Host Peptidic Hormones Affecting Bacterial Biofilm Formation and Virulence

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Abstract

Bacterial biofilms constitute a critical problem in hospitals, especially in resuscitation units or for immunocompromised patients, since bacteria embedded in their own matrix are not only protected against antibiotics but also develop resistant variant strains. In the last decade, an original approach to prevent biofilm formation has consisted of studying the antibacterial potential of host communication molecules. Thus, some of these compounds have been identified for their ability to modify the biofilm formation of both Gram-negative and Gram-positive bacteria. In addition to their effect on biofilm production, a detailed study of the mechanism of action of these human hormones on bacterial physiology has allowed the identification of new bacterial pathways involved in biofilm formation. In this review, we focus on the impact of neuropeptidic hormones on bacteria, address some future therapeutic issues, and provide a new view of inter-kingdom communication.

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Introduction

Biofilms are defined as complex microbial communities of cells that are attached to a substratum (biotic or abiotic), an interface, or to each other and embedded into a matrix of self-produced extracellular polymeric compounds [1]. Biofilm formation endows bacteria with a high tolerance to antimicrobials and host immune defense mechanisms, thus enabling the pathogens to survive in hostile environments and disperse and colonize

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new niches. In addition, a small percentage of persister cells developing within the biofilm is known to be highly tolerant to antibiotics and has typically been involved in causing the relapse of infections [2]. The medical consequence for the infected host is the persistency of chronic infections in organs in which the biofilm is established [3]. While the molecular mechanisms that allow Gram-negative bacteria to resist to antibiotics within biofilms are well documented, this is not yet the case for Gram-positive bacteria [4, 5]. Based on naturally occurring antimicrobial and host defense peptides, the design of small synthetic peptides with broad-spectrum antibiofilm activity has provided a glimmer of hope in the fight against pan-antibiotic-resistant bacteria, the so-called superbugs [6, 7], providing a really promising start for the development of new antibacterial agents [8]. These peptides have been tested either alone [9] or in association with antibiotics since they could act as biofilm-dispersive agents to improve the action of antibiotics [10]. Some of these peptides, whether endogenous or synthetic (i.e., LL-37 and peptide 1018), do not require a specific binding to a bacterial target such as a sensor protein [9]. However, as for antibiotics, some cationic antimicrobial peptides (AMP) such as LL-37 or magainin II induce the emergence of bacterial resistance often through the activation of 2-component systems (TCS) [11–13].

In the search for new strategies or bacterial targets to counteract bacterial infection and/or biofilm formation, the potential as antimicrobials of eukaryotic communication factors released constantly by host cells (hormones and cytokines) during a surgery (norepinephrine) [14] or during infections (natriuretic peptides, substance P [SP], or neuropeptide Y) [15–17] has emerged. This concept originated in 1930, when it was suspected that epinephrine could favor bacterial infections [18]. This hypothesis was validated by the studies of Lyte and Ernst [19], which showed that epinephrine and more generally catecholamines enhance bacterial virulence. This work opened a new research field named "microbial endocrinology" [20, 21] that refers to bacterial sensitivity to host hormones. The discovery that the bacterial response to epinephrine and norepinephrine was not only restricted to *Escherichia coli* [22] suggested that this phenomenon was widely encountered in host-bacteria relationships. In addition, since in humans these catecholamines act through specific eukaryotic receptors, it was tempting to explore the possibility that the bacterial sensitivity to these hormones was relayed via specific bacterial sensors. In this context, a crucial step in the comprehension of this mechanism was achieved by the studies of Sperandio

et al. [23] and Clarke et al. [24], since they identified QseC as the bacterial sensor for catecholamines. In addition, this sensor further appeared to be widespread since homologs of *E. coli* QseC have been referenced in 24 bacterial species [25]. Interestingly, it was observed that bacteria expressed several subtypes of catecholamine sensors [26], as in human cells where numerous receptor subtypes exist. In parallel to the characterization of catecholamine effects on bacteria, several studies have focused on the impact of eukaryotic communication compounds (i.e., neurotransmitters, cytokines, and hormones) and a clear consensus emerged that numerous eukaryotic molecules have a physiological effect on a large variety of bacteria [27, 28] and that these activities are quite often dependent on binding to a bacterial sensor. For instance, it was shown that interferon-γ led to the induction of the *Pseudomonas aeruginosa* lectin PA-I via activation of the quorum-sensing network that is required for the production of virulence factors [29]. Noticeably, this effect was shown to be triggered by direct binding of interferon-γ on OprF [29], which is a multifunctional outer membrane porin [30] that is required for *P. aeruginosa* virulence regulation [31] and involved in regulation of biofilm formation [32]. It has also been observed that dynorphin A, an endogenous opioid, modifies *P. aeruginosa* physiology [33]. It is interesting to mention that opioid receptor agonists are able to mimic the effect of dynorphin A on *P. aeruginosa*, especially by enhancing bacterial adhesion properties [33], suggesting that the effect of this human peptide is relayed by a bacterial sensor showing a pharmacological profile similar to that of its eukaryotic counterpart [33]. In the same vein, it was shown that morphine can trigger *P. aeruginosa* virulence [34]. This seemingly exotic idea that bacteria would express sensor proteins presenting pharmacological activities similar to that of the eukaryote receptors was already suggested for a human hormone by Yamashita et al. [35]. In this case, somatostatin, which binds to receptors localized on the eukaryotic cell membrane that are coupled to adenylate or guanylate cyclases [36], is able to modify *Helicobacter pylori* physiology by enhancing the cAMP (cyclic adenosine monophosphate) and/or cGMP (cyclic guanosine monophosphate) intrabacterial concentration [35]. This concept, in which human hormones may have an impact on bacteria through bacterial cyclases, was also verified in *Pseudomonas* species. Indeed, natriuretic peptides were shown to modify the virulence of *P. aeruginosa* [37] and *P. fluorescens* [38] after modulation of cAMP or cGMP intrabacterial concentration.

