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A new host cell internalisation pathway for SadA-expressing staphylococci triggered by excreted neurochemicals

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Abstract

Staphylococcus aureus is a facultative intracellular pathogen that invades a wide range of professional and nonprofessional phagocytes by triggering internalisation by interaction of surface-bound adhesins with corresponding host cell receptors. Here, we identified a new concept of host cell internalisation in animal-pathogenic staphylococcal species. This new mechanism exemplified by *Staphylococcus pseudintermedius* ED99 is not based on surface-bound adhesins but is due to excreted small neurochemical compounds, such as trace amines (TAs), dopamine (DOP), and serotonin (SER), that render host cells competent for bacterial internalisation. The neurochemicals are produced by only one enzyme, the staphylococcal aromatic amino acid decarboxylase (SadA). Here, we unravelled the mechanism of how neurochemicals trigger internalisation into the human colon cell line HT-29. We found that TAs and DOP are agonists of the α2-adrenergic receptor, which, when activated, induces a cascade of reactions involving a decrease in the cytoplasmic cAMP level and an increase in F-actin formation. The signalling cascade of SER follows a different pathway. SER interacts with 5HT receptors that trigger F-actin formation without decreasing the cytoplasmic cAMP level. The neurochemical-induced internalisation in host cells is independent of the fibronectin-binding protein pathway and has an additive effect. In a *sadA* deletion mutant, ED99Δ*sadA*, internalisation was decreased approximately threefold compared with that of the parent strain, and treating *S. aureus* USA300 with TAs increased internalisation by approximately threefold.

KEYWORDS

actin, cAMP, dopamine, SadA, serotonin, *Staphylococcus*, trace amines, α2-adrenergic receptors

Abbreviations: 5-HTP, 5-hydroxytryptophan; AAAs, aromatic amino acids; cAMP, cyclic adenosine monophosphate; DOP, dopamine; EPI, epinephrine (synonym of adrenaline), endogenous agonist of α2-adrenergic receptors; Fn, fibronectin; FnBP, fibronectin-binding protein; G proteins, guanine nucleotide-binding proteins forming a family of proteins that act as molecular switches inside cells; Gi proteins, inhibitory G proteins; L-DOPA, dihydroxy phenylalanine; NPPC, nonprofessional phagocytic cells; PEA, phenethylamine; Phe, phenylalanine; PTL, phentolamine; *sadA*, gene encoding staphylococcal aromatic amino acid decarboxylase; SadA, staphylococcal aromatic amino acid decarboxylase; SER, serotonin; TAs, trace amines; Trp, tryptophan; TRY, tryptamine; TYM, tyramine; Tyr, tyrosine; α2-ARs, α2-adrenergic receptors

1 | INTRODUCTION

Staphylococcus aureus is a facultative intracellular pathogen that triggers internalisation by nonphagocytic cells such as endothelial, epithelial, and mammary cells; fibroblasts; and osteoclasts and can persist intracellularly for various periods of time (Dziewanowska et al., 1999; Garzoni & Kelley, 2009; Lowy, 2000; Sinha et al., 2000). Upon internalisation, *S. aureus* can escape phagosomal membranes by the actions of phenol-soluble modulin alpha (PSMα) and a cyclic dipeptide, phevalin, produced by a nonribosomal peptide synthetase (NRPS; Blättner et al., 2016; Zimmermann & Fischbach, 2010). Triggering the internalisation of pathogenic bacteria into host cells is an advantage because the pathogens are largely protected from the immune defence system and antibiotics. It is also assumed that internalisation favours persistence and chronicity of an infection, particularly if cells adapt to small-colony variants, which show an increased persistence within host cells (Sendi & Proctor, 2009; von Eiff et al., 1997). Internalised staphylococci may induce apoptosis or autophagy or exist free within the cytoplasm (Mauthe et al., 2012; Menzies & Kourteva, 1998).

One of the key players in triggering internalisation into the host cell is the fibronectin-binding proteins (FnBPs), which are anchored in the cell wall of *S. aureus* (Burke, Di Poto, Speziale, & Foster, 2011; Jonsson, Signas, Müller, & Lindberg, 1991). FnBPs bind fibronectin, which in turn interacts with the α5ß1 integrin of host cells, thus triggering internalisation (Fowler, Johansson, Wary, & Hook, 2003; Sinha et al., 1999). This mechanism is the FnBP-Fn-α5β1 integrin-mediated pathway for adherence and internalisation into nonprofessional phagocytic cells (NPPCs). In addition to this pathway, several other surface proteins have been identified that boost internalisation into host cells. One protein is the major autolysin, Atl and AtlE from *S. aureus* and *Staphylococcus epidermidis*, respectively, that interacts with the heat shock cognate protein Hsc70 as a host cell receptor to trigger internalisation (Hirschhausen et al., 2010). This Atl-mediated internalisation mechanism may represent a "back-up" mechanism in *S. aureus* internalisation, whereas it may represent the major or even sole mechanism involved in the internalisation of coagulase-negative staphylococci and thus may play an important role in the pathogenesis of chronic and relapsing infections with these pathogens. Another class of proteins involved in internalisation comprises the lipoproteins (Lpls) encoded in an *S. aureus* pathogenicity island, vSaα (Nguyen et al., 2015). Some other staphylococcal surface proteins involved in internalisation were reviewed recently (Josse, Laurent, & Diot, 2017).

However, it must not always be a surface-bound protein that triggers internalisation into host cells. Recently, a completely new mechanism of internalisation has been uncovered in mainly animal-pathogenic staphylococcal species (Luqman et al., 2018). They produce the so-called trace amines (TAs) by decarboxylation of aromatic amino acids: Phenylalanine is converted to phenethylamine (PEA), tryptophan to tryptamine (TRY), and tyrosine to tyramine (TYM). These staphylococcal strains are also able to decarboxylate dihydroxyphenylalanine and 5-hydroxytryptophan to the neurotransmitters dopamine (DOP) and serotonin (SER), respectively. All of these neurochemicals are generated by only one enzyme, the staphylococcal aromatic amino acid (AAA) decarboxylase (SadA). Interestingly, AAAs are mainly taken up by the cells, they are then decarboxylated by SadA in a pyridoxal phosphate-dependent reaction, and the products are secreted without being further metabolised (Table S2). This seemingly useless and energy-consuming reaction gained importance when it was revealed that TAs and the neurotransmitters DOP and SER significantly increase the internalisation of staphylococci in host cells (Luqman et al., 2018).

To study this mechanism in more detail, we used *Staphylococcus pseudintermedius* ED99 as a model strain because its genome was first sequenced (Ben Zakour, Bannoehr, van den Broek, Thoday, & Fitzgerald, 2011); it is representative of this species that causes deep pyodermas and atopic dermatitis in canine and feline pets (Bannoehr et al., 2011; Fitzgerald, 2009; Hill et al., 2006; Kadlec et al., 2010). To prevent relapsing infections and transmission to humans, which occurs occasionally (Paul, Damborg, & Guardabassi, 2014; Pottumarthy et al., 2004), an aggressive antibiotic therapy is recommended (Guardabassi, Schwarz, & Lloyd, 2004; Scott, Peters, & Miller, 2006). A number of virulence factors have been identified in this organism, including the FnBPs SpsD and SpsL, which, similar to their *S. aureus* counterparts, support invasion into canine epithelial cells (Bannoehr et al., 2011; Pietrocola et al., 2015).

Although we know that the α2-adrenergic receptor on the host side plays a crucial role in TA-mediated internalisation, the molecular mechanism of how these neurochemicals boost bacterial internalisation in host cells is unknown. We show that these neurochemicals use two different pathways, TAs and DOP, by activating the α2-adrenergic receptor, whereas SER is an agonist of the 5HT receptors, to increase F-actin formation and increase the internalisation readiness of the host cells; therefore, bacterial internalisation is an active process of the host cell.

2 | EXPERIMENTAL PROCEDURES

2.1 | Bacterial strains and growth conditions

Staphylococcus strains were cultivated in basic medium (BM; 1% soy peptone, 0.5% yeast extract, 0.5% NaCl, 0.1% glucose, and 0.1% K_2HPO_4, pH 7.2) at 37°C with continuous shaking at 150 rpm. When appropriate, the medium was supplemented with 10 μg ml^{-1} chloramphenicol for *Staphylococcus* strains. The staphylococcal strains used in this study are listed in Table S1.

2.2 | Ethics statement

The use of human stool samples was approved by the Ethics Commission of the University of Tübingen (Approval no. 320/2017BO2). Stool samples were obtained from 19 adult probands (ages 20–70). The samples were anonymised, and the probands provided written consent. The C57BL6 mice used in the experiments were kept at the Institute of Pharmacy and used according to the rules of the animal welfare and ethical committee.

2.3 | Neurochemical quantification in human stool samples

Stool samples were diluted in phosphate-buffered saline (PBS) and centrifuged at 5,000 *g* for 20 min, and the supernatants were subjected to HPLC analysis for quantification of neurochemicals. HPLC analyses were conducted using reversed-phase HPLC (RP-HPLC) on an Eclipse XDB-C18 column (Agilent) with a 15 min linear gradient of 0.1% phosphoric acid to acetonitrile at a flow rate of 1.5 ml min^{-1}. Quantification of neurochemicals was calculated using a standard curve (Figure S2).

2.4 | Internalisation assay in HT-29 cell line

HT-29 cells, a human colon adenocarcinoma cell line purchased from DSMZ (DSMZ no. ACC 299), were seeded in 24-well plates (5×10^5 cells per well) in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) and an antibiotic mix and incubated at 37°C in 5% CO_2 for 48 hr prior to the addition of bacteria. Overnight cultures of bacteria were washed twice in DPBS and added to HT-29 cells at a multiplicity of infection (MOI) of 30. To study which 5HT receptor plays a role in internalisation, 50 μg ml^{-1} SER and 5HT antagonists were added to the wells upon internalisation. Subsequently, the cell culture was incubated at 37°C in 5% CO_2 for 2 hr. Bacterial internalisation into HT-29 cells was carried out as described previously (Nguyen et al., 2015). For the combination of SER and antagonists, the antagonists were added 30 min prior to the addition of SER.

2.5 | Internalisation assay in primary epithelial cells isolated from mouse colons

C57BL6 mice were killed by cervical dislocation prior to opening the abdomen. The colon was removed, cut into three pieces, and kept on ice. The colon was cleared from faeces by flushing it three times with ice-cold PBS using a syringe. After opening the colon longitudinally, the epithelial layer was removed by scratching with a plastic spatula, the tissue was disintegrated through homogenisation with a pipette, and the cells were suspended in ice-cold PBS. Primary epithelial colon cells were then counted and seeded into a 24-well plate in DMEM with 10% FBS. We then added antagonists (phentolamine [PTL], ondansetron, and SB-207266) at a final concentration of 50 μg ml^{-1} and incubated the cells for 30 min at 37°C in 5% CO_2. TRY, epinephrine (EPI), and SER were then added into the wells separately, and the cell culture was incubated further at 37°C in 5% CO_2 for 30 min. We then added *S. pseudintermedius* ED99Δ*sadA* at an MOI of 100 and incubated the cells at 37°C in 5% CO_2 for 2 hr. Bacterial internalisation into HT-29 cells was carried out as described previously (Nguyen et al., 2015).

2.6 | Cytotoxicity assay with human cell lines

We used HT-29 cells to investigate the toxicity of adrenergic receptor antagonists and 5HT receptor antagonists on cells. Prior to the cytotoxicity assay, HT-29 cells were seeded in flat-bottom 96-well plates with 10^5 cells per well and incubated overnight in DMEM with 10% FBS and an antibiotic mix at 37°C in 5% CO_2. The host cells were treated with various concentrations (25, 50, 100, and 200 μg ml^{-1}) of PTL, WAY100635, SB207266, ondansetron, GR127935, and SB224289 for 3 hr. The cytotoxicity assay was performed using Cell Proliferation Kit I (MTT; Roche, Germany). At 570 and 690 nm (reference), the absorbance of the formed formazan was determined.

