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Original Paper

In Silico and *in Vitro* Study of Trace Amines (TA) and Dopamine (DOP) Interaction with Human Alpha 1-Adrenergic Receptor and the Bacterial Adrenergic Receptor QseC

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Key Words
Trace amines • Dopamine • QseC • α1-adrenergic receptor

Abstract
Background/Aims: Trace amines (TA) are small organic compounds that have neuromodulator activity due to their interaction with some neuron-related receptors, such as trace amine associated receptors (TAARs), α2-adrenergic receptor (α2-AR) and ß-adrenergic receptor (ß-AR). However, there is little information on whether TA and dopamine (DOP) can interact with other adrenergic receptors (ARs) such as the mammalian α1-AR and the bacterial counterpart QseC, which is involved in quorum sensing of some Gram-negative pathogens. The aim of this study was to investigate the interaction of TA and DOP with α1-AR and QseC. ***Methods:*** We performed an *in silico* study using 3D structure from SWISS MODEL and analyzed the protein interaction via molecular docking using PyMol, PoseView and PyRX 8.0. For the *in vitro* study, we investigated the QseC kinase activity by measuring the remaining ATP in a reaction containing QseC-enriched membrane incubated together with purified QseB and EPI, TA, DOP, or PTL respectively. We also measured the intracellular Ca++ levels, which represents the α1-AR activation, in LNCAP (pancreatic cell line) cells treated with EPI, TA, DOP and PTL respectively using a fluorescence-based assay. The LNCAP cell proliferation was measured using an MTT-based assay. ***Results:*** Our *in silico* analysis revealed that TAs and DOP have high binding affinity to the human α1-AR and the bacterial adrenergic receptor (QseC), comparable to epinephrine (EPI). Both are membrane-bound kinases. Experimental studies with pancreatic cell line (LNCAP) showed that the TAs and DOP act as α1-AR antagonist by counteracting the

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effect of EPI. In the presence of EPI, TA and DOP trigger an increase of the intracellular Ca++ levels in the LNCAP cells leading to an inhibition of cell proliferation. Although *in silico* data suggest an interaction of TA and DOP with QseC, they do not inhibit the kinase activity of QseC, a histidine kinase receptor involved in quorum sensing which is also sensitive to EPI. ***Conclusion:*** Our study showed that the TAs and DOP act as α1-AR antagonist but no effect was observed for QseC.

Introduction

Trace amines (TA) are monoamines which are regarded as undesirable by-products of food and beverage fermentation [1-4]. However, they also play a role as a neurotransmitter in invertebrates such as insects [5-7], or as neuromodulators in vertebrates where they are ligands of the trace amine associated receptors (TAARs) [8-10]. The term 'trace' is based on their low concentrations in the central nervous system (CNS) in comparison to the endogenous catecholamines, such as serotonin, epinephrine (EPI) or dopamine (DOP) [11]. According to some reports, activation of TAAR1 in the brain by TA might contribute to the generation of schizophrenia, depression and other neurodegenerative and neuropsychiatric diseases [12, 13]. The psychotic syndromes might be triggered by TAAR1 activation via TA that inhibits the expression of transporters for neurotransmitters and consequently their re-uptake is affected [14].

TA, DOP and EPI behave differently for certain ARs. On α2-AR, TA and DOP act as agonist like EPI, whereas on α2-AR, TA and DOP act as partial allosteric antagonists in contrast to EPI [15-17]. Since a number of commensal skin bacteria produce TA, we asked the question whether bacteria communicate with their host via TA. Indeed, it has been shown that the TA and DOP increase bacterial internalization in the intestinal epithelia via an agonistic effect on α2-AR [18, 19]. Furthermore, skin commensals producing TAs such as *Staphylococcus epidermidis*, accelerates wound healing by antagonizing the effect of EPI which inhibits cell motility by β2-adrenergic receptor (β2-AR) activation [20]. As β2-AR antagonists, TA and dopamine (DOP) abrogate the effect of EPI, thus accelerating wound healing both *in vitro* and in a mouse model.