The data presented above show that the activation of bacterial sensors by host communication compounds results in the modulation of bacterial virulence, adhesion, and/or biofilm formation. Next to these physiological observations, identification of the putative bacterial sensors for these human hormones and the deciphering of the mechanisms of action of these hormones on bacteria could serve the scientific community to identify new bacterial targets to counteract bacterial virulence and biofilm formation. The originality of this review, which is focused on the neuropeptide hormone family, lies in the fact that it describes not only the impact of these compounds on biofilm formation and on virulence regulation but also the corresponding bacterial sensors and the mechanisms of action triggered by the binding of neuropeptide hormones on their target(s). This review will begin with the sensitivity of Gram-negative bacteria to host signal compounds and it will continue with the response of Gram-positive bacteria.

Effects of Peptidic Hormones on Gram-Negative Bacteria

Dynorphins and P. aeruginosa
Hormone Presentation

Dynorphins are involved among others in pain and stress responses after binding to the κ-opioid receptor family. Dynorphin A (1–17), dynorphin B (1–13) (Table 1), and α-neoendorphin are generated from the common precursor prodynorphin by proteolytic cleavage and share a highly conserved *N*-terminal sequence and charge distribution [39]. Since opioids have been shown to accumulate during inflammation [40], this highlights that bacteria can be exposed to dynorphins during infection.

Impact of Dynorphins on *P. aeruginosa*

It has been shown that *P. aeruginosa* virulence increases after exposure to opioid agonists or dynorphins [33]. Indeed, the contact of *P. aeruginosa* with these compounds results in activation of the *Pseudomonas* quinolone signal (PQS) quorum-sensing system, pyocyanin (PCN) production, and consequently activation of bacterial virulence. More interestingly, exposure of *P. aeruginosa* to various concentrations of dynorphin A (100–400 μM) resulted in a dose-dependent effect on PCN production [33]. The biological relevance of this finding was further highlighted using a *Caenorhabditis elegans* nematode infection model, which showed enhanced virulence when *P. aeruginosa* cells were treated with dynorphins.
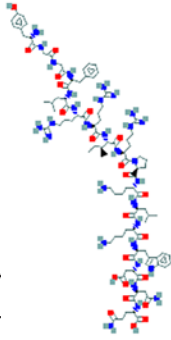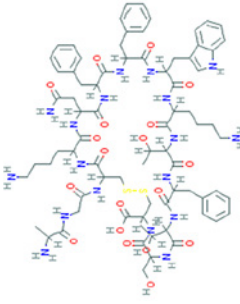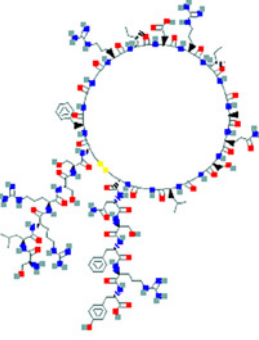
Since the 3 quorum-sensing-related signal compounds PQS, 4-hydroxy-2-heptylquinoline (HHQ), and 4-hydroxy-2-heptylquinoline-*N*-oxide (HQNO) were overproduced, the authors investigated the levels of PQS-regulated PA-I lectin mRNA [41, 42]. Since the authors observed that the synthetic κ-agonist U-50,488 mimicked the dynorphin effects on *P. aeruginosa* PCN and quorum-sensing quinolone signaling molecule production and on expression of the *pqsABCDE* operon, it has been speculated that, like U-50,488, dynorphins would increase the expression of lectin PA-I in *P. aeruginosa* by increasing the PQS level. This is underlined by the facts that: (1) the productions of lectin PA-I and PCN were described to be very similarly regulated, (2) PQS was previously shown to enhance the attachment of *P. aeruginosa* to stainless steel coupons [42], and (3) lectin PA-I is involved in attachment to surfaces and subsequent biofilm formation [43]. The effect of dynorphins was also assayed on preformed biofilm, but neither induction of rhamnolipids nor induction of the PQS system could be observed in this condition [44].
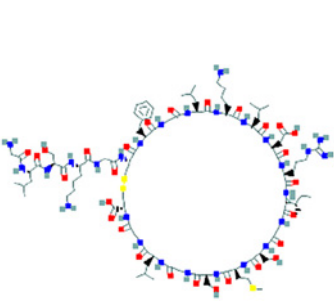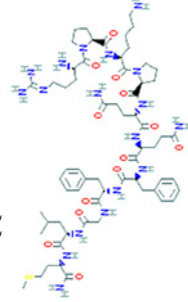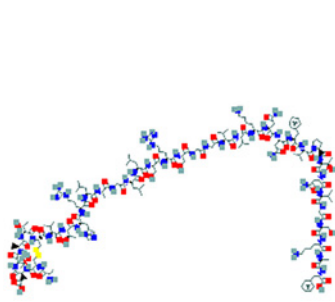
Hormone Bacterial Targets and Mechanism of Action

The virulence and PQS production regulator MvfR was first suggested to be the bacterial sensor for dynorphins [33] (Fig. 1). This hypothesis is backed by the observation that MvfR is associated with the cytoplasmic membrane during the exponential phase of growth before being released into the cytoplasm by proteolytic degradation [45]. Recently, the effect of dynorphins on *P. aeruginosa* was suggested to be triggered by the TCS ParRS rather than by MvfR, independently of the quorum-sensing system [46]. Interestingly, ParRS TCS has been involved in AMP resistance [47] since ParRS was shown to be required for induction of the lipopolysaccharide (LPS) remodeling *arn* operon by the cationic cyclolipopeptide polymyxin B [47]. Two other mechanisms of multidrug resistance are attributed to the ParRS system: overproduction of the MexXY efflux pump and downregulation of the porin *oprD* gene, resulting in an active efflux of antibiotics and a decreased membrane permeability to imipenem, respectively [48] (Fig. 1). Since dynorphin was shown to bind directly the inner membrane (IM) sensor ParS leading to activation of the cytoplasmic regulator ParR, these data suggest that *P. aeruginosa* could perceive this host factor as a classical AMP and therefore adapt its physiological answer.