2.7 | Intracellular cAMP level measurement of HT-29 cells

Prior to cAMP measurement, HT-29 cells were seeded in a black flat-bottom 96-well plate with 1×10^5 cells per well and incubated overnight in DMEM with 10% FBS and an antibiotic mix at 37°C in 5% CO_2. Cells were then treated with TAs and/or antagonists for 3 hr before cAMP measurement. For the combination of TAs and antagonists, the antagonists were added 30 min prior to addition of the TAs. For treatment with bacteria, we used wild-type (WT) ED99, Δ*sadA*, and a complemented mutant at an MOI of 30 and AAAs, L-DOPA, and 5-HTP separately at a concentration of 100 μg ml^{-1} and incubated the cells for 3 hr at 37°C in 5% CO_2. Lysostaphin was then added to the cell cultures, and cells were incubated further for 30 min. Cells were then washed three times, and cAMP levels were measured using a cAMP Glo™ Assay (Promega) according to the protocol provided by the company.

2.8 | F-actin measurement

Prior to the cAMP measurement, HT-29 cells were seeded in a black flat-bottom 96-well plate with 5×10^4 cells per well and incubated for 48 hr in DMEM with 10% FBS and an antibiotic mix at 37°C in 5% CO_2. Cells were then treated with TAs and/or antagonists for 3 hr before F-actin measurement. For the combination of TAs and antagonists, the antagonists were added 30 min prior to addition of the TAs. For treatment with bacteria, we used WT ED99, Δ*sadA*, and a complemented mutant at MOI of 30 and AAAs, L-DOPA, and 5-HTP separately at a concentration of 100 μg ml^{-1} and incubated the cells for 3 hr at 37°C in 5% CO_2. Lysostaphin was then added to the cell cultures, and cells were incubated further for 30 min. The treated cells were washed with DPBS, permeabilised with 0.1% (v/v) Triton X-100, stained with ActinGreen™ 488 ReadyProbes® (Thermo Fischer) for 30 min and washed again three times with DPBS, and the relative fluorescence intensity was measured at 495 nm for the excitation and 518 nm for the emission using a Tecan Infinite M200.

2.9 | Microscopy analysis

HT-29 cells were seeded in glass-bottom cell culture dish plates (Greiner) with 1.5×10^6 cells per plate and incubated for 48 hr in DMEM with 10% FBS and an antibiotic mix at 37°C in 5% CO_2. Cells were then treated with either TRY or SER at a concentration of

50 μg ml^{-1} and incubated for 2 hr at 37°C in 5% CO_2. Prior to microscopy, the treated cells were washed with DPBS, fixed with 0.4% paraformaldehyde, permeabilised with 0.1% (v/v) Triton X-100, stained with ActinGreen™ 488 ReadyProbes® (Thermo Fisher) for 30 min and washed again three times with DPBS. Confocal microscopy was performed using a Zeiss Axio Observer Z1 LSM800 equipped with an Airyscan detector and C Plan-Apo 63x/1.4 Oil DIC objective (Zeiss). Images were acquired via the ZEN 2.3 image analysis software package (Zeiss). Because the changes in fluorescence intensity cannot be sufficiently distinguished simply by eye, ImageJ software was used to calculate the fluorescence intensity. We calculated the fluorescence intensity by subtracting the background signal of the selected area from the total area signal, as previously described (Gavet & Pines, 2010).

2.10 | Growth curve and viability assay

We inoculated *S. pseudintermedius* ED99 in BM and incubated the suspension overnight at 37°C with 150 rpm shaking as a preculture. For the growth curve, we inoculated the preculture into fresh BM in the presence of 50 μg ml^{-1} antagonist (PTL, ondansetron, SB-207266, or WAY100265). We measured the OD_{578} every 2 hr from 0 to 24 hr. We also carried out a viability assay by inoculating with the preculture to a final OD_{578} of 0.06 in DMEM in 24-well plates in the presence of antagonists (50 μg ml^{-1}) to mimic the conditions in the internalisation assay and incubated at 37°C with 5% CO_2 for 2 hr. We plated the medium at 0 and 2 hr and compared the colony-forming units (CFU) on BM agar.

2.11 | Statistical significance

Multiple comparisons were analysed using one-way analysis of variance with Dunnett's posttest. Normal distributions were analysed by Student's *t* test. Statistical analyses were performed with GraphPad Prism or Minitab software, with significance defined as $P < .05$; *n* represents the number of independent biological replicates.

3 | RESULTS

3.1 | Neurochemicals prime host cells to increase internalisation activity

It has been demonstrated that SadA-expressing staphylococci are distinguished by significantly higher internalisation frequency due to the production of TAs, DOP, and SER, and such staphylococcal strains are prevalent in human faeces (Luqman et al., 2018). We carried out internalisation assays with the human colon epithelial cell line HT-29 at an MOI of 30 and investigated the internalisation frequency of various staphylococcal strains that do not produce TAs: *S. pseudintermedius* ED99Δ*sadA*, *S. epidermidis* RP62A, *S. aureus* USA300, and *S. aureus* 2F8, an *S. aureus* strain isolated from human stool samples. All strains responded with a roughly threefold increase in internalisation upon addition of TRY and SER (final concentration 50 μg ml^{-1}). Interestingly, when we preincubated HT-29 cells for 30 min with TRY and SER and removed the neurochemicals by a wash step before adding the bacterial cells, there was still an observed increase in internalisation (Figure 1). This finding shows that the HT-29 cells are primed by the neurochemicals to increase internalisation readiness.

In addition to performing assays with the HT-29 cells, we also performed an internalisation assay using primary colon cells isolated from mice. As reported in the literature, the α2-adrenergic receptor is expressed in the colon of rats (Schultheiss & Diener, 2000), and the gastrointestinal tract of mice appears to be similar to that of rats

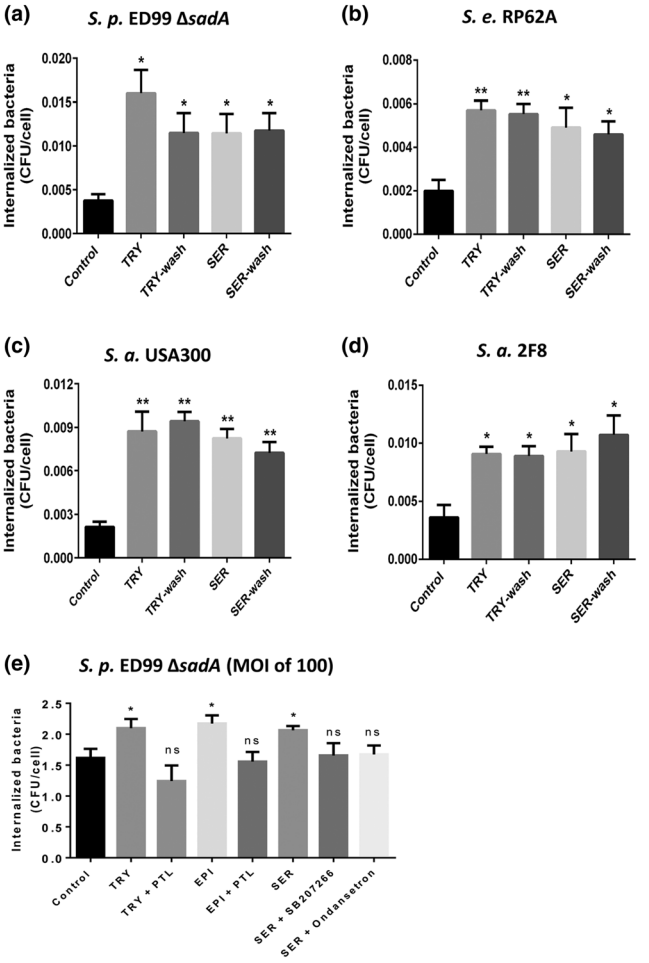

FIGURE 1 TRY and SER increase the internalisation of staphylococci into HT-29 cell. (a) HT-29 cells were preincubated with TRY and SER (50 μg ml^{-1}) separately for 30 min prior the addition of bacteria with washing step to get rid of the added TRY and SER and without washing. Different staphylococcal species were added at MOI of 30 into the cell culture. In these assays, we used *Staphylococcus pseudintermedius* ED99 Δ*sadA*; (b) *Staphylococcus epidermidis* RP62A; (c) *Staphylococcus aureus* USA300; and (d) *S. aureus* 2F8, a strain isolated from human stool sample. The addition of TRY and SER with and without washing step significantly increased the internalisation of staphylococci into HT-29 cells. (e) TRY and SER also increased the internalisation of *S. pseudintermedius* ED99 Δ*sadA* in primary epithelial cells isolated from mouse colon. The graphs show representative data from at least three independent experiments. Each data point is the mean value ± *SEM*, **P* < .05; ***P* < .01; ****P* < .001; and *****P* < .0001, by students *t* test

(Chen, Brown, Xie, Green, & Lyte, 2003). The mouse colon also expresses the 5HT3 and 5HT4 receptors (Chetty, Irving, & Coupar, 2009; Hoffman et al., 2012). We used *S. pseudintermedius* ED99Δ*sadA* as the bacterial model at an MOI of 100 and in the presence of TRY, EPI, and SER with and without antagonists (PTL, ondansetron, and SB207266; final concentration of 50 μg ml^{-1}). The experiments showed that TRY, EPI, and SER increased internalisation significantly, which was nullified by the antagonists (Figure 1e). This result suggests that activation of α2-adrenergic and 5HT receptors increases the internalisation of bacteria into host cells and that this effect is nullified by the antagonists. In a control experiment, we showed that the antagonists had no significant effect on the growth and viability of *S. pseudintermedius* ED99 (Figure S5).

3.2 | The neurochemical-triggered internalisation pathway does not interfere with the FnBP-Fn-α5β1 integrin pathway

As it is well known that the FnBP-Fn-$\alpha_5\beta_1$ integrin-mediated pathway plays a major role in internalisation into NPPCs, we asked the question of whether there is an interference of the pathway with neurochemical-induced internalisation. All four strains tested above (Figure 1) produce fibronectin-binding proteins that may trigger internalisation into host cells (Pietrocola et al., 2015; Sinha et al., 2000); even *S. epidermidis* has such a protein, named Embp (Williams, Henderson, Sharp, & Nair, 2002). Therefore, it is impossible to verify interference between the two pathways. To answer this question, we carried out internalisation assays with *S. aureus* 8325-4 and *S. aureus* 8325-4Δ*fnbAB* as well as with *Staphylococcus carnosus* TM300 and *S. carnosus* TM300 (pFnBA4). In *S. aureus* 8325-4Δ*fnbAB*, the two fibronectin-binding protein A- and B-encoding genes are deleted, and *S. carnosus* TM300 does not have fibronectin-binding proteins (Rosenstein et al., 2009); therefore, we cloned the *S. aureus* FnBPA-encoding gene, *fnbA*, into TM300. Internalisation assays with all four strains in HT-29 cells showed that the addition or preincubation of TRY and SER increased the internalisation frequency approximately threefold (Figure 2). The impact of FnBP on internalisation is observed if one compares *S. aureus* 8325-4 with *S. aureus* 8325-4Δ*fnbAB*; in the latter clone, the internalisation frequency was approximately 10-fold lower than that in the former clone. In contrast, in *S. carnosus*, TM300 (pFnBA4) internalisation was approximately 10-fold higher than that of the parent strain (Figure 2). These results indicate that neurochemical-induced internalisation does not interfere with the FnBP-Fn-α5β1 integrin pathway, thus representing a separate pathway.

