all the *S epidermidis* strains tested; below this proportion growth was inconsistent and biofilm formation unreliable. The data shown in figure 3 were obtained from a single experiment for each condition, one on each day of a 2-day period, owing to the limitation in the amount of plasma that could be obtained from a single donor as allowed under the institutional review board.

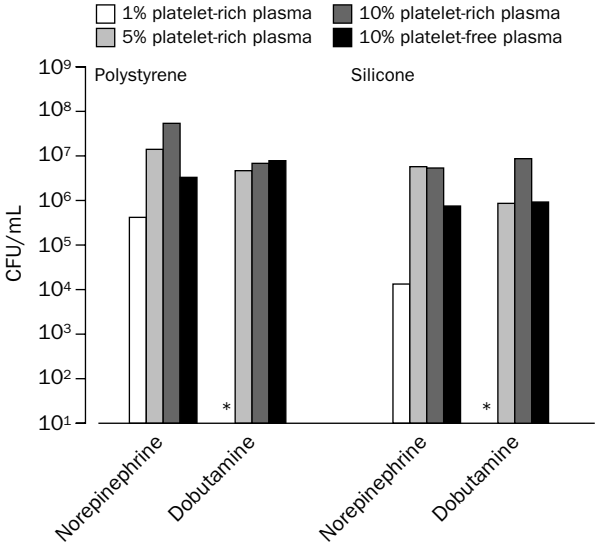

Figure 3: **Effect of plasma concentration on *S epidermidis* biofilm formation (strain F1580)**
Cultures were harvested at 48 h. Platelet-free and platelet-rich plasma were prepared from the blood of the same individual. Polystyrene and silicone cultures were done on separate days. Results are presented on a logarithmic scale and represent data obtained from a single experiment for each surface condition. *No growth was obtained. Essentially the same results were obtained for strains RP62A, F1423, and F1553.

Catecholamine	Mean (SE) growth, CFU/mL	Mean (SE) ^{55}Fe uptake, cpm/mL
Strain F1423		
None	1·1 (0·5)×10^4	22 (10)
Norepinephrine, 10^{-4} mol/L	6·3 (0·6)×10^6	1330 (8)
Norepinephrine, 10^{-3} mol/L	30·0 (1·0)×10^6	7695 (263)
Dobutamine, 10^{-4} mol/L	4·5 (0·6)×10^6	1312 (107)
Dobutamine, 10^{-3} mol/L	20·4 (1·3)×10^6	5001 (176)
Strain F1553		
None	1·2 (0·4)×10^4	15 (3)
Norepinephrine, 10^{-4} mol/L	4·3 (0·5)×10^6	515 (35)
Norepinephrine, 10^{-3} mol/L	17·1 (1·1)×10^6	3726 (172)
Strain F1580		
None	1·5 (0·4)×10^4	32 (13)
Norepinephrine, 10^{-4} mol/L	9·9 (0·8)×10^6	3356 (183)
Norepinephrine, 10^{-3} mol/L	33·1 (1·4)×10^6	13 840 (256)

Values for CFU and cpm represent means for results obtained from four or five separate experiments done on separate days.

Supply of transferrin-bound iron to *S epidermidis* mediated by catecholamine inotropes

Iron removal from transferrin by catecholamine inotropes

Catecholamine hormones and inotropes support the growth of coagulase-negative staphylococci in liquid culture in serum-supplemented medium by facilitating delivery of iron from the host iron-binding protein transferrin.[10,17] To investigate whether the same mechanism explains the stimulation of biofilm formation in the presence of platelet-rich or platelet-free plasma, we incubated *S epidermidis* strains in plasma-supplemented SAPI medium to which ^{55}Fe-labelled transferrin had been added. Inotrope-induced stimulation of bacterial growth was accompanied by increased incorporation of transferrin-derived ^{55}Fe by bacterial cells (table).

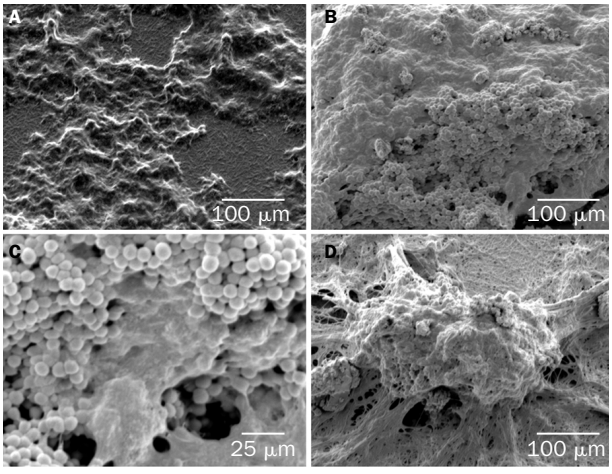

Figure 4: **Analysis by scanning electron microscopy of *S epidermidis* (strain RP62A) biofilm growth in the presence of catecholamine inotropes**
Cultures were harvested at 24 h. A: control culture. B: incubated with dobutamine. C: incubated with dobutamine. D: incubated with norepinephrine.