Although the dynorphin bacterial target and the associated mechanism of action of this peptide on *P. aerugi-*

Table 1. List of the eukaryotic peptide hormones that have an effect on bacteria
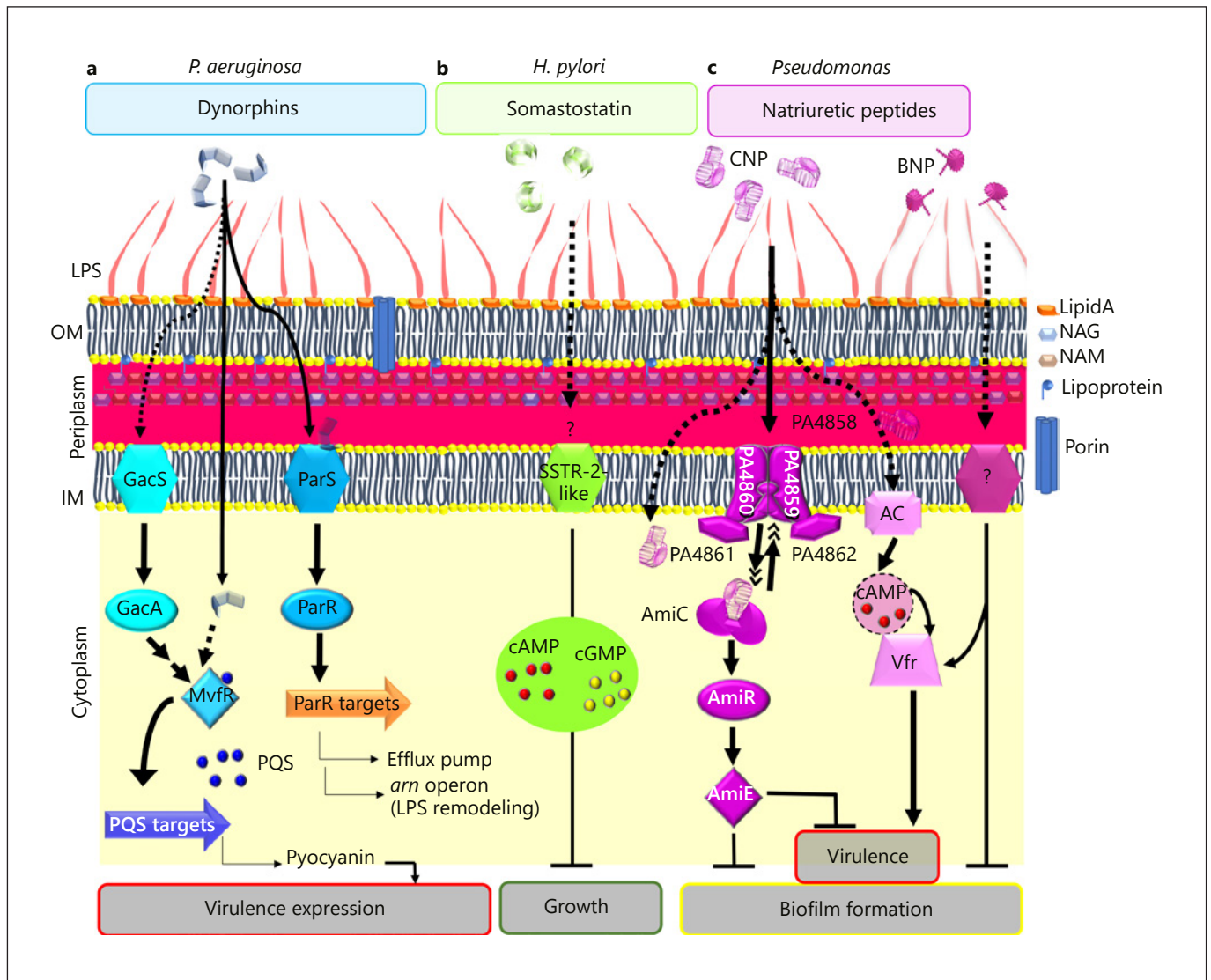
Peptides	Sequence and structure	UniProt/PubChem (CID)[a] accession numbers	Organs and cells that produce the peptide hormone	Bacteria encountered in the producing organ	Bacteria susceptible to the peptide (molecular target)	Key references
Dynorphin A and dynorphin B	17 AA or 13 AA YGGFLRRIRPKLKWDNQ Dynorphin A structure 	P01213/13133805/ 25081093	Cerebellum[b] Lymphoid node[b] Skeletal muscle[b] Gall bladder[b] Pancreas[b] Oral mucosa[b] Stomach[b] Intestine[b] Kidney[b] Testis[b] Placenta[b]	Bacteria from the oral microbiota	*P. aeruginosa* (MvfR or ParS)	33, 39, 46
Somatostatin SST-14 and SST-28	14 AA (disulfide bridge 3–14] or 28 AA (disulfide bridge 17–28) SANSNPAMAPRERKAGCKNFFWKTFTSC SST-14 structure 	P61278/16129681/ 16133849	Hypothalamus[b] Cerebral cortex[b] Adrenal cells[b] Pancreas[b] Stomach[b] Duodenum[b] Small intestine[b] Colon[b] Rectum[b]	Bacteria from the intestinal microbiota	*H. pylori* (SSTR-2-like protein)	35, 51
ANP	28 AA (disulfide bridge 7–23) SLRRSSCFGGRMDRIGAQSGLGCNSFRY 	P01160 (human)/ 16166338 (rat)	Heart[b] Skin	Bacteria involved in endocarditis Bacteria from the skin microbiota	*S. epidermidis* (molecular target not yet identified) *S. aureus* (molecular target not yet identified)	110, 117–119
BNP	32 AA (disulfide bridge 10–26) SPKMVQGSGCFGRKMDRISSSSGLGCKVLRRH 	P16860/71308561	Heart Skin	Bacteria involved in endocarditis	*P. aeruginosa* (molecular target not yet identified)	37, 117,118

Table 1 (continued)