3.3 | SadA-expressing staphylococci decrease cytoplasmic cAMP levels in HT-29 cells

Although we know that, on the host side, the α2-adrenergic receptor plays a role in the adherence and internalisation of SadA-expressing staphylococci, nothing is known regarding the underlying mechanism and what happens downstream of the α2-adrenergic receptor. In most

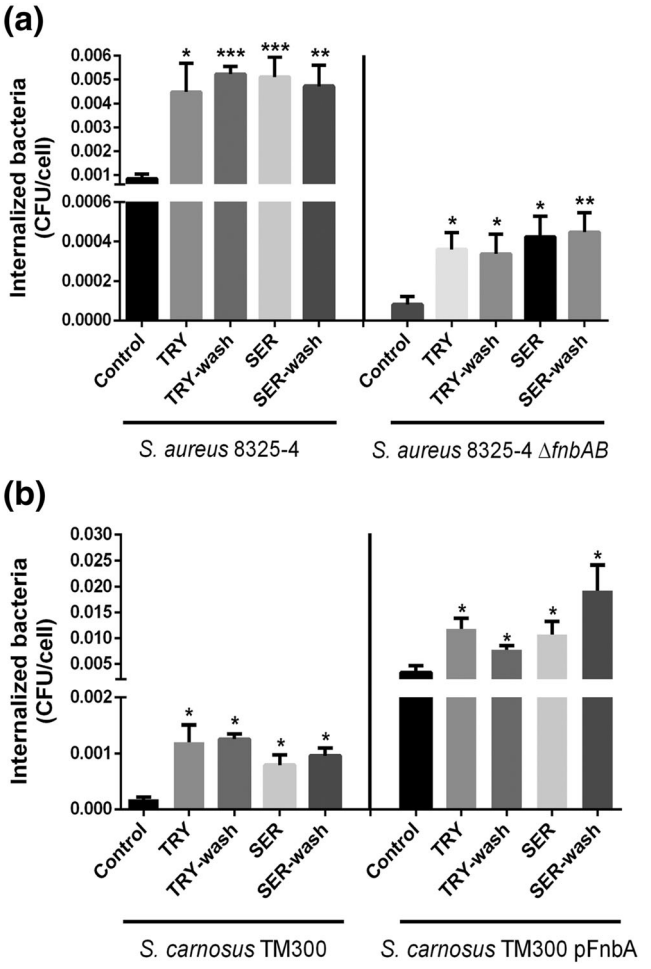

FIGURE 2 TRY and SER increase the internalisation independent of FnBP. (a) We incubated HT-29 cells with *Staphylococcus aureus* 8325-4 WT and Δ*fnbAB* and (b) it resulted that the antagonists we used *S. carnosus* TM300 WT and p*fnbA*4 in the presence of TRY and SER at concentration 50 μg ml^{-1} with and without washing. The addition of TRY and SER significantly increased the internalisation three to four times higher than the control even in the absence of FnBP. The graphs show representative data from at least three independent experiments. Each data point is the mean value ± *SEM*, **P* < .05; ***P* < .01; ****P* < .001; and *****P* < .0001, by students *t* test

human cell types, the α_{2A}-adrenergic receptor is linked to a G_i protein, and thus receptor occupation inhibits adenylate cyclase (AC) activity. For example, EPI (synonym of adrenaline) activates thrombocytes by interacting with the α2-adrenergic receptor, which causes activation of $G_{i\alpha 2}$ followed by AC inhibition and consequently a decrease in cytosolic cAMP and cAMP-dependent protein kinases (Aktories & Jakobs, 1981; Brass, Woolkalis, & Manning, 1988; Keularts, van Gorp, Feijge, Vuist, & Heemskerk, 2000). Intrigued by these findings, we wondered whether TAs, DOP, and SER have an effect on the cytosolic cAMP level in HT-29 cells. Therefore, we examined the effect of *S. pseudintermedius* ED99, ED99Δ*sadA*, and ED99Δ*sadA*com (*sadA*-complemented mutant) on cytosolic cAMP production in HT-29. To allow the production of TAs and DOP, we fed the bacteria with the AAAs (Phe, Trp, and Tyr) as well as with L-DOPA and 5-HTP at a final concentration of 100 μg ml^{-1} each, thus enabling the production of

TAs as well as DOP and SER. The cytoplasmic cAMP level in HT-29 cells was significantly decreased with only ED99 and ED99Δ*sadA*com in the presence of AAAs and L-DOPA but not in the presence of 5-HTP. The cAMP level was even higher in the ED99Δ*sadA*-treated cells than in the control cells (Figure 3a). These results indicate that the TAs (TRY, PEA, and TYM) and DOP produced by ED99 activate the α2-adrenergic receptor, which causes a decrease in cytosolic cAMP. This outcome is similar to that described above for the EPI effect. In contrast to TAs and DOP, 5-HTP, the precursor of SER, did not affect the cytoplasmic cAMP level significantly (Figure 3a).

3.4 | TAs and DOP decrease cytoplasmic cAMP levels in HT-29 cells

To confirm our results obtained with the ED99 clones, we investigated whether TAs, DOP, SER, and EPI (each at a final concentration of 50 μg ml^{-1}) affect cAMP production in HT-29 cells. As shown in Figure 3b, TRY, PEA, TYM, DOP, and EPI caused a significant decrease in cAMP production. The highest decrease was observed with EPI, which is known to be the true ligand of adrenergic receptors and was used as a positive control. The next highest decrease was observed with DOP followed by TRY, PEA, and TYM. SER did not decrease cAMP production, which confirms the results with ED99 and 5-HTP (Figure 3a). Using TRY as an example, we demonstrated that the decrease in cytoplasmic cAMP is concentration dependent (Figure 3c); at 25 μg ml^{-1} of TRY, a significant decrease in the cAMP level in HT-29 cells was observed.

3.5 | *SadA*-expressing staphylococci increase F-actin formation in HT-29 cells

It has been shown that a decrease in cytoplasmic cAMP levels in host cells is correlated with increasing polymerisation of actin to F-actin (Ganguly, Saxena, & Chattopadhyay, 2011). Therefore, we investigated the effect of ED99, ED99Δ*sadA*, or ED99Δ*sadA*com on F-actin formation. As shown in Figure 4a, ED99- and ED99Δ*sadA*com-induced F-actin formation was significantly increased compared with that induced by ED99Δ*sadA*. These results were confirmed when

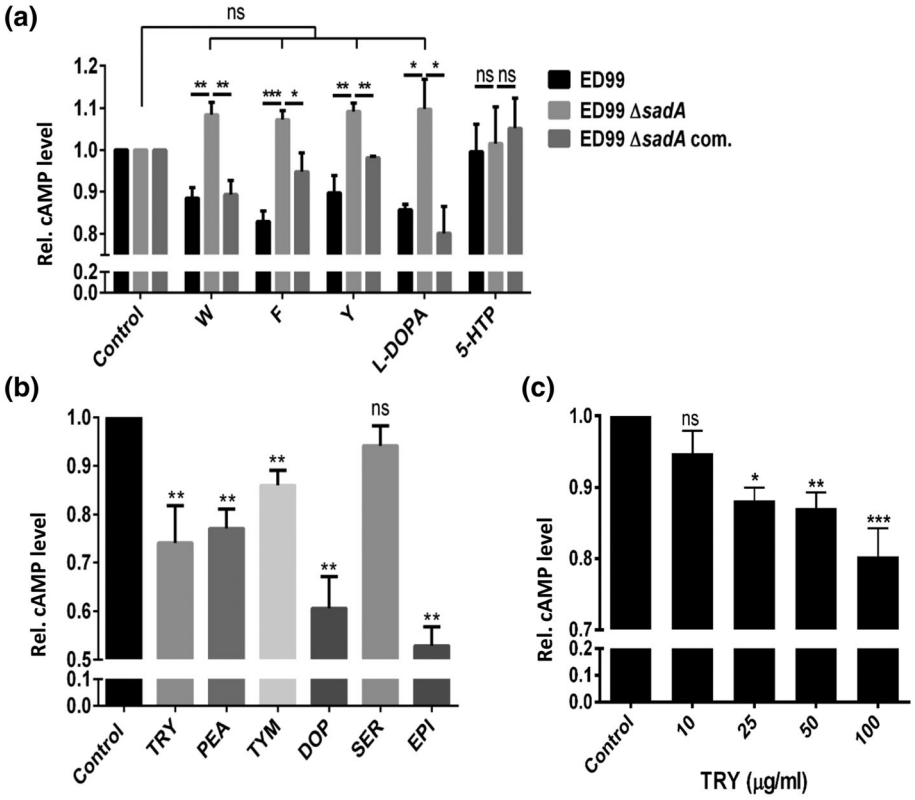

FIGURE 3 TAs and DOP decrease the intracellular cAMP level of HT-29 cells. (a) HT-29 cells were incubated with ED99 (parent strain), ED99Δ*sadA*, and complemented mutant (ED99Δ*sadA*comp) together with the AAAs (W, F, Y), L-DOPA, or 5-HTP, each in a final concentration 100 μg ml^{-1}. Addition of AAAs and L-DOPA decreased the intracellular cAMP level of HT-29 cells significantly when they were treated with ED99 and ED99ΔsadAcomp, whereas with ED99Δ*sadA* the cAMP level was increased. The cAMP levels of HT-29 cells incubated with ED99Δ*sadA* were not significantly different compared with the control. (b) HT-29 cells were treated with different neurochemicals at final concentration 50 μg ml^{-1}. Intracellular cAMP level of HT-29 cells were significantly decreased in the presence of TRY, PEA, TYM, and DOP but not with SER. We used EPI (epinephrine) as positive control as it is an endogenous agonist of α2-adrenergic receptors. (c) The decrease of intracellular cAMP level in HT-29 cells when incubated with TRY was dose dependent ($P \leq .0001$). The graphs show representative data from at least three independent experiments. Each data point is the mean value ± *SEM*, **P* < .05; ***P* < .01; ****P* < .001; and *****P* < .0001, by students *t* test for (a) and (b) and by one-way analysis of variance with Dunnett posttest for (c). All cAMP levels were normalised to control