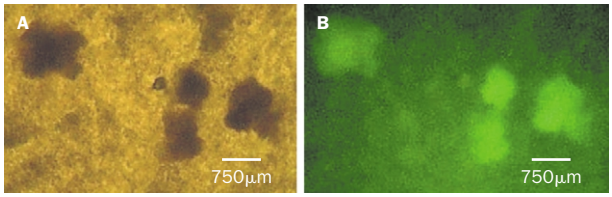

Figure 5: **PIA production in *S epidermidis* (strain RP62A) biofilms grown in the presence of norepinephrine**
Cultures were harvested at 20 h and stained with fluorescent antibody to PIA. A: brightfield image, shows several large bacterial clusters adhering to the polystyrene surface. B: visualisation under ultraviolet light, shows intense fluorescence from the same bacterial clusters demonstrating the presence of large amounts of PIA.

Electronmicroscopy

Scanning electronmicroscopy provided visual confirmation that growth of *S epidermidis* stimulated by catecholamine inotropes in medium containing platelet-rich plasma resulted in the formation of extensive biofilms. Control cultures (with no inotrope supplementation, figure 4, A) showed little biofilm formation with only small amounts of exopolysaccharide, as would be expected in a nutritionally poor medium containing a low infective dose of bacteria. Both dobutamine and norepinephrine, however, induced extensive biofilm production with large amounts of exopolysaccharide clearly visible (figure 4, B–D), although the PIA (slime) layer appeared to be more complete in the dobutamine culture. These images show the effects of 24 h incubation, but experiments in which norepinephrine-supplemented cultures were incubated for up to 44 h showed continued production of slime that eventually completely engulfed the biofilm (data not shown). Essentially the same results were obtained in medium containing platelet-free plasma (data not shown). We also used critical-point drying to prepare samples for scanning electronmicroscopy but found that it was less useful than tetramethylsilane for observing biofilm formation because the preparative steps resulted in disruption of the exopolysaccharide layer.

Antibodies against PIA were used to confirm the presence of exopolysaccharide. Catecholamine-stimulated *S epidermidis* biofilm showed high-intensity staining with fluorescein-labelled antibodies to PIA: thus, the slime layer observed on scanning electronmicroscopy was PIA and not other biological material such as fibrin (figure 5). Although these results show the ability of catecholamine inotropes to affect PIA production, many environmental factors could also affect the expression of PIA when the *icaADBC* gene complex is present.[19]

Discussion

This study shows that catecholamine inotropes commonly used in the intensive-care setting can stimulate *S epidermidis* growth as biofilms on medically relevant materials. The process occurs in the presence of human plasma and involves the acquisition by bacteria of iron from the serum protein transferrin. Large clumps of bacteria seen by scanning electronmicroscopy to be adherent to biomaterial surfaces and covered with an extensive PIA (slime) layer are reminiscent of *S epidermidis* biofilms first described nearly 30 years ago on infected cerebrospinal-fluid shunts from children.[20]

Clinical evidence for a role of catecholamines in promoting infectious disease has existed since the early 1930s after the first isolation of epinephrine and its subsequent therapeutic use in human beings. Reports associating the use of contaminated needles for administration of epinephrine with the development of rapidly disseminating infections appeared almost immediately,[21] leading to a series of experiments examining the role of catecholamines in bacterial pathogenesis.[22,23] These experiments demonstrated the ability of epinephrine to increase in-vivo growth and subsequent virulence of various gram-positive and gram-negative bacterial species. Although no mechanism of action was conclusively proposed at that time, the potentially lethal combination of bacteria with a catecholamine inotrope led to warnings about the need for thorough cleansing of non-disposable glass syringes and needles before use.