Peptides	Sequence and structure	UniProt/PubChem (CID)[a] accession numbers	Organs and cells that produce the peptide hormone	Bacteria encountered in the producing organ	Bacteria susceptible to the peptide (molecular target)	Key references
CNP	22 AA (disulfide bridge 10–26) GLSKGCFGLKLDRIGSMSGLGC	P23582/16179407	Prostate (RNA) Retina (RNA) Testis (RNA) Brain Bone Testis Granulosa cells Thyroid Thymus Skin Endothelial cells	*P. aeruginosa* *S. aureus* Bacteria from the skin microbiota	*P. aeruginosa* (AmiC) *S. epidermidis* (molecular target not yet identified) *S. aureus* (molecular target not yet identified)	37, 68, 69, 77, 110, 117, 118, 120–123
Substance P	11 AA RPKPQQFFGLM	P20366/36511	Cerebral cortex[b] Thyroid gland[b] Testis[b] Adrenal gland[b] Spleen[b] Bronchus[b] Nasopharynx[b] Pancreas[b] Salivary gland[b] Stomach[b] Intestine[b] Skin[b]	Bacteria from the skin microbiota	*B. cereus* (EfTu) *S. epidermidis* (EfTu) *S. aureus* (EfTu) *P. fluorescens* (molecular target not yet identified)	83, 88, 97, 99, 124
CGRP	37 AA ACDTATCVTHRLAGLLSRSGGVVKNNFVPTNVGSKAF	P06881/56841902	Thyroid gland[b] Skin	Bacteria from the skin microbiota	*S. epidermidis* (DnaK)	83, 106, 109, 124

The amino sequence and the 2-D structure of each peptide, the UniProt accession number, and the PubChem accession number of the corresponding peptide, the organs and cells that produce the peptide (protein form or RNA), the family of bacteria encountered in the organs producing the corresponding peptide hormone, and the bacteria sensitive to the corresponding peptide and the bacterial molecular targets are shown. a Data retrieved from the National Center for Biotechnology Information PubChem Compound Database (https://pubchem.ncbi.nlm.nih.gov, accessed September 3, 2018) [125]. b Data retrieved from the Human Protein Atlas (https://www.proteinatlas.org/, accessed September 3, 2018) [126].

Fig. 1. Host peptidic hormones and Gram-negative bacteria. Model of the regulatory pathways involved in the effects of dynorphins on *P. aeruginosa* (**a**), somatostatin on *H. pylori* (**b**) and natriuretic peptides on *P. aeruginosa* (**c**). Arrows represent positive regulation and T-bar-finishing lines indicate negative regulation. OM, outer membrane; AC, adenylate cyclase; NAG, *N*-acetylglucosamine; NAM, *N*-acetylmuramic acid.

nosa remain unclear, it appears that bacteria are able to sense the opioid dynorphin peptides, resulting in a complex bacterial response.

Therapeutics Future

Opioid compounds are widely used in human health and affect the immune host defense [49]. A growing concern is related to the use of these compounds in humans as, like in animals, opioid use is associated with an increased risk of bacterial infections like pneumococcal dis-

eases [50]. A better understanding of how endogenous opioids as well as synthetic ones interact with bacterial cells may help in the identification of potential bacterial targets and the development of dual-therapy in high-risk patients. Knowing the structure-activity relationships between the bacterial sensor and opioids would lead to the development of compounds that might not be recognized by bacteria but that would keep their helpful effect on pain release in humans.

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Somatostatin and H. pylori

Hormone Presentation

Somatostatins belong to a neuropeptide family that has numerous functions, with effects on nearly every tissue or organ of the human body, with a special mention of the gastrointestinal tract. Somatostatins are produced after proteolytic processing of a pre-pro-somatostatin precursor. This process generates 2 cyclic peptides named according to their length, i.e., somatostatin-14 (SST-14) and somatostatin-28 (SST-28), that are characterized by a 12-amino acid loop [51] (Table 1). Somatostatins exert their biological effects after binding 1 of the 5 somatostatin receptor (SSTR) subtypes (SSTR-1 to SSTR-5) expressed in humans [52, 53], which are coupled to an adenylate cyclase through a G-protein.

Impact of Somatostatin on *H. pylori*

H. pylori is a bacterium that colonizes stomach and intestine mucosa where peptidic hormones such as somatostatin and gastrin are released. Gastrin peptides exist in 2 active forms (gastrin-17 and gastrin-34). A study conducted by Yamashita et al. [35] showed that the SST-14 somatostatin exerted a dose-dependent (1 μM to 10 pM) inhibition of *H. pylori* growth. In the same study, the authors concluded that the somatostatin effect on *H. pylori* was highly specific since gastrin peptides had no activity against *H. pylori* while somatostatin did not affect *E. coli* growth [35]. Nevertheless, this specificity should be confirmed since another study showed that gastrin could bind to a non-defined *H. pylori* sensor, increasing the bacterial growth rate [54]. Since somatostatin effects on human cells are relayed mainly by adenylate cyclases and occasionally by guanylate-cyclases modifying cAMP and cGMP intracellular concentrations, respectively, the impact of 2 cell-permeable analogs of cAMP and cGMP on *H. pylori* has been evaluated. Surprisingly, it has been observed that 8-bromo-cGMP fully mimicked the somatostatin inhibition of *H. pylori* growth while di-butyryl cAMP stimulated *H. pylori* growth, whereas cAMP and cGMP intrabacterial concentrations were both enhanced when *H. pylori* was exposed to somatostatin (10 pM; 48 h) [35]. These discrepancies suggested nonetheless that somatostatin acts on *H. pylori* through a specific sensor target.

Hormonal Bacterial Targets and Mechanism of Action

With the aim of characterizing the *H. pylori* somatostatin sensor, binding studies using radiolabeled SST-14 were performed. This enabled the identification of a saturable site presenting a dissociation constant (K_D) of 0.31 nmol/L [35], confirming the presence of a specific target on *H. pylori* for SST-14. In the same vein, using immunoglobulins directed against the SSTR subtypes, it has been observed that IgG against SSTR-2 are able to block somatostatin effects on *H. pylori* whereas IgG against SSTR-1 have no impact [35]. Although these data remain fragmentary since the bacterial target was not identified at that time, they suggest the presence of a bacterial somatostatin sensor presenting functional similarities with its human counterpart SSTR-2 (Fig. 1).