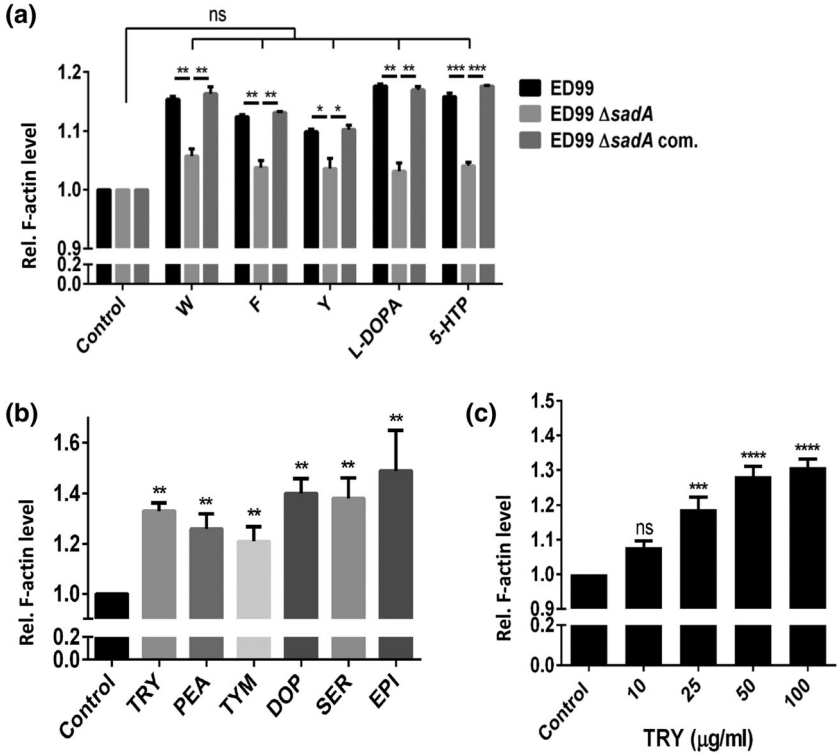

FIGURE 4 Neurochemicals induce F-actin formation in HT-29 cells. (a) HT-29 cells were incubated with ED99 WT, Δ*sadA*, and complemented mutant and AAAs, L-DOPA, and 5-HTP separately (final concentration 100 μg ml^{-1}). Addition of AAAs and L-DOPA increased the F-actin level of HT-29 cells in WT and complemented mutant significantly. The F-actin levels of HT-29 cells incubated with ED99Δ*sadA* were not significantly different compared with the control. (b) HT-29 cells were treated with different neurochemicals at final concentration 50 μg ml^{-1}. F-actin level of HT-29 cells were significantly increased in the presence of TRY, PEA, TYM, DOP, and SER. We used EPI as positive control because it is known as the true ligand for adrenergic receptor. (c) The increasing of F-actin level in HT-29 cells when incubated with TRY were dose dependent manner ($P \leq .0001$). Representative data from at least three independent experiments are shown. For all graphs, each data point is the mean value ± *SEM*, **P* < .05; ***P* < .01; ****P* < .001; and *****P* < .0001, by students *t* test for (a) and (b) and by one-way analysis of variance with Dunnett posttest for (c). All F-actin levels were normalised to control

HT-29 cells were treated with TAs, DOP, SER, and EPI (the latter as a positive control). Again, EPI induced the highest F-actin formation, followed by DOP, SER, TRY, PEA, and TYM (Figure 4b). Surprisingly, SER, which hardly decreased cAMP levels, as shown in Figure 3a,b, induced F-actin formation. Using TRY as an example, we showed that actin polymerisation was dose dependent (Figure 4c); at only 25 μg ml^{-1} TRY, a significant increase in actin polymerisation was achieved in HT-29 cells. The results show that TAs, DOP, and SER produced by ED99 or ED99Δ*sad*Acom increased F-actin formation in HT-29. F-actin formation was also illustrated by microscopic analyses of TRY- and SER-treated HT-29 cells by determining fluorescence intensity using ImageJ software; increased F-actin formation was indicated by increased fluorescence intensity compared with that of control cells (Figures 5 and S3).

3.6 | Neurochemicals affect the cytoplasmic cAMP and F-actin levels of HT-29 cells via the α2-adrenergic receptor and 5HT receptors

We also raised the question of whether activation of the α2-adrenergic and 5HT-receptors by TAs, DOP, EPI (the latter was used as a positive control) and SER cause a decrease in cAMP levels and an increase in F-actin formation. To answer this question, we tested these neurochemicals in the presence of PTL. PTL is a competitive antagonist of α1- and α2-adrenergic receptors, leading to muscle relaxation and a widening of the blood vessels (Mostaghim, Thomas, & Ramwell, 1988; Saeed, Sommer, Holtz, & Bassenge, 1982). If the α2-adrenergic receptor plays a role in the decrease in cAMP, the decrease should be reversed in the presence of PTL. This effect is exactly what we observed for TAs, DOP, and EPI (Figure 6a). However, not only cytoplasmic cAMP but also F-actin formation were reversed in the presence of PTL (Figure 6b). As HT-29 cells show only a low expression of α1-adrenergic receptor (Agrawal, Wildrick, & Boman, 1992), it is likely that the α2-adrenergic receptor controls the cytoplasmic cAMP and F-actin levels upon stimulation by neurochemicals.

3.7 | SER did not significantly affect the cytoplasmic cAMP level but increased F-actin formation in HT-29 cells

HT-29 cells have four 5HT (SER) receptors: 5HT1A, 5HT1B (Ataee, Ajdary, Zarrindast, Rezayat, & Hayatbakhsh, 2010), 5HT3, and 5HT4 (Ataee et al., 2010). To investigate which receptors are involved, we tested different specific antagonists of the various 5HT receptors: WAY100635 (5HT1A antagonist [Forster et al., 1995]), SB207266 (5HT4 antagonist [Fedouloff et al., 2001; Wardle et al., 1996]), and ondansetron (5HT3 antagonist [Butler, Hill, Ireland, Jordan, & Tyers, 1988]). We also tested two 5HT1B antagonists, SB224289 and GR127935 (Lucas, Segu, & Hen, 1997; Selkirk et al., 1998); however, these two compounds were toxic to HT-29 and HaCaT cells (Figure S1). Therefore, we did not use these two compounds for further experiments. Although WAY100635 is reported to be a potent

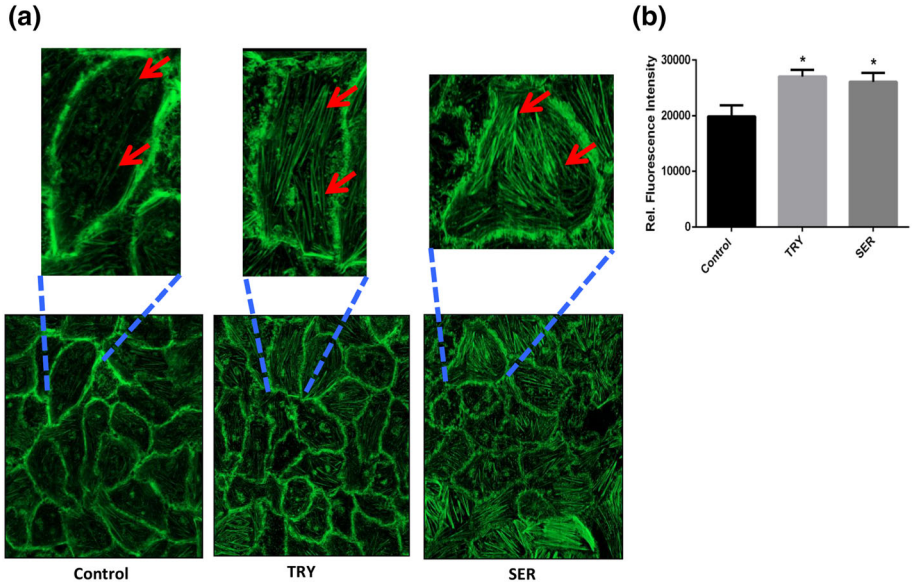

FIGURE 5 TRY and SER increase F-actin formation in HT-29 cells. (a) We specifically imaged F-actin formation in HT-29 cells treated with TRY, SER, and DMSO (as a control) using ActinGreen fluorescence dye. On average, HT-29 cells treated with TRY and SER (each 50 μg ml^{-1}) appeared to have more F-actin (indicated with red arrows) compared with control cells. (b) ImageJ analyses calculating the fluorescence intensity of confocal images indicate a higher amount of F-actin in TRY- and SER-treated cells. The fluorescence intensity was calculated from at least five different observation areas for each treatment. For the graph, each data point is the mean value ± *SEM*, **P* < .05 by students *t* test

dopamine D4 receptor agonist (Chemel, Roth, Armbruster, Watts, & Nichols, 2006), it did not influence our results, as this receptor is most likely not expressed in HT-29 cells. When testing SER together with 5HT antagonists, only the 5HT1A antagonist WAY100635 but not the 5HT3 and 5HT4 antagonists caused a significant increase in cAMP levels (Figure 6c). All three antagonists decreased F-actin formation, but only the WAY100635 and SB207266 antagonists decreased F-actin formation below that of the control (Figure 6d), indicating that the two antagonists have an inverse agonist effect. The addition of ondansetron alone or together with SER did not affect cAMP or F-actin formation. These results suggest that SER-induced upregulation of F-actin formation is triggered by the 5HT1A, 5HT3, and 5HT4 receptors.

We investigated whether SER-induced F-actin formation also triggers an increased invasion frequency. To avoid interference from the AAAs present in the medium, we carried out invasion studies with the *sadA* mutant, ED99Δ*sadA*. SER always caused an increase in internalisation. In the presence of SER and the 5HT1A antagonist (WAY100635), 5HT3 antagonist (ondansetron), or 5HT4 antagonist (SB207266), the effect of SER was nullified (Figure 7a–c). Internalisation was decreased below that of the control with only the 5HT1A antagonist (WAY100635; Figure 7a). In conclusion, these results show that SER increases bacterial internalisation by activating 5HT1A, 5HT3, and 5HT4.

If the antagonists were used alone in the absence of SER, only WAY100635 (5HT1A antagonist) and SB207266 (5HT4 antagonist) caused a decrease in internalisation below that of the control. Such an inverse agonist effect has also been reported previously (Claeysen et al., 2000; Cosi & Koek, 2000; Newman-Tancredi, Conte, Chaput, Spedding, & Millan, 1997; Figure 7a,c).

3.8 | Neurochemicals are present in human stool samples

We also addressed the question of how relevant our in vitro results are in relation to the in vivo situation. Therefore, we tested stool samples from 19 adults by HPLC analysis to determine the abundance and concentration of TRY, PEA, TYM, DOP, and SER. In 80–100% of the samples, we detected TRY, TYM, DOP, and SER; PEA was only ≈25% less prevalent than the other neurochemicals (Figure S4a). Regarding the concentration, TYM was present in high concentrations (ranging from 7.6 to 621 μg g^{-1} of faeces), followed by DOP, TRY, and SER, whereas PEA was the least abundant, ranging from 0.8 to 12 μg g^{-1} (Figure S4b). In 50% of the samples, the total concentration of neurochemicals reached >100 μg g^{-1}, and in one case, it reached >600 μg g^{-1}, suggesting that neurochemicals are highly abundant in the intestine and might affect bacterial colonisation and internalisation as shown in in vitro assays.

4 | DISCUSSION

Bacterial pathogens can be classified as exclusively extracellular, dually intracellular/extracellular and exclusively intracellular bacteria based on their infective lifestyle in the host (Silva, 2012). *S. aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, and *Escherichia coli* are typical examples of bacteria that have been regarded as extracellular pathogens causing, for example, wound infections, osteomyelitis, scarlet fever, certain forms of pneumonia, and urinary tract infections. However, there are now numerous examples in which all these species use an intracellular phase (Casadevall, 2008; Silva, 2012). *S. aureus* is