To our knowledge, there are no reports on how TA and DOP act on α1-AR. Such an investigation of the interaction of TA and DOP with α1-AR might provide more insight on the significance of TA, if any, in vertebrates. Since ARs are expressed in many parts of the human body, they are important receptors that can affect the body's metabolism. Recently, some Gram-negative bacteria have also been reported to have an AR-like receptor involved in quorum sensing (QS) regulation. This receptor, named QseC, is one of the quorum sensing receptors first reported in *Escherichia coli*. QseC is a sensor kinase that can be activated by either the autoinducer-3, but also by EPI and norepinephrine [21]. The activation of this QseC receptor leads to an upregulation of various virulence factors in pathogenic *E. coli*, such as flagella, shiga toxins, intimin, and locus of enterocyte effacement (LEE) island [21, 22]. Therefore, it would be interesting to investigate the interaction of other potential compounds such as TA and DOP on QseC. Here we performed *in silico* and *in vitro* interaction studies of TA and DOP with α1-AR and QseC. TA and DOP showed high binding affinity towards α1-AR and QseC. TA and DOP share some interaction sites with the known agonist (EPI) *in silico*. In the *in vitro* studies, however, TA and DOP differed in their effect on α1-AR and QseC.

Materials and Methods

In silico study

Ligand and protein interaction. We modeled the α1-AR and QseC using SWISS MODEL software [23] to obtain the 3D structure of protein receptors. We then analyzed the ligand and resulted 3D structure of the protein interactions using PyMol. We further analyzed the ligand-receptor interaction for more information

about the type of chemical interaction and its interaction sites namely the position and type of chemical bond interactions such as hydrophobic, hydrogen, or phi-alkyl using PoseView [24, 25]. The blind docking method was used in this study because the active site of the target protein was unknown, therefore the grid was directed at the entire surface of the target protein [26].

Molecular docking. We used PyRx 0.8 software to perform molecular docking between the ligands (TRY, PEA, TYM, DOP, PTL, EPI and Autoinducer-3) and the receptors, α1-AR and QseC. The molecular docking study was conducted to obtain the binding affinity value between the ligand and the receptor. For this analysis, a blind docking was carried out to reveal the possible affinity of the ligands to all sites of the receptor [26].

In vitro study

Preparation of QseB and QseC-enriched membrane. Plasmids pET28a-qseC and pET28a-qseB were constructed for the expression and purification of QseC and QseB in *E: coli* BL21. *qseC* and *qseB* genes were amplified from *E. coli* K12 using specific primers (Supplementary Table S1) and inserted into HindIII-linearized pET28a plasmid using Hi-Fi DNA Assembly Master Mix (New England Biolabs), and the construct was transformed into *E. coli* DC10B (for all supplementary material see www.cellphysiolbiochem.com). Clones were selected on 30 µg/mL kanamycin-containing Luria-Bertani (LB) agar. The constructed plasmid from selected clones were isolated and transformed into *E. coli* BL21; clones were selected on 30 µg/mL kanamycin containing LB agar. Selected clones containing pET28a-qseC and pET28a-qseB were grown in 30 µg/mL kanamycin-containing LB broth overnight at 37°C with 150 rpm shaking. The grown cultures were reinoculated into fresh 30 µg/mL kanamycin-containing LB medium and the protein expression was induced using a final concentration of 0.5 mM of Isopropyl β-D-1-thiogalactopyranoside (IPTG). The cells were pelleted and resuspended in PBS-containing protease inhibitor cocktail. Cells were disintegrated with glass beads (Roth, Karlsruhe, Germany) using FastPrep instrument (MP Biomedicals). Cell lysate from *E. coli* BL21 pET28a-qseB was subjected to QseB purification using Ni-NTA resin (Qiagen). Cell lysate from *E. coli* BL21 pET28a-qseC was subjected to be pelleted using ultracentrifuge at 235,000 x g for 30 min. The pellet was collected and resuspended in 20 mM Tris pH 7.5 with 1 mM $MgCl_2$. This QseC-enriched membrane was subjected to *in vitro* kinase assay. Membrane fractions from non-induced culture of *E. coli* BL21 pET28a-qseC was used as negative control.

In vitro kinase activity assay. The *in vitro* assays were carried out using the prepared QseC-enriched membrane fraction and purified QseB in the presence of ATP (5 mM), $MgCl_2$ (25 mM) in HNG buffer (HEPES 100 mM, NaCl 300 mM, Glycerol 20%). To induce the kinase reaction, we added EPI (1 mM). We also added TAs, DOP, PTL (1 mM) or water (as negative control) to the reaction to investigate the kinase inhibition. The *in vitro* assays were perfomed in room temperature for 30 min. After 30 min, it was centrifuged at 5000 g for 10 min and the supernatant was taken to measure the remaining ATP using Kinase Glo-Max (Promega). The luminescent intensity is inversely correlated with the kinase activity. The values from negative control were considered as baseline for zero kinase activity and was used to calculate the kinase activity for the reactions with the addition of TAs, DOP, and PTL.