Therapeutics Future

H. pylori biofilm establishment in the stomach is a source of gastritis and peptic ulcers. In the search for natural compounds able to block *H. pylori* biofilm formation, curcumin has shown interesting potential [55]. However, it is important to keep in mind that curcumin is able to reduce both somatostatin and gastrin release [55]. Regarding the impact of these hormones on *H. pylori* described above, it is important to mention that all of these parameters must be taken into account in the race to discover natural compounds able to prevent stomach disease.

Natriuretic Peptides and P. aeruginosa

Hormone Presentation

Natriuretic peptides constitute a family of hormones first identified for their cardiovascular and osmoregulation activities [56]. In addition to these well characterized functions, numerous activities have been attributed to these peptides [57]. The 3 main members of this family are: atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), the C-type natriuretic peptide (CNP), which are mainly released in human blood by cardiomyocytes (ANP and BNP) and endothelial cells (CNP). This family of peptides is completed by urodilatin, uroguanylin, and guanylin, which are released by epithelial cells in the intestine or in the kidney lumen, and dendroaspis natriuretic peptide, which is a component of snake venom [58]. These peptides are synthesized as pre-pro-hormones that are cleaved in the cytoplasm first as pro-hormones and finally as the biologically active form by the action of a membrane enzyme during the releasing process [57, 59–61]. The length of these peptides is between 22 and 38 amino acids [57] (Table 1), and their structure is characterized by a disulfide bridge leading to a 17 amino-acid loop conferring an omega form. This structure is encountered in some cationic AMP such as rhesus θ-defensin-1 from the macaque or tyrocidin from *Bacillus* [62–65].

These data added to the clinical observations showing that *N*-terminal-pro-BNP release is enhanced in the presence of *E. coli* or LPS in blood [15], and in vitro studies demonstrating that LPS added to cultured endothelial cells stimulated CNP release by these cells [66] have prompted some laboratories to study in more detail the impact of natriuretic peptides on the physiology of Gram-negative bacteria.

Impact of Natriuretic Peptides on *P. aeruginosa*

The first work dedicated to the impact of natriuretic peptides on bacteria was performed by Krause et al. [67]. In that study, it was shown that, among the natriuretic peptides tested, human BNP (hBNP) possesses antimicrobial activity against both Gram-positive and Gram-negative bacteria and that hANP and urodilatin are active as well, but with a weaker activity, while hCNP does not show any direct activity against bacteria [67]. This work constituted a starting point to evaluate the antimicrobial potential of natriuretic peptides, even if the minimum inhibitory concentration measured for hBNP was around 5 μM against Gram-positive bacteria and around 30–35 μM against Gram-negative bacteria [67], which are concentrations still quite far above their physiological concentration in human blood [56].

In this context, study of the impact of lower concentrations of natriuretic peptides on bacterial physiology was required to evaluate their real potential. It was observed that, whereas hBNP and hCNP have no impact at 1 and 0.1 μM on *P. aeruginosa* growth [37, 68], bacterial exposure to these peptides at 1 μM stimulates the virulence of *P. aeruginosa* and *P. fluorescens* [37, 38]. In addition, both hBNP and hCNP at 0.1 μM strongly prevented *P. aeruginosa* biofilm formation [69]. The most active peptide was hCNP, which was able to impair biofilm formation in a dose-dependent manner, and which was still able at 10 nM to reduce by a half the biofilm formation by *P. aeruginosa* [68].

Hormone Bacterial Targets and Mechanism of Action

In *P. aeruginosa*, production of virulence factors and biofilm establishment are inversely regulated at least partly by intracellular cyclic nucleotides [70]. The pro-virulent effect of CNP on *P. aeruginosa* was shown to result from the increase in the cAMP concentration [37], which activates the virulence factor regulator Vfr [71] (Fig. 1). In addition, in human cells, CNP was shown to bind both on the natriuretic peptide receptor (NPR) subtypes B (NPR-B) and C (NPR-C) [72], regulating cGMP and cAMP intracellular concentrations, respectively [72, 73]. Altogether, these data suggested that a putative bacterial CNP sensor could be present in *P. aeruginosa*. This hypothesis was reinforced by a study showing that isatin, an antagonist of NPR-A and NPR-C receptors in rats, totally blocked the antibiofilm activity of CNP [69]. In this context, it was clearly suspected that the *P. aeruginosa* sensor for CNP could present some similarities with the eukaryotic NPR. An in silico search for protein structure similarities between the 3 types of human NPR (NPR-A, NPR-B, and NPR-C) and the *P. aeruginosa* whole proteome (www.pseudomonas.com) [74] was then performed. The best score was obtained for the acetamide sensor AmiC [75]. Comparisons of human NPR-C and *P. aeruginosa* AmiC in terms of amino acid distribution and 3-D model structures based on molecular docking approaches allowed the proposal of AmiC as a putative bacterial sensor for CNP [69]. This hypothesis was validated by microscale thermophoresis assays using purified AmiC, in which CNP was shown to directly bind AmiC with a K_D of 2 μM [69]. It is interesting to note that this binding was highly specific since: (1) BNP displayed no affinity for AmiC, suggesting that the antibiofilm effects of BNP are mediated by another pathway in bacteria (Fig. 1), and (2) NPR-C but not NPR-A agonists can bind AmiC [69]. Altogether these data proved that a bacterial sensor for a hormone and its eukaryotic receptor counterpart could have similar pharmacological profiles.