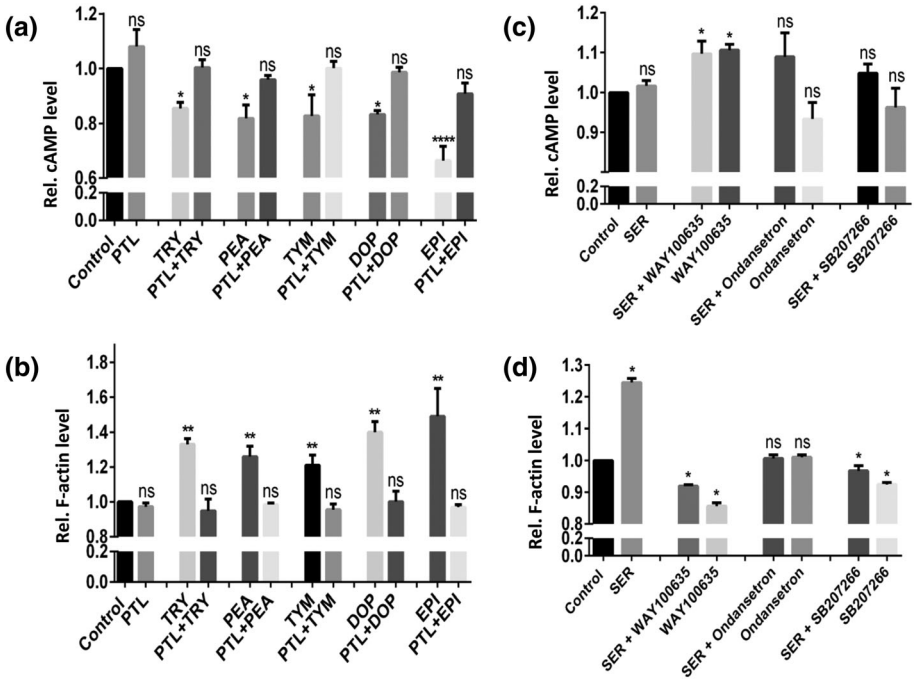

FIGURE 6 TAs and DOP can bind to α-adrenergic receptor and SER can bind to 5HT receptors to induce host cells response. (a) We treated HT-29 with PTL (α-adrenergic receptor antagonist) before addition of neurochemicals in order to block the receptor and prove that TRY, PEA, TYM, and DOP can interact to α-adrenergic receptor in HT-29 and lead to induce further host cells response. We also used EPI as positive control. Treatment with PTL (final concentration 50 μg ml^{-1}) showed that intracellular cAMP level did not decrease significantly in the presence of TRY, PEA, TYM, DOP, and EPI (final concentration 50 μg ml^{-1}). (b) HT-29 cells treated with PTL alone (final concentration 50 μg ml^{-1}) did not significantly affect F-actin level. Meanwhile, when we added PTL prior the addition of TRY, PEA, TYM, DOP, and EPI (final concentration 50 μg ml^{-1}), PTL nullified the enhancement effect of those compounds in F-actin formation to the control. (c) We treated HT-29 with 5HT receptor antagonists (final concentration 50 μg ml^{-1}) before addition of SER (final concentration 50 μg ml^{-1}) in order to block the receptor and measured the intracellular cAMP level of HT-29 cells. No significant changes in the intracellular cAMP level in addition of 5HT antagonists and SER except WAY100635. Addition of WAY100635 alone and together with SER increased the intracellular cAMP level of HT-29 cells significantly compared with control. (d) The addition of ondansetron (final concentration 50 μg ml^{-1}) prior the addition of SER (final concentration 50 μg ml^{-1}) nullified the increasing effect of SER in F-actin formation to the control level. The addition of WAY100635 and SB207266 alone and together with SER even decreased the F-actin formation in HT-29 cells significantly compared with the control. Representative data from at least three independent experiments are shown. For all graphs, each data point is the mean value ± *SEM*, $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$; and $^{****}P < .0001$, by students *t* test. All cAMP and F-actin levels were normalised to control

one such facultative intracellular pathogen invading a wide range of host cells, both professional (macrophages) and nonprofessional phagocytes such as epithelial, endothelial cells, osteoblasts, fibroblasts, and keratinocytes (Löffler, Tuchscherr, Niemann, & Peters, 2014). The advantage of an intracellular lifestyle is long-term persistence in the host tissue, as bacteria are largely protected against antimicrobial treatments and the host immune system (Sendi & Proctor, 2009).

It is well known that a crucial early step in infection development is tight adherence of the pathogen to host matrices or host cells. In particular, *S. aureus* expresses an exceptionally large variety of surface proteins called adhesins (Clarke & Foster, 2006) that can be divided into proteins that are covalently bound to bacterial peptidoglycan, the MSCRAMMs (Foster & Hook, 1998); rebinding secreted proteins, the SERAMs (Chavakis, Wiechmann, Preissner, & Herrmann, 2005); or membrane-anchored lipoproteins (Shahmirzadi, Nguyen, & Götz, 2016). Some of these adhesins trigger invasion by interacting with a cognate receptor on host cells. For example, FnBPs bind to the host cell integrin α5ß1 through cellular or soluble fibronectin as a bridging molecule (Sinha et al., 1999); FnBPs can also bind to human Hsp60 (Dziewanowska et al., 2000); the major autolysin, Atl, binds to Hsc70 and to the α5ß1 integrin (Hirschhausen et al., 2010); extracellular adherence protein, Eap, contributes to internalisation by epithelial cells and fibroblasts via an unknown receptor (Haggar et al., 2003); and the Lpls mediate internalisation by human keratinocytes and mouse skin also via a still-unknown receptor (Nguyen et al., 2015).

Exemplified of *S. pseudintermedius* ED99, we identified a new concept of host cell internalisation that plays a role especially in animal-pathogenic staphylococcal species expressing SadA or nonaureus staphylococcal strains (Maali et al., 2016; Valour et al., 2013). In contrast to all published pathways, this pathway does not involve a staphylococcal surface protein that interacts with a corresponding host cell receptor to trigger invasion.

We unravelled two internalisation pathways in the colon carcinoma cell line HT-29 (Rousset, 1986; Turner, Ray-Prenger, & Bylund, 1985). One pathway is triggered by TAs and DOP. Binding of these

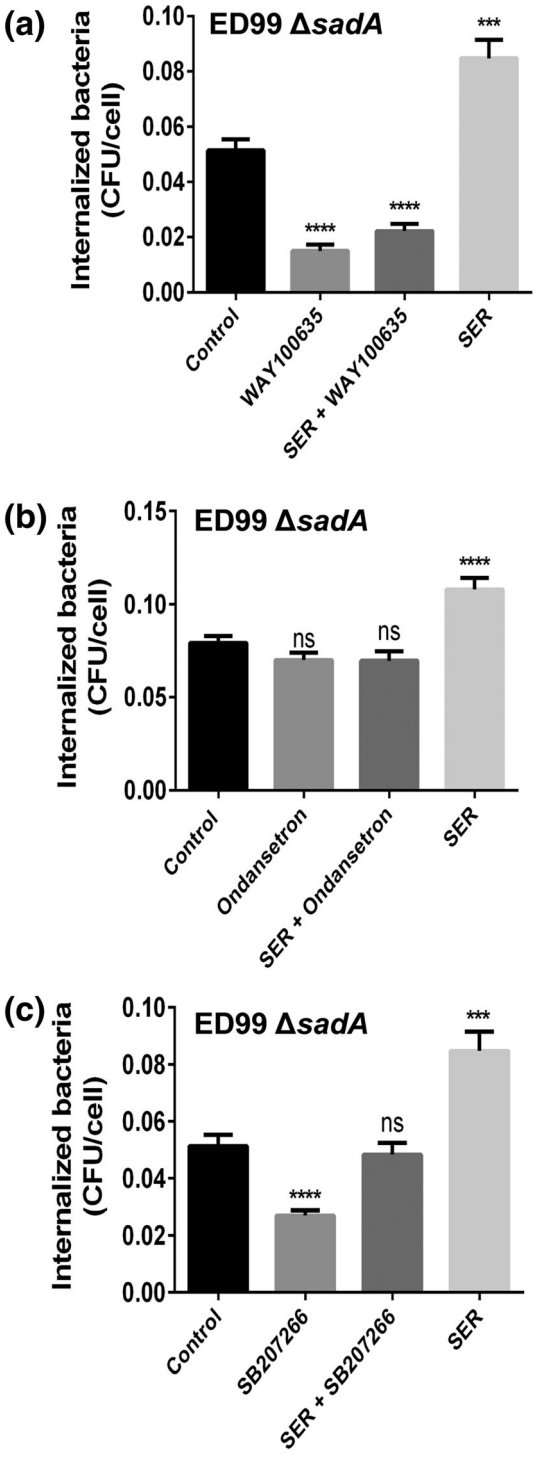

FIGURE 7 The blockage of 5HT receptors by antagonists leads to the decreasing of staphylococcal internalisation. Internalisation of ED99Δ*sadA* was enhanced around twofold in the presence of SER (25 μg ml^{-1} as final concentration) but was decreased to control level (10% DMSO) when SER was applied together with 25 μg ml^{-1} antagonists. We used (a) WAY100635 as 5HT1A antagonist, (b) ondansetron as 5HT3 antagonist, and (c) SB207266 as 5HT4 antagonist. Representative data from at least three independent experiments are shown. For all graphs, each data point is the mean value ± *SEM*, **P* < .05; ***P* < .01; ****P* < .001; and *****P* < .0001, by students *t* test

neurochemicals to the α2-adrenergic receptor triggers repression of cytoplasmic cAMP production, and low cAMP levels activate polymerisation of G-actin to F-actin. The cytoplasmic cAMP level in HT-29 cells was significantly decreased in the presence of AAAs and L-DOPA in the presence of only ED99 and the complemented mutant, ED99Δ*sadA*com, but not in the presence of 5-HTP (Figure 3a,b). The other pathway is triggered by SER. Although SER does not alter the cAMP level, it also increases F-actin formation (Figure 6d). By using various 5HT antagonists, we showed that SER interacts with at least three 5HT receptors in HT-29 cells. This interaction triggers G-actin polymerisation to F-actin without significantly affecting the cytoplasmic cAMP level (Figure 8).

The α2-adrenergic receptor-mediated effect of TAs and DOP, namely, lowering cytoplasmic cAMP levels and increasing F-actin formation, is the same as that observed with EPI (adrenaline), a well-known agonist of the α2-adrenergic receptor (Tian, Duzic, Lanier, & Deth, 1994; Figure 4). Based on our knowledge of the EPI-mediated pathway, we propose the following downstream reactions after activation of the α2-adrenergic receptor by TAs, DOP, and EPI. Activation of the α2-adrenergic receptor causes dissociation of the alpha subunit of G protein-Gi, and this subunit binds to AC, causing its inactivation (Turner et al., 1985) and a decrease in the intracellular cAMP level (Figure 6a). The decreased cAMP level induces F-actin formation (Whelan & Senger, 2003; Figure 6b), which leads to the formation of pedestal-like structures that are known to play a role in bacterial adherence and internalisation (Bhavsar, Guttman, & Finlay, 2007; Carabeo, 2011; dos Reis & Horn, 2010; Finlay, Rosenshine, Donnenberg, & Kaper, 1992; Finlay, Ruschkowski, & Dedhar, 1991), in the host cells and an approximately threefold increase in adherence and internalisation of staphylococci by HT-29 cells. The activation of the α-adrenergic receptor in colonic mucosa also increases the internalisation of *E. coli* O157:H7 and *Salmonella choleraesuis* (which has no SadA homologue and does not produce TAs), as reported by (Green, Lyte, Kulkarni-Narla, & Brown, 2003).