Intracellular Ca++ measurement assay. We seeded the LNCaP cells in 96-well microtiter black flat bottom plate with $1x10^5$ cells/well and incubated for 24 h at 37°C in 5% CO_2. The LNCAP cells were treated with the neurochemicals (TRY, PEA, TYM and DOP) and PTL (50 µg/ml) with and without EPI (50 µg/ml). EPI was added 5 min after neurochemicals, incubated further for 12 min and the Ca++ levels were measured every 3 min. The intracellular calcium measurement assays were performed using Fluo-8 Calcium Flux Assay Kit – No Wash (Abcam) according to the protocol provided by the company.

Cell proliferation assay. Prior the cell proliferation assay, LNCaP cells were seeded in a 96-well microtiter flat bottom plate with $1x10^5$ cells/well and incubated for 24 h at 37°C in 5% CO_2. The LNCAP cells were treated with the neurochemicals (TRY, PEA, TYM and DOP) and PTL with final concentration 50 µg/ml with and without EPI (50 µg/ml). The cytotoxicity assay was performed using the Cell Proliferation Kit I (MTT; Roche, Germany) according to the protocol provided by the company.

Statistical significance

Multiple comparisons were analyzed using one-way ANOVA with Dunnett post-test. Normal distributions were analyzed by Student's t test, with the significance defined as $p < 0.05$ where n represents independent biological replicates.

Results

TA and DOP have similar binding affinity as EPI on α1-AR and QseC in silico

It has been reported that TA and DOP act as agonists on α2-AR and as partial antagonists on ß2-AR [15-17]. For this reason, we wondered whether TA and DOP can also affect other types of AR, such as α1-AR and QseC. In order to answer this question, we first performed *in silico* analyses of the binding affinity of TA and DOP on α1-AR and QseC. We used EPI and PTL as controls because they were reported to act as agonists (EPI) and antagonist (PTL) on both α1-AR and QseC. We also used autoinducer-3 (AI-3), a bacterial endogenous QseC agonist, as an additional positive control since the structure has been characterized recently [27]. Indeed, we could show that TA and DOP have similar binding affinity to α1-AR and QseC as EPI. The binding affinity of TA and DOP are even stronger than AI-3 to QseC (Table 1). This results lead to the suggestion that TA and DOP are probably able to interact with α1-AR and QseC.

TA and DOP have similar binding sites as EPI and PTL on α1-AR in silico

Next, we analyzed the binding sites of TA and DOP on α1-AR and QseC. EPI and PTL were included in these analyses for comparison. In particular, we investigated the chemical interactions, such as hydrophobic, hydrogen and π interaction between the ligands and the receptors. Our *in silico* studies showed that TRY, TYM, EPI and PTL have an overlapping binding region at α1-AR, while PEA and DOP interacted with another site (Fig. 1). Although TA and DOP have a similar binding affinity to α1-AR, they bind to different sites of α1-AR (Table 1). The binding studies suggest that TRY and TYM interact with α1-AR at the same orthosteric site, while PEA and DOP interact with α1-AR at an allosteric site (Table 2 and Fig. 1).

Table 1. The binding affinity of the compounds of interest to α1-AR and QseC. *nd: not determined

Compound	PubChem ID	Molecular weight (g/mol)	Binding affinity to α1-AR (kcal/mol)	Binding affinity to QseC (kcal/mol)
EPI	5816	183,207	-5,7	-5,8
PTL	5775	281,359	-8,5	-7,8
TRY	1150	160,220	-6,6	-6,2
PEA	1001	121,183	-5,9	-5,8
TYM	5610	137,182	-5,8	-6,0
DOP	681	153,181	-5,8	-6,0
AI-3	-	124,141	nd	-5.0