The *amiC* gene belongs to the *ami* operon which contains 4 or 5 genes depending on the *P. aeruginosa* strain (www.pseudomonas.com) [74]. In the absence of acetamide, AmiC binds and sequesters the anti-terminator factor regulator AmiR [76]. When acetamide [76] or CNP [69] binds to AmiC, AmiR is released and the whole operon is overexpressed. It is tempting to propose that the aliphatic amidase AmiE, which is the final product of the *ami* operon, could be responsible for the antibiofilm effect of CNP on *P. aeruginosa*. This hypothesis was recently reinforced since it was demonstrated that overproduction of AmiE impaired *P. aeruginosa* biofilm formation [77] (Fig. 1). One outstanding issue remains: how and where does CNP bind AmiC in vivo? Up to now, AmiC was classified as being a cytoplasmic protein (www.pseudomonas.com) [74]. However, this protein possesses a periplasmic signature in its sequence, suggesting that AmiC could move to the IM. It has therefore been suggested that during CNP exposure *P. aeruginosa* would address a pool of AmiC close to or within the bacterial IM [69] (Fig. 1). In addition, it has been shown that the presence of a protein named PA4858, which presents an ami-

no acid sequence closely related to AmiC, is essential to seeing the inhibitory effect of CNP on biofilm [69]. In this context, AmiC could interact with CNP through the putative CNP periplasmic binding protein PA4858 [78]. It is speculated that the protein PA4858 could finally facilitate binding between AmiC and CNP [69], probably through a multiprotein complex including PA4859 to PA4862 (Fig. 1).

Therapeutics Future

It was recently shown that the CNP concentration used to affect *P. aeruginosa* physiology is important, since CNP at 1 µM induced both specific effects through the AmiC sensor and nonspecific effects likely through its interaction with the bacterial membranes, triggering virulence and/or antibiofilm activities [68] (Fig. 1). Conversely, CNP concentrations of 0.1 µM or less inhibited biofilm formation via binding to AmiC without an apparent virulence increase [68]. These data suggest that CNP or CNP derivatives could become interesting drugs for inhibition of *P. aeruginosa* biofilm formation either alone or in association with antibiotics, provided that a low hormone concentration is used. Among CNP derivatives, the osteocrin peptide, which displayed an affinity for AmiC in the nanomolar range, could be an interesting candidate [69]. In any case, the enhancement of the bacterial AmiE concentration induced after binding of CNP to AmiC must also be taken into account since it was shown that AmiE overproduction converts *P. aeruginosa* into a non-virulent bacterium using an acute lung infection mouse model [77]. Finally, we can speculate that the development of such drugs could be facilitated by the fact that natriuretic peptides are already used as therapeutic drugs. Indeed, nesiritide (BNP) has been used since 2001 in patients with acute heart failure [79] and an agonist for natriuretic peptide receptors is actually in clinical trials (phase IIa) for future use as a bronchodilatory drug [80] and this could serve as a launch pad for delivery of natriuretic peptides in lung of patients suffering from *P. aeruginosa* chronic infections.

Effects of Peptidic Hormones on Gram-Positive Bacteria

The interest shown in the investigations on the effects of neuropeptides on Gram-positive bacteria was driven by the development of cosmetic sciences and the 1223UE regulation which required, since 2009, a complete scientific demonstration of all claims of the absence of adverse effects (regulation 1223/2009). In this regard, the pressure from consumers for preservative-free cosmetics or cosmetic containing a minimum of preservatives was of major concern since, in the absence of preservatives, any substance could modulate the development, virulence, and/or biofilm formation activity of skin-associated bacteria. The skin microbiota, which is the second most important one in the human body (i.e., 10^{12} microorganisms including more than 80% of bacteria) [81], was initially regarded as a potential issue due to the presence of numerous opportunistic pathogens. At this time, the whole skin microbiota is considered as a potential new target of cosmetic compounds. Cutaneous bacteria are continuously exposed to sweat and a mean of 25% are localized deep in the skin through hair follicles and sebaceous or sweat glands [82]. Human skin represents the largest neuroendocrine organ of the human body [83], and cutaneous bacteria are continuously exposed to skin neuropeptides diffusing in sweat and though the tissue matrix [84, 85].
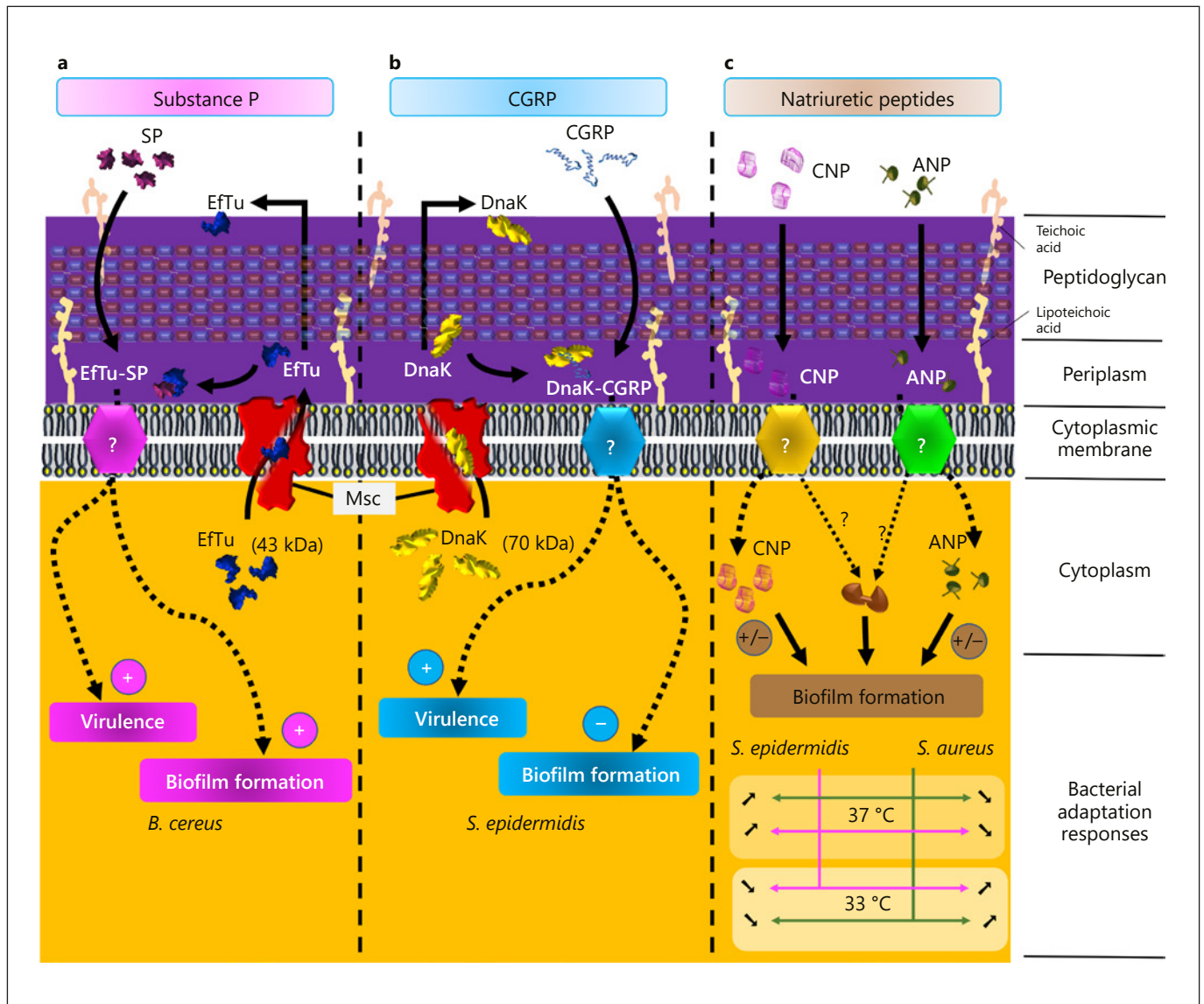
SP and Bacteria
Hormone Presentation