SER follows a different pathway, although with the same outcome. SER did not decrease the intracellular cAMP level (Figure 6c) but did increase the F-actin level (Figure 6d). This finding is explainable because studies by Ataee et al. (Ataee, Ajdary, Rezayat, et al., 2010; Ataee, Ajdary, Zarrindast, et al., 2010) showed that HT-29 cells express various 5HT receptors: 5HT1A, 5HT1B, 5HT3, and 5HT4. 5HT1A is a G protein-coupled receptor (Raymond, Mukhin, Gettys, & Garnovskaya, 1999) with many effectors, and one of them is AC. 5HT1A activation induces activation of protein kinase C (PKC), Src kinase, and mitogen-activated protein (MAP) kinases and inhibition of AC (Raymond et al., 2001), thus causing a decrease in the cytoplasmic cAMP level (Aune, McGrath, Sarr, Bombara, & Kelley, 1993; Fargin et al., 1989). Both 5HT3 and 5HT4 activation induce F-actin formation by Ca2+- and PKC-dependent pathways (Gill et al., 2008). Therefore, in contrast to 5HT1A, 5HT4 activation increases the intracellular cAMP level (Kuemmerle, Murthy, Grider, Martin, & Makhlouf, 1995) by activating AC (Raymond et al., 2001). Because of the opposing effects of 5HT1A and 5HT4

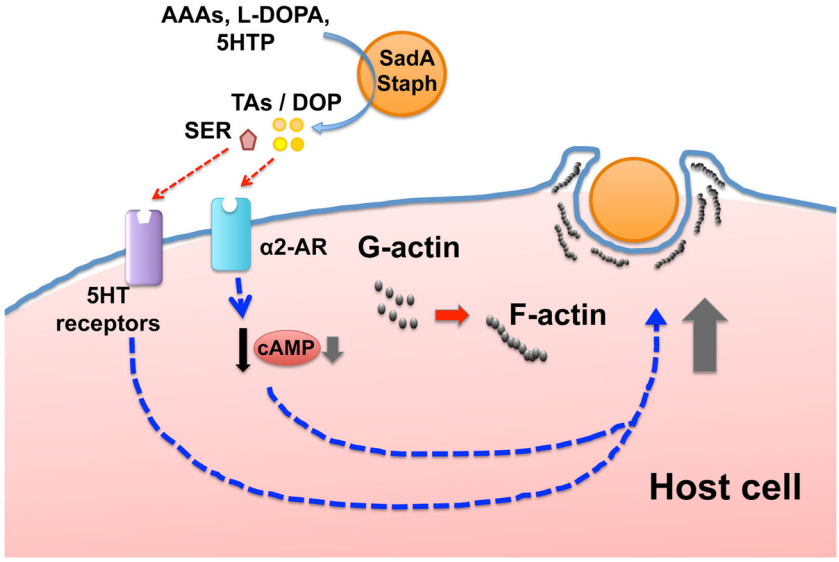

FIGURE 8 The neurochemicals TAs, DOP, and SER produced by SadA-expressing staphylococci enhance bacterial internalisation into host cells. SadA-expressing staphylococci convert aromatic amino acids (AAAs), L-DOPA, and 5HTP into corresponding trace amines (TAs), dopamine (DOP), and serotonin (SER), respectively. TAs and DOP interact with α2-adrenergic receptor (α2-AR) while SER with 5HT receptors. The signaling of TAs and DOP via α2-AR leads to a decrease of cytoplasmic cAMP level and actin polymerisation. SER signals via interaction with 5HT receptors leading also to actin polymerisation, however, without affecting the cytoplasmic cAMP level. In the presence of SadA or neurochemicals internalisation into host cell is boosted about two to three fold

activation, their effect is nullified, and as a consequence, we observed no change in the intracellular cAMP level of HT-29 cells treated with SER. However, blocking 5HT1A with the inhibitor WAY100635 increased cAMP levels, decreased F-actin formation (Figure 6c,d), and decreased internalisation of ED99Δ*sadA* (Figure 7 a) because WAY100635 has an inverse agonist effect (Cosi & Koek, 2000; Newman-Tancredi et al., 1997). Similar effects were also observed when we treated HT-29 cells with the 5HT4 antagonist (SB207226) and the 5HT3 antagonist (ondansetron); in both cases, F-actin formation and internalisation of ED99Δ*sadA* were decreased. In conclusion, activation of 5HT1A, 5HT3, and 5HT4 by SER increases staphylococcal internalisation in HT-29 cells.

The bacterial invasion process and the subsequent infection course in different cell types have in common that the uptake mechanism requires an intact cytoskeleton and is an active process of the host cells (Ellington et al., 1999; Josse et al., 2017; Kahl et al., 2000; Mempel et al., 2002; Menzies & Kourteva, 1998). Directly after infection, bacteria are usually located within phagosomes, but they can later escape to the cytoplasm, which is often associated with apoptosis of host cells (Grosz et al., 2014; Löffler et al., 2014).

The final questions are about the relevance of neurochemical-triggered internalisation in vivo and whether there are sufficient amounts of AAAs in the various body fluids to play a role in infection. In blood, there is approximately 60–80 μM each AAA, totaling 200 μM (Forteschi et al., 2015). In the kidneys, there are comparable amounts (Li, Tang, & Mu, 2011). Here, we show that in 50% of the human stool samples, the total neurochemicals reached >100 μg g^{-1} and, in some cases, >600 μg g^{-1}, suggesting that neurochemicals are highly abundant in the intestine and might influence bacterial colonisation and internalisation, as shown in vitro.

In the case of the neurochemical level in human stool samples, *SadA*-expressing staphylococci might contribute to the neurochemical level. However, there are many other factors that also play a role, for example, the intestine epithelial cells, the enterochromaffin cells (Pettersson, Newson, Ahlman, & Dahlstrom, 1980), and various other bacteria that can produce neurochemicals (Williams et al., 2014). It is difficult, if not impossible, to determine the origin of TAs in the human intestine.

5 | CONCLUSION

In this study, we wanted to determine the pathway of neurochemical-triggered internalisation into host cells. We identified a new mechanism of how pathogenic staphylococci can increase their internalisation into host cells. Not a cell surface protein but rather excreted neurochemicals, TAs, DOP, and SER, boost actin cytoskeletal remodelling to increase the internalisation rate by approximately threefold. The aromatic amino acid decarboxylase gene, *sadA*, is responsible for the production of the neurochemicals and is present in most animal-pathogenic staphylococcal species. The internalisation can be subdivided into three steps: actin recruitment, engulfment, and actin disassembly as a driving force for internalisation (Carabeo, 2011). It is most likely that the *sadA* gene increases the virulence potential (internalisation, persistence, and cytotoxicity) of staphylococci. As the neurochemicals are diffusible, the internalisation of *S. aureus* can be boosted by coinfecting bacteria that produce these compounds.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

FG and AL designed the study. AL, PE, SR, CH, CK, PR and FG designed the experiments. AL performed all experiments except the microscopy analyses, which were carried out by PS. CK prepared the primary epithelial colon cells from mice. FG, PS, PR and AL wrote the manuscript.

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REFERENCES

Agrawal, D. K., Wildrick, D. M., & Boman, B. M. (1992). Characteristics of alpha-adrenoceptors in two human colorectal cancer cell lines. *Biochemical and Biophysical Research Communications*, *185*, 176–184. https://doi.org/10.1016/S0006-291X(05)80972-6

Aktories, K., & Jakobs, K. H. (1981). Epinephrine inhibits adenylate cyclase and stimulates a GTPase in human platelet membranes via alpha-adrenoceptors. *FEBS Letters*, *130*, 235–238. https://doi.org/10.1016/0014-5793(81)81128-3

Ataee, R., Ajdary, S., Rezayat, M., Shokrgozar, M. A., Shahriari, S., & Zarrindast, M. R. (2010). Study of 5HT3 and HT4 receptor expression in HT29 cell line and human colon adenocarcinoma tissues. *Archives of Iranian Medicine*, *13*, 120–125.

Ataee, R., Ajdary, S., Zarrindast, M., Rezayat, M., & Hayatbakhsh, M. R. (2010). Anti-mitogenic and apoptotic effects of 5-HT1B receptor antagonist on HT29 colorectal cancer cell line. *Journal of Cancer Research and Clinical Oncology*, *136*, 1461–1469. https://doi.org/10.1007/s00432-010-0801-3

Aune, T. M., McGrath, K. M., Sarr, T., Bombara, M. P., & Kelley, K. A. (1993). Expression of 5HT1a receptors on activated human T cells. Regulation of cyclic AMP levels and T cell proliferation by 5-hydroxytryptamine. *Journal of Immunology*, *151*, 1175–1183.

Bannoehr, J., Ben Zakour, N. L., Reglinski, M., Inglis, N. F., Prabhakaran, S., Fossum, E., … Fitzgerald, J. R. (2011). Genomic and surface proteomic analysis of the canine pathogen *Staphylococcus pseudintermedius* reveals proteins that mediate adherence to the extracellular matrix. *Infection and Immunity*, *79*, 3074–3086. https://doi.org/10.1128/IAI.00137-11

Ben Zakour, N. L., Bannoehr, J., van den Broek, A. H., Thoday, K. L., & Fitzgerald, J. R. (2011). Complete genome sequence of the canine pathogen *Staphylococcus pseudintermedius*. *Journal of Bacteriology*, *193*, 2363–2364. https://doi.org/10.1128/JB.00137-11

Bhavsar, A. P., Guttman, J. A., & Finlay, B. B. (2007). Manipulation of host-cell pathways by bacterial pathogens. *Nature*, *449*, 827–834. https://doi.org/10.1038/nature06247

Blättner, S., Das, S., Paprotka, K., Eilers, U., Krischke, M., Kretschmer, D., et al. (2016). *Staphylococcus aureus* exploits a non-ribosomal cyclic dipeptide to modulate survival within epithelial cells and phagocytes. *PLoS Pathogens*, *12*, e1005857. https://doi.org/10.1371/journal.ppat.1005857

Brass, L. F., Woolkalis, M. J., & Manning, D. R. (1988). Interactions in platelets between G proteins and the agonists that stimulate phospholipase C and inhibit adenylyl cyclase. *The Journal of Biological Chemistry*, *263*, 5348–5355.

Burke, F. M., Di Poto, A., Speziale, P., & Foster, T. J. (2011). The A domain of fibronectin-binding protein B of *Staphylococcus aureus* contains a novel fibronectin binding site. *The FEBS Journal*, *278*, 2359–2371. https://doi.org/10.1111/j.1742-4658.2011.08159.x

Butler, A., Hill, J. M., Ireland, S. J., Jordan, C. C., & Tyers, M. B. (1988). Pharmacological properties of GR38032F, a novel antagonist at 5-HT3 receptors. *British Journal of Pharmacology*, *94*, 397–412. https://doi.org/10.1111/j.1476-5381.1988.tb11542.x

Carabeo, R. (2011). Bacterial subversion of host actin dynamics at the plasma membrane. *Cellular Microbiology*, *13*, 1460–1469. https://doi.org/10.1111/j.1462-5822.2011.01651.x

Casadevall, A. (2008). Evolution of intracellular pathogens. *Annual Review of Microbiology*, *62*, 19–33. https://doi.org/10.1146/annurev.micro.61.080706.093305

Chavakis, T., Wiechmann, K., Preissner, K. T., & Herrmann, M. (2005). *Staphylococcus aureus* interactions with the endothelium: the role of bacterial "secretable expanded repertoire adhesive molecules" (SERAM) in disturbing host defense systems. *Thrombosis and Haemostasis*, *94*, 278–285. https://doi.org/10.1160/TH05-05-0306

Chemel, B. R., Roth, B. L., Armbruster, B., Watts, V. J., & Nichols, D. E. (2006). WAY-100635 is a potent dopamine D4 receptor agonist. *Psychopharmacology*, *188*, 244–251. https://doi.org/10.1007/s00213-006-0490-4

Chen, C., Brown, D. R., Xie, Y., Green, B. T., & Lyte, M. (2003). Catecholamines modulate *Escherichia coli* O157:H7 adherence to murine cecal mucosa. *Shock*, *20*, 183–188. https://doi.org/10.1097/01.shk.0000073867.66587.e0

Chetty, N., Irving, H. R., & Coupar, I. M. (2009). Activation of 5-HT3 receptors in the rat and mouse intestinal tract: A comparative study. *British Journal of Pharmacology*, *148*, 1012–1021. https://doi.org/10.1038/sj.bjp.0706802

Claeysen, S., Sebben, M., Becamel, C., Eglen, R. M., Clark, R. D., Bockaert, J., & Dumuis, A. (2000). Pharmacological properties of 5-Hydroxytryptamine(4) receptor antagonists on constitutively active wild-type and mutated receptors. *Molecular Pharmacology*, *58*, 136–144. https://doi.org/10.1124/mol.58.1.136