Many studies to investigate the mechanism of *S epidermidis* attachment to surfaces have used rich microbiological media and large bacterial inocula, which do not reflect in-vivo conditions. We have shown that *S epidermidis* can form biofilms on biomaterial surfaces in plasma-supplemented minimum medium even at low cell densities. We found that platelets are not essential for the formation of biofilms, by contrast with previous findings[14,15] of decreased ability of *S epidermidis* to adhere to surfaces in the presence of platelet-poor plasma. The earlier studies, however, did not use catecholamine inotropes in the experimental design. Direct interactions of platelets with bacteria (although not with *S epidermidis*) have been noted,[24] and although the inotropes used in our study are well known to affect the function of platelets, with their ability to aggregate,[18] the results of our studies in the absence of platelets show that such interactions are not essential for *S epidermidis* biofilm formation.

We chose to use methods that have been extensively used by other laboratories[11,19,25,26] to address the crucial medical issue of whether inotropic drugs can themselves contribute to bacterial biofilm formation on surfaces of indwelling medical devices. The methods used, coupled with scanning electronmicroscopy, have provided evidence of the existence of channels within inotrope-induced biofilms, which is considered a hallmark of biofilm formation.[9] Another feature was the use of a low bacterial inoculum to mimic more closely in-vivo conditions. However, a range of approaches can be used to study biofilm formation, and one limitation of this study is that it does not provide information on the rate of biofilm formation. Future investigations of biofilm formation induced by catecholamine inotropes should use flow-cell and image analysis, with confocal microscopy, to gain additional insight into the development of biofilms over time.

Effects of catecholamine inotropes on bacteria are not restricted to the coagulase-negative staphylococci. Studies[13,16,17,27] have shown that species of the gram-negative family Enterobacteriaceae, including strains pathogenic for human beings, such as *Escherichia coli* O157:H7, can also respond to catecholamines. Increases in both growth and production of virulence-associated factors such as adhesins[16] have been observed on stimulation with catecholamines, but not with compounds that have undergone physiological inactivation through modification of the catechol moiety, such as norepinephrine-3-*O*-sulphate.[17]

One mechanism suggested for the growth-stimulatory activity of catecholamines is their ability to supply iron to bacteria through interaction with iron-laden transferrin or lactoferrin.[17] These proteins have a key role in defence against infectious microorganisms by maintaining concentrations of free iron within the body below the absolute requirement for microbial growth.[28] Our findings are consistent with the hypothesis of iron acquisition put forward in previous studies on the interaction of catecholamine inotropes with both gram-positive and gram-negative bacteria.[10,17] The most likely explanation therefore for the ability of catecholamine inotropes to affect growth and biofilm formation of *S epidermidis* may be the

overcoming of iron restriction through the facilitated removal of iron from transferrin (and lactoferrin) and subsequent acquisition by bacterial cells. Inotrope-induction of growth alone may not be the sole explanation for the effects of these substances on biofilm formation. Singh and colleagues[29] showed that lactoferrin in concentrations too low to cause iron-restriction was still able to inhibit biofilm formation,[29] an interesting observation in light of our observation that catecholamine inotropes are able to interact with lactoferrin.

The finding that norepinephrine-3-*O*-sulphate does not affect bacterial growth or biofilm formation accords with the experimental demonstration that catecholamine inotropes are able to increase bacterial growth and biofilm formation because they can facilitate the transfer of iron from transferrin to the bacterium. Our previous studies have shown that norepinephrine-3-*O*-sulphate, which is the primary form of circulating, inactivated norepinephrine, cannot facilitate such a transfer.[17] Since the sulphation of catecholamines occurs very rapidly in the body, this result further indicates that the parent drug brings about the observed effect on biofilm formation.

About 50% of patients in intensive care receive inotropic support.[30] The results of this study raise the possibility that the administration of catecholamine inotropes to critically ill patients may predispose them to development of catheter-related bloodstream infection through stimulation of *S epidermidis* growth and biofilm formation. The clinical relevance of this in-vitro study is emphasised by the fact the inotropes were tested at concentrations at or below those at which they are used clinically, in conjunction with low bacterial inocula that mimic bacterial burdens likely to be present at the initiation of an opportunistic infection. Furthermore, the use of diluted plasma in the assay system also approximates the in-vivo milieu, in which the aqueous drug solution mixes with the blood on entry into the body. Experiments are under way to investigate whether the effects of catecholamines are achieved solely through stimulation of bacterial growth or whether additional phenotypic changes, such as increased production of PIA, which may contribute to increased antibiotic resistance,[9] are also induced by exposure to these drugs.

Contributors

M Lyte, P Freestone, R Haigh, and C Neal contributed to study design, did experiments, analysed data, and wrote and edited the report. P Williams contributed to study design, analysed data, and wrote and edited the report. R Bayston contributed to study design, characterised and supplied clinical strains, antibodies, and biomaterials, analysed data, and edited the report. B A Olson did experiments and analysed data.

Conflict of interest statement

None declared.

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