SP was the first skin neuropeptide whose activity towards cutaneous bacteria was investigated. SP is an undecapeptide of the tachykinin family (Table 1), which is released in the skin by sensory C-type fibers [83]. It was the first skin neuropeptide to be identified and it is also the most abundant. Its role is not limited to neurotransmission and it is well known as a causative factor of neurogenic/antidromic inflammation, characterized by local plasma extravasion and edema in the absence of any exogenous signal [86]. In eukaryotes, SP is recognized by NK-1 receptors, although 2 other types of NK receptors with a lower affinity also exist [87].

Impact of SP on Bacteria

The first study of the effect of SP on bacteria was performed using a cutaneous strain of *Bacillus cereus* as a model [88]. This species was not selected because of it abundance in skin, although strains of *B. cereus* are part of the commensal cutaneous flora [89], but because of its potential virulence. Exposure of *Bacillus cereus* to SP was associated with a rapid (5 min) and important (400 %) increase in virulence that was associated with a significant rise of biofilm thickness (+63% after 5 h) [88]. The sensitivity of *B. cereus* to SP was considered high, since these values were obtained at micromolar concentrations and with a nanomolar threshold. The biofilm structure itself was also affected by the exposure to the neuropeptide

Fig. 2. Host peptidic hormones and Gram-positive bacteria. Model of the regulatory pathways involved in the effects of SP on *B. cereus* (**a**), CGRP on *S. epidermidis* (**b**), and natriuretic peptides on staphylococci (**c**).

with the formation of mushroom-like structures and a decrease in biofilm stability. The reversed sequence peptide of SP and neurokinin A, the natural ligand of human NK-2 receptors, did not show any activity, demonstrating the specificity of the action of SP peptides, suggesting the presence of a specific SP sensor in *B. cereus*.

Hormone Bacterial Targets and Mechanism of Action

The ligand of SP in *B. cereus* was identified by immunoprecipitation, Western blot and MALDI-TOF/TOF

analysis as the thermo unstable ribosomal elongation factor (EfTu) (Fig. 2). EfTu is produced in excess (4- to 14-fold) with regard to its stoichiometric association with ribosomes [90]. It is a member of a family of molecules identified as "moonlighting" proteins, i.e., proteins which possess alternative functions alongside their principal function. These different functions must be exercised by the same polypeptide chain and they must be realized into 2 different compartments of the cell. In this way, numerous enzymes or chaperones could be secreted outside of the cell or inserted into the membrane where they acquire

generally the role of environmental sensor [91–93]. EfTu fits perfectly with this definition since this elongation factor is not only intracellular but it was also clearly found on the cell membrane [94–96], suggesting that in addition to its role in translation EfTu has a variety of other functions. SP was found to display similar effects on biofilm formation in the case of *Staphylococcus epidermidis* and *S. aureus*, with an increase in thickness after exposure to SP (1 μM) [97]. Like for *B. cereus*, EfTu was identified as the SP sensor in *Staphylococcus* [97]. EfTu is a highly conserved protein which is expressed in all bacteria as well as in archaea and eukaryotes (in mitochondria) [98]. It was thus tempting to hypothesize that SP is also capable of acting on other bacterial species, including Gram-negative ones. So far, the only Gram-negative bacterium on which the effect of SP has been investigated is *P. fluorescens* and it was observed that, as in Gram-positive species, SP stimulated biofilm formation [99]. The target of SP in *P. fluorescens* was not identified but it is interesting to note that in *Pseudomonas*, including *P. aeruginosa*, EfTu was also identified as a sensor for the neurotransmitter γ-aminobutyric acid (GABA) [100, 101].