Clarke, S. R., & Foster, S. J. (2006). Surface adhesins of *Staphylococcus aureus*. *Advances in Microbial Physiology*, *51*, 187–224. https://doi.org/10.1016/S0065-2911(06)51004-5

Cosi, C., & Koek, W. (2000). The putative 5-HT(1A) receptor antagonist, WAY 100635, has inverse agonist properties at cloned human 5-HT(1A) receptors. *European Journal of Pharmacology*, *401*, 9–15. https://doi.org/10.1016/S0014-2999(00)00410-6

dos Reis, R. S., & Horn, F. (2010). Enteropathogenic *Escherichia coli*, *Samonella*, *Shigella* and *Yersinia*: Cellular aspects of host-bacteria interactions in enteric diseases. *Gut Pathog*, *2*, 8. https://doi.org/10.1186/1757-4749-2-8

Dziewanowska, K., Carson, A. R., Patti, J. M., Deobald, C. F., Bayles, K. W., & Bohach, G. A. (2000). Staphylococcal fibronectin binding protein interacts with heat shock protein 60 and integrins: Role in internalization by epithelial cells. *Infection and Immunity*, *68*, 6321–6328. https://doi.org/10.1128/IAI.68.11.6321-6328.2000

Dziewanowska, K., Patti, J. M., Deobald, C. F., Bayles, K. W., Trumble, W. R., & Bohach, G. A. (1999). Fibronectin binding protein and host cell tyrosine kinase are required for internalization of *Staphylococcus aureus* by epithelial cells. *Infection and Immunity*, *67*, 4673–4678.

von Eiff, C., Heilmann, C., Proctor, R. A., Woltz, C., Peters, G., & Götz, F. (1997). A site-directed *Staphylococcus aureus hemB* mutant is a small-colony variant which persists intracellularly. *Journal of Bacteriology*, *179*, 4706–4712. https://doi.org/10.1128/jb.179.15.4706-4712.1997

Ellington, J. K., Reilly, S. S., Ramp, W. K., Smeltzer, M. S., Kellam, J. F., & Hudson, M. C. (1999). Mechanisms of *Staphylococcus aureus* invasion of cultured osteoblasts. *Microbial Pathogenesis*, *26*, 317–323. https://doi.org/10.1006/mpat.1999.0272

Fargin, A., Raymond, J. R., Regan, J. W., Cotecchia, S., Lefkowitz, R. J., & Caron, M. G. (1989). Effector coupling mechanisms of the cloned 5-HT1A receptor. *The Journal of Biological Chemistry*, *264*, 14848–14852.

Fedouloff, M., Hossner, F., Voyle, M., Ranson, J., Powles, J., Riley, G., & Sanger, G. (2001). Synthesis and pharmacological activity of metabolites of the 5-HT(4) receptor antagonist SB-207266. *Bioorganic & Medicinal Chemistry*, *9*, 2119–2128. https://doi.org/10.1016/S0968-0896(01)00120-1

Finlay, B. B., Rosenshine, I., Donnenberg, M. S., & Kaper, J. B. (1992). Cytoskeletal composition of attaching and effacing lesions associated with enteropathogenic *Escherichia coli* adherence to HeLa cells. *Infection and Immunity*, *60*, 2541–2543.

Finlay, B. B., Ruschkowski, S., & Dedhar, S. (1991). Cytoskeletal rearrangements accompanying *Salmonella* entry into epithelial-cells. *Journal of Cell Science*, *99*, 283.

Fitzgerald, J. R. (2009). The *Staphylococcus intermedius* group of bacterial pathogens: Species re-classification, pathogenesis and the emergence of meticillin resistance. *Veterinary Dermatology*, *20*, 490–495. https://doi.org/10.1111/j.1365-3164.2009.00828.x

Forster, E. A., Cliffe, I. A., Bill, D. J., Dover, G. M., Jones, D., Reilly, Y., & Fletcher, A. (1995). A pharmacological profile of the selective silent 5-HT1A receptor antagonist, WAY-100635. *European Journal of Pharmacology*, *281*, 81–88. https://doi.org/10.1016/0014-2999(95)00234-C

Forteschi, M., Sotgia, S., Assaretti, S., Arru, D., Cambedda, D., Sotgiu, E., … Carru, C. (2015). Simultaneous determination of aromatic amino acids in human blood plasma by capillary electrophoresis with UV-absorption detection. *Journal of Separation Science*, *38*, 1794–1799. https://doi.org/10.1002/jssc.201500038

Foster, T. J., & Hook, M. (1998). Surface protein adhesins of *Staphylococcus aureus*. *Trends in Microbiology*, *6*, 484–488. https://doi.org/10.1016/S0966-842X(98)01400-0

Fowler, T., Johansson, S., Wary, K. K., & Hook, M. (2003). Src kinase has a central role in in vitro cellular internalization of *Staphylococcus aureus*. *Cellular Microbiology*, *5*, 417–426. https://doi.org/10.1046/j.1462-5822.2003.00290.x

Ganguly, S., Saxena, R., & Chattopadhyay, A. (2011). Reorganization of the actin cytoskeleton upon G-protein coupled receptor signaling. *Biochimica et Biophysica Acta*, *1808*, 1921–1929. https://doi.org/10.1016/j.bbamem.2011.04.001

Garzoni, C., & Kelley, W. L. (2009). *Staphylococcus aureus*: New evidence for intracellular persistence. *Trends in Microbiology*, *17*, 59–65. https://doi.org/10.1016/j.tim.2008.11.005

Gavet, O., & Pines, J. (2010). Progressive activation of CyclinB1-Cdk1 coordinates entry to mitosis. *Developmental Cell*, *18*, 533–543.

Gill, R. K., Shen, L., Turner, J. R., Saksena, S., Alrefai, W. A., Pant, N., … Dudeja, P. K. (2008). Serotonin modifies cytoskeleton and brush-border membrane architecture in human intestinal epithelial cells. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, *295*, G700–G708. https://doi.org/10.1152/ajpgi.90362.2008

Green, B. T., Lyte, M., Kulkarni-Narla, A., & Brown, D. R. (2003). Neuromodulation of enteropathogen internalization in Peyer's patches from porcine jejunum. *Journal of Neuroimmunology*, *141*, 74–82. https://doi.org/10.1016/S0165-5728(03)00225-X

Greene, C., McDevitt, D., Francois, P., Vaudaux, P. E., Lew, D. P., & Foster, T. J. (1995). Adhesion properties of mutants of Staphylococcus aureus defective in fibronectin-binding proteins and studies on the expression of fnb genes. *Molecular Microbiology*, *17*, 1143–1152. https://doi.org/10.1111/j.1365-2958.1995.mmi_17061143.x

Grosz, M., Kolter, J., Paprotka, K., Winkler, A. C., Schäfer, D., Chatterjee, S. S., … Fraunholz, M. (2014). Cytoplasmic replication of *Staphylococcus aureus* upon phagosomal escape triggered by phenol-soluble modulin alpha. *Cellular Microbiology*, *16*, 451–465. https://doi.org/10.1111/cmi.12233

Guardabassi, L., Schwarz, S., & Lloyd, D. H. (2004). Pet animals as reservoirs of antimicrobial-resistant bacteria. *The Journal of Antimicrobial Chemotherapy*, *54*, 321–332. https://doi.org/10.1093/jac/dkh332

Haggar, A., Hussain, M., Lonnies, H., Herrmann, M., Norrby-Teglund, A., & Flock, J. I. (2003). Extracellular adherence protein from *Staphylococcus aureus* enhances internalization into eukaryotic cells. *Infection and Immunity*, *71*, 2310–2317. https://doi.org/10.1128/IAI.71.5.2310-2317.2003

Hill, P. B., Lo, A., Eden, C. A., Huntley, S., Morey, V., Ramsey, S., et al. (2006). Survey of the prevalence, diagnosis and treatment of dermatological conditions in small animals in general practice. *The Veterinary Record*, *158*, 533–539. https://doi.org/10.1136/vr.158.16.533

Hirschhausen, N., Schlesier, T., Schmidt, M. A., Götz, F., Peters, G., & Heilmann, C. (2010). A novel staphylococcal internalization mechanism involves the major autolysin Atl and heat shock cognate protein Hsc70 as host cell receptor. *Cellular Microbiology*, *12*, 1746–1764. https://doi.org/10.1111/j.1462-5822.2010.01506.x

Hoffman, J. M., Tyler, K., MacEachern, S. J., Balemba, O. B., Johnson, A. C., Brooks, E. M., … Mawe, G. M. (2012). Activation of colonic mucosal 5-HT(4) receptors accelerates propulsive motility and inhibits visceral hypersensitivity. *Gastroenterology*, *142*, 844–854 e844. https://doi.org/10.1053/j.gastro.2011.12.041

Jonsson, K., Signas, C., Müller, H. P., & Lindberg, M. (1991). Two different genes encode fibronectin binding proteins in *Staphylococcus aureus*. The complete nucleotide sequence and characterization of the second gene. *European Journal of Biochemistry*, *202*, 1041–1048. https://doi.org/10.1111/j.1432-1033.1991.tb16468.x

Josse, J., Laurent, F., & Diot, A. (2017). Staphylococcal adhesion and host cell invasion: Fibronectin-binding and other mechanisms. *Frontiers in Microbiology*, *8*, 2433. https://doi.org/10.3389/fmicb.2017.02433

Kadlec, K., Schwarz, S., Perreten, V., Andersson, U. G., Finn, M., Greko, C., et al. (2010). Molecular analysis of methicillin-resistant *Staphylococcus pseudintermedius* of feline origin from different European countries and North America. *The Journal of Antimicrobial Chemotherapy*, *65*, 1826–1828. https://doi.org/10.1093/jac/dkq203

Kahl, B. C., Goulian, M., van Wamel, W., Herrmann, M., Simon, S. M., Kaplan, G., … Cheung, A. L. (2000). *Staphylococcus aureus* RN6390 replicates and induces apoptosis in a pulmonary epithelial cell line. *Infection and Immunity*, *68*, 5385–5392. https://doi.org/10.1128/IAI.68.9.5385-5392.2000

Keularts, I. M., van Gorp, R. M., Feijge, M. A., Vuist, W. M., & Heemskerk, J. W. (2000). α_{2A}-Adrenergic receptor stimulation potentiates calcium release in platelets by modulating cAMP levels. *The Journal of Biological Chemistry*, *275*, 1763–1772. https://doi.org/10.1074/jbc.275.3.1763

Kuemmerle, J. F., Murthy, K. S., Grider, J. R., Martin, D. C., & Makhlouf, G. M. (1995). Coexpression of 5-HT2A and 5-HT4 receptors coupled to distinct signaling pathways in human intestinal muscle cells. *Gastroenterology*, *109*, 1791–1800. https://doi.org/10.1016/0016-5085(95)90745-9

Li, Y., Tang, A. G., & Mu, S. (2011). HPLC-FLD determination of serum aromatic amino acids: Application in chronic kidney disease patients. *Clinica Chimica Acta*, *412*, 1032–1035. https://doi.org/10.1016/j.cca.2011.02.015

Löffler, B., Tuchscherr, L., Niemann, S., & Peters, G. (2014). *Staphylococcus aureus* persistence in non-professional phagocytes. *International Journal of Medical Microbiology*, *304*, 170–176. https://doi.org/10.1016/j.ijmm.2013.11.011

Lowy, F. D. (2000). Is *Staphylococcus aureus* an intracellular pathogen? *Trends in Microbiology*, *8*, 341–343. https://doi.org/10.1016/S0966-842X(00)01803-5

Lucas, J. J., Segu, L., & Hen, R. (1997). 5-Hydroxytryptamine1B receptors modulate the effect of cocaine on c-fos expression: Converging evidence using 5-hydroxytryptamine1B knockout mice and the 5-hydroxytryptamine1B/1D antagonist GR127935. *Molecular Pharmacology*, *51*, 755–763. https://doi.org/10.1124/mol.51.5.755

Luqman, A., Nega, M., Nguyen, M.-T., Ebner, P., & Götz, F. (2018). SadA-expressing staphylococci in the human gut show increased cell adherence and internalization. *Cell Reports*, *22*, 535–545. https://doi.org/10.1016/j.celrep.2017.12.058

Maali, Y., Martins-Simoes, P., Valour, F., Bouvard, D., Rasigade, J. P., Bes, M., … Trouillet-Assant, S. (2016). Pathophysiological mechanisms of staphylococcus non-aureus bone and joint infection: Interspecies homogeneity and specific behavior of *S. pseudintermedius*. *Frontiers in Microbiology*, *7*, 1063.