Therapeutics Future

Different complementary studies revealed that the effect of SP on biofilm formation can be inhibited by high-ionic-force thermal water, probably through chelation, and by cosmetic products able to block the access of SP to the membrane [88]. The concentration of SP in skin and/or sweat can be increased during inflammation, stress, or even a nervous breakdown [84, 85] (these events are known to be frequently associated with skin disorders). Cutaneous bacteria, whose virulence and biofilm formation activities appear to be closely regulated by SP, are likely to take part in skin alterations. This concept was at the origin of the development of new cosmetic products, as well as new drugs with dermatological clinical applications [102]. Indeed, it was shown that, in reconstructed skin, SP-treated *S. epidermidis* promotes overexpression of the keratinocytes integrin-α-5 and chemokine ligand 10, which are both typical markers of psoriasis, suggesting that SP, in addition to its direct activity on skin cells [103], should be somehow involved in the development of psoriasis through its action on skin bacteria [97]. In addition, it has been observed that *S. aureus* biofilm formation and its persistence are associated with numerous skin disorders such as folliculitis decalvans [104], psoriasis, acne, and rosacea [105], suggesting that the impact of SP on bacteria biofilm should be evaluated in relation to these skin disorders.

Calcitonin Gene-Related Peptide and Bacteria
Hormone Presentation

Calcitonin gene-related peptide (CGRP) is a 37 amino-acid peptide (Table 1) cosecreted with SP by skin nerve terminals and it presents functions quite similar to those of SP in skin [83]. Considering its concomitant secretion with SP, CGRP was logically the second neuropeptide whose potential action on cutaneous bacteria was investigated.

Impact of CGRP on Bacteria

Until now, the effect of CGRP had only been investigated in *S. aureus* and *S. epidermidis*, but the 2 Staphylococci behaved differently. Whereas *S. aureus* was not sensitive to CGRP with no modification of bacterial virulence regulation and biofilm formation even at the highest concentration tested (1 μM), *S. epidermidis* showed an extreme sensitivity to CGRP with a nanomolar threshold [106]. In *S. epidermidis*, CGRP induced an increase in virulence of the same range as that observed with SP, which was followed by increased surface hydrophobicity and a decrease in biofilm formation at least in dynamic conditions [106] (Fig. 2).

Hormone Bacterial Targets and Mechanism of Action

Unexpectedly, the effects of SP and CGRP on *S. epidermidis* were not additive but antagonistic, suggesting that both peptides were acting on a common and easily saturable bacterial target. Surprisingly, this common step in the mechanism of action of SP and CGRP was not the recognition site, as CGRP was found to act through another moonlighting protein, i.e., the chaperone DnaK [106] (Fig. 2). However, the SP sensor EfTu and the CGRP sensor DnaK share the fact that they do not have export sequence signals and that, as shown in *E. coli*, these proteins should be exported through the cytoplasmic membrane by mechanosensitive channels (Msc) [107], presumably following transport by an MreB-dependent process [108]. In line with the involvement of Msc channels, blocking them using the specific inhibitor gadolinium chloride inhibited both EfTu and DnaK export and markedly inhibited the effects of SP and CGRP on *S. epidermidis* [106]. The apparent absence of sensitivity of *S. aureus* to CGRP remains a puzzling question. It should be noted that DnaK is a much larger protein than EfTu (70 vs. 43 kDa) and that the pores formed by Msc channels in *S. epidermidis* and *S. aureus* are not identical in terms of width. Since the binding of SP and CGRP to their sensor should occur in the periplasmic space (Fig. 2), DnaK cannot go

through Msc in *S. aureus* while EfTu can cross the pore because of it smaller size, explaining why the bacterium should be sensitive to SP (via EfTu) and not to CGRP as experimentally observed [109].

Therapeutics Future

Until now the effect of CGRP on *S. epidermidis* has not been a matter of cosmetic or clinical applications, presumably as these observations were recent, but these data confirmed that skin neuropeptides should play a central role in the control of cutaneous bacterial behavior.

Natriuretic Peptides and Gram-Positive Bacteria

We can consider that all known neuropeptides can be found in skin, although they should be only present in specific niches. This is the case of natriuretic peptides (see above), which are produced by endothelial cells of capillary vessels and can diffuse from blood at a close distance to capillaries. In this regard, natriuretic peptides should be particularly concentrated in a specific region of the hair follicle, i.e., the bulge, where capillary vessels are abundant. However, this region of the hair follicle is rich in sebum and it is colonized by bacteria such as *S. aureus* and *Cutibacterium (Propionibacterium) acnes* because of their lipophilic characteristic and tolerance to low oxygen local concentrations [81].

Impact of Natriuretic Peptides on Gram-Positive Bacteria

It was recently demonstrated that the effects of natriuretic peptides on *S. aureus* were highly dependent on the growth temperature. At 37 °C, the biofilm formation activity of *S. aureus* was inhibited by ANP and CNP, whereas that of *S. epidermidis* was increased [110]. The opposite effect was observed at 33 °C (Fig. 2). These results should be correlated to the skin microenvironment since, while in depth the temperature stabilizes at 37 °C, the surface temperature is generally about 33 °C. Very recent data suggest that the biofilm formation activity of *C. acnes* should be submitted to even more complex temperature regulation, or probably medium- and oxygen-dependent regulation by natriuretic peptides [111], as was observed for *S. aureus*.

Therapeutics Future

Natriuretic peptides, SP, and CGRP are known as key factors of neurogenic antidromic inflammation in skin. This process should be essential in atopic dermatitis [112], psoriasis [113], rosacea [114], and a large range of inflammation-related troubles [115]. The discovery of the effects of SP, CGRP, and natriuretic peptides on Gram-positive bacteria led to a new vision of neurogenic inflammation which involves the microbial skin flora as a central relay in the inflammation process but also as a potential new target for the treatment of these diseases [116].

Conclusion

Taken together, the original new field of research consisting of the study of the impact of eukaryotic communication signals on bacteria in general and biofilms especially suggests future applications mainly in cosmetology for Gram-positive bacteria and preferentially in the clinical field for Gram-negative bacteria. This review will encourage crossing of data obtained in these 2 application fields (i.e., cosmetic and clinical) in order to enlarge the spectrum of action of these peptides on bacteria. In addition, deciphering the mechanism of action of human hormones on bacteria will allow indirect discovery of new functions for bacterial proteins in term of virulence regulation or biofilm formation often far from the main functions for which they were primarily characterized, extending the concept of moonlighting proteins.

Disclosure Statement

The authors have no conflict of interests to declare.

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