Mauthe, M., Yu, W., Krut, O., Kronke, M., Götz, F., Robenek, H., & Proikas-Cezanne, T. (2012). WIPI-1 positive autophagosome-like vesicles entrap pathogenic *Staphylococcus aureus* for lysosomal degradation. *International Journal of Cell Biology*, *2012*, 179207.

Mempel, M., Schnopp, C., Hojka, M., Fesq, H., Weidinger, S., Schaller, M., … Abeck, D. (2002). Invasion of human keratinocytes by *Staphylococcus aureus* and intracellular bacterial persistence represent haemolysin-independent virulence mechanisms that are followed by features of necrotic and apoptotic keratinocyte cell death. *The British Journal of Dermatology*, *146*, 943–951. https://doi.org/10.1046/j.1365-2133.2002.04752.x

Menzies, B. E., & Kourteva, I. (1998). Internalization of *Staphylococcus aureus* by endothelial cells induces apoptosis. *Infection and Immunity*, *66*, 5994–5998.

Mostaghim, R., Thomas, G., & Ramwell, P. W. (1988). Endothelial potentiation of relaxation response to phentolamine in rat thoracic aorta. *The Journal of Pharmacology and Experimental Therapeutics*, *244*, 475–478.

Newman-Tancredi, A., Conte, C., Chaput, C., Spedding, M., & Millan, M. J. (1997). Inhibition of the constitutive activity of human 5-HT1A receptors by the inverse agonist, spiperone but not the neutral antagonist, WAY 100,635. *British Journal of Pharmacology*, *120*, 737–739. https://doi.org/10.1038/sj.bjp.0701025

Nguyen, M. T., Kraft, B., Yu, W., Demicrioglu, D. D., Hertlein, T., Burian, M., … Götz, F. (2015). The vSaα specific lipoprotein like cluster (lpl) of *S. aureus* USA300 contributes to immune stimulation and invasion in human cells. *PLoS Pathogens*, *11*, e1004984. https://doi.org/10.1371/journal.ppat.1004984

Novick, R. (1967). Properties of a cryptic high-frequency transducing phage in *Staphylococcus aureus*. *Virology*, *33*, 155–166. https://doi.org/10.1016/0042-6822(67)90105-5

Paul, N. C., Damborg, P., & Guardabassi, L. (2014). Dam-to-offspring transmission and persistence of *Staphylococcus pseudintermedius* clones within dog families. *Veterinary Dermatology*, *25*, 3–e2. https://doi.org/10.1111/vde.12090

Pettersson, G. B., Newson, B., Ahlman, H., & Dahlstrom, A. (1980). In vitro studies of serotonin release from rat enterochromaffin cells: Studies of gut serotonin release. *The Journal of Surgical Research*, *29*, 141–148. https://doi.org/10.1016/0022-4804(80)90032-3

Pietrocola, G., Gianotti, V., Richards, A., Nobile, G., Geoghegan, J. A., Rindi, S., … Speziale, P. (2015). Fibronectin binding proteins SpsD and SpsL both support invasion of canine epithelial cells by *Staphylococcus pseudintermedius*. *Infection and Immunity*, *83*, 4093–4102. https://doi.org/10.1128/IAI.00542-15

Pottumarthy, S., Schapiro, J. M., Prentice, J. L., Houze, Y. B., Swanzy, S. R., Fang, F. C., & Cookson, B. T. (2004). Clinical isolates of *Staphylococcus intermedius* masquerading as methicillin-resistant *Staphylococcus aureus*. *Journal of Clinical Microbiology*, *42*, 5881–5884. https://doi.org/10.1128/JCM.42.12.5881-5884.2004

Raymond, J. R., Mukhin, Y. V., Gelasco, A., Turner, J., Collinsworth, G., Gettys, T. W., … Garnovskaya, M. N. (2001). Multiplicity of mechanisms of serotonin receptor signal transduction. *Pharmacology & Therapeutics*, *92*, 179–212. https://doi.org/10.1016/S0163-7258(01)00169-3

Raymond, J. R., Mukhin, Y. V., Gettys, T. W., & Garnovskaya, M. N. (1999). The recombinant 5-HT1A receptor: G protein coupling and signalling pathways. *British Journal of Pharmacology*, *127*, 1751–1764. https://doi.org/10.1038/sj.bjp.0702723

Rosenstein, R., Nerz, C., Biswas, L., Resch, A., Raddatz, G., Schuster, S. C., & Götz, F. (2009). Genome analysis of the meat starter culture bacterium *Staphylococcus carnosus* TM300. *Applied and Environmental Microbiology*, *75*, 811–822. https://doi.org/10.1128/AEM.01982-08

Rousset, M. (1986). The human colon carcinoma cell lines HT-29 and Caco-2: Two in vitro models for the study of intestinal differentiation. *Biochimie*, *68*, 1035–1040. https://doi.org/10.1016/S0300-9084(86)80177-8

Saeed, M., Sommer, O., Holtz, J., & Bassenge, E. (1982). Alpha-adrenoceptor blockade by phentolamine causes beta-adrenergic vasodilation by increased catecholamine release due to presynaptic alpha-blockade. *Journal of Cardiovascular Pharmacology*, *4*, 44–52. https://doi.org/10.1097/00005344-198201000-00008

Schultheiss, G., & Diener, M. (2000). Adrenoceptor-mediated secretion across the rat colonic epithelium. *European Journal of Pharmacology*, *403*, 251–258. https://doi.org/10.1016/S0014-2999(00)00487-8

Schumacher-Perdreau, F., Heilmann, C., Peters, G., Gotz, F., & Pulverer, G. (1994). Comparative analysis of a biofilm-forming *Staphylococcus epidermidis* strain and its adhesion-positive, accumulation-negative mutant M7. *FEMS Microbiology Letters*, *117*, 71–78. https://doi.org/10.1111/j.1574-6968.1994.tb06744.x

Scott, D. W., Peters, J., & Miller, W. H. Jr. (2006). Efficacy of orbifloxacin tablets for the treatment of superficial and deep pyoderma due to *Staphylococcus intermedius* infection in dogs. *The Canadian Veterinary Journal*, *47*, 999–1002.

Selkirk, J. V., Scott, C., Ho, M., Burton, M. J., Watson, J., Gaster, L. M., … Price, G. W. (1998). SB-224289—A novel selective (human) 5-HT1B receptor antagonist with negative intrinsic activity. *British Journal of Pharmacology*, *125*, 202–208. https://doi.org/10.1038/sj.bjp.0702059

Sendi, P., & Proctor, R. A. (2009). *Staphylococcus aureus* as an intracellular pathogen: The role of small colony variants. *Trends in Microbiology*, *17*, 54–58. https://doi.org/10.1016/j.tim.2008.11.004

Shahmirzadi, S. V., Nguyen, M. T., & Götz, F. (2016). Evaluation of *Staphylococcus aureus* lipoproteins: Role in nutritional acquisition and pathogenicity. *Frontiers in Microbiology*, *7*, 1404.

Silva, M. T. (2012). Classical labeling of bacterial pathogens according to their lifestyle in the host: Inconsistencies and alternatives. *Frontiers in Microbiology*, *3*, 71.

Sinha, B., Francois, P., Que, Y. A., Hussain, M., Heilmann, C., Moreillon, P., … Herrmann, M. (2000). Heterologously expressed *Staphylococcus aureus* fibronectin-binding proteins are sufficient for invasion of host cells. *Infection and Immunity*, *68*, 6871–6878. https://doi.org/10.1128/IAI.68.12.6871-6878.2000

Sinha, B., Francois, P. P., Nusse, O., Foti, M., Hartford, O. M., Vaudaux, P., … Krause, K. H. (1999). Fibronectin-binding protein acts as *Staphylococcus aureus* invasin via fibronectin bridging to integrin alpha5beta1. *Cellular Microbiology*, *1*, 101–117. https://doi.org/10.1046/j.1462-5822.1999.00011.x

Tenover, F. C., & Goering, R. V. (2009). Methicillin-resistant *Staphylococcus aureus* strain USA300: Origin and epidemiology. *The Journal of Antimicrobial Chemotherapy*, *64*, 441–446. https://doi.org/10.1093/jac/dkp241

Tian, W. N., Duzic, E., Lanier, S. M., & Deth, R. C. (1994). Determinants of alpha 2-adrenergic receptor activation of G proteins: Evidence for a precoupled receptor/G protein state. *Molecular Pharmacology*, *45*, 524–531.

Turner, J. T., Ray-Prenger, C., & Bylund, D. B. (1985). Alpha 2-adrenergic receptors in the human cell line, HT29. Characterization with the full agonist radioligand [3H]UK-14,304 and inhibition of adenylate cyclase. *Molecular Pharmacology*, *28*, 422–430.

Valour, F., Trouillet-Assant, S., Rasigade, J. P., Lustig, S., Chanard, E., Meugnier, H., … Lyon BJI Study Group (2013). *Staphylococcus epidermidis* in orthopedic device infections: The role of bacterial internalization in human osteoblasts and biofilm formation. *PLoS ONE*, *8*, e67240. https://doi.org/10.1371/journal.pone.0067240

Wagner, E., Doskar, J., & Gotz, F. (1998). Physical and genetic map of the genome of *Staphylococcus carnosus* TM300. *Microbiology*, *144* (*Pt 2*, 509–517. https://doi.org/10.1099/00221287-144-2-509

Wardle, K. A., Bingham, S., Ellis, E. S., Gaster, L. M., Rushant, B., Smith, M. I., & Sanger, G. J. (1996). Selective and functional 5-hydroxytryptamine4 receptor antagonism by SB 207266. *British Journal of Pharmacology*, *118*, 665–670. https://doi.org/10.1111/j.1476-5381.1996.tb15452.x

Whelan, M. C., & Senger, D. R. (2003). Collagen I initiates endothelial cell morphogenesis by inducing actin polymerization through suppression of cyclic AMP and protein kinase A. *The Journal of Biological Chemistry*, *278*, 327–334. https://doi.org/10.1074/jbc.M207554200

Williams, B. B., Van Benschoten, A. H., Cimermancic, P., Donia, M. S., Zimmermann, M., Taketani, M., … Fischbach, M. A. (2014). Discovery and characterization of gut microbiota decarboxylases that can produce the neurotransmitter tryptamine. *Cell Host & Microbe*, *16*, 495–503. https://doi.org/10.1016/j.chom.2014.09.001

Williams, R. J., Henderson, B., Sharp, L. J., & Nair, S. P. (2002). Identification of a fibronectin-binding protein from *Staphylococcus epidermidis*. *Infection and Immunity*, *70*, 6805–6810. https://doi.org/10.1128/IAI.70.12.6805-6810.2002

Zimmermann, M., & Fischbach, M. A. (2010). A family of pyrazinone natural products from a conserved nonribosomal peptide synthetase in *Staphylococcus aureus*. *Chemistry & Biology*, *17*, 925–930. https://doi.org/10.1016/j.chembiol.2010.08.006

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