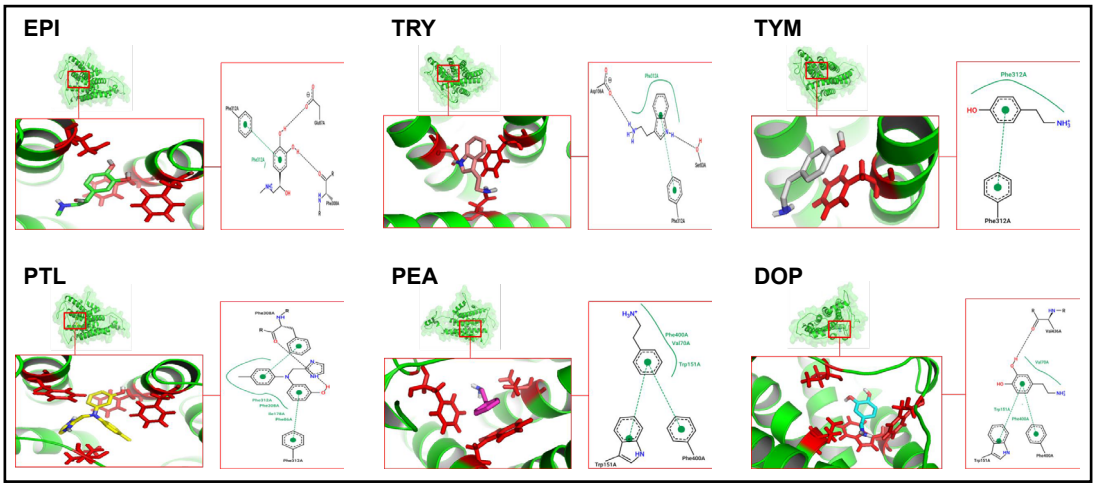

Fig. 1. Visualization of the predicted chemical interaction between ligands and α_1-AR. The 3D structure of the protein was modeled using SWISS MODEL. The ligand and receptor interactions were analyzed using PyMol and PoseView. The green dotted lines represent hydrophobic interactions, the black dotted lines represent hydrogen interactions and the green lines with green font for amino acids represents the π interactions. The visualization was drawn using ChemSketch.

TA and DOP have similar binding sites as EPI and PTL on QseC in silico

TA and DOP, like EPI and PTL, have similar interactions sites on QseC (Fig. 2). They share at least three similar chemical interaction sites on QseC (Table 2), suggesting that TA and DOP bind to a common orthosteric motif. The recently elucidated endogenous agonist, AI-3, is also interacting with QseC at orthostheric site at position Tyr367 and Arg366, similar to EPI, PTL, TA and DOP (Supplementary Fig. S1 and Table 2). Due to the high binding affinity of TA and DOP to QseC and the high similarity of their interaction sites in QseC compared to the known agonists (EPI) and antagonists (PTL), we postulate that TA and DOP might have an effect on the activity of QseC.

TA and DOP act as α1-AR antagonist in vitro

Since TAs and DOP have a high affinity to α1-AR, we performed *in vitro* experiments to confirm the interaction and to study the effect of TAs and DOP on α1-AR. In this experiment, we used LNCaP as a model cell line that expresses α1a-AR [28]. To address this, we measured the intracellular Ca++ level of the treated LNCaP cells, since activation of α1-AR leads to an increase of the intracellular Ca++ level due to the activation of Ca++ channel. We could show that the addition of EPI (50 µg/mL) alone significantly increased intracellular Ca++ levels compared to the control after 3 min of incubation. If EPI (50 µg/mL) is added together with DOP (50 µg/mL), the Ca++ level decreased to a level similar to the control. Treatment with TAs and DOP without EPI did not show a significant effect on Ca++ levels (Fig. 3A). Cell

Table 2. The chemical interaction prediction between the compounds of interest and α1-AR or QseC. *nd: not determined

Compound	Chemical Interaction with α1-AR			Chemical Interaction with QseC		
	Hydrophobic	Hydrogen	π	Hydrophobic	Hydrogen	π
EPI	Phe312	Glu87, Phe308	Phe312	Pro407, Ile400	Tyr376, Asn363	-
PTL	Phe312, Phe308, Ile178, Phe86	Phe308	Phe312, Phe308	Pro407, Ile400, Val392, Tyr367, Phe443	Asp387	Tyr367
TRY	Phe312	Asp106, Ser83	Phe312	Pro407, Ile400	Ile400, Asn363	Tyr367
PEA	Phe400, Val70, Trp151	-	Trp151, Phe400	Ile400, Val392, Tyr367	Asn363, Tyr367	Tyr367
TYM	Phe312	-	Phe312	Tyr367, Val392, Ile400	Asn363, Pro390	Tyr367
DOP	Val70	Val436	Trp151, Phe400	Tyr367, Val392, Ile400	Asn363, Pro390, Tyr367	Tyr367
AI-3	nd	nd	nd	-	Tyr367, Arg366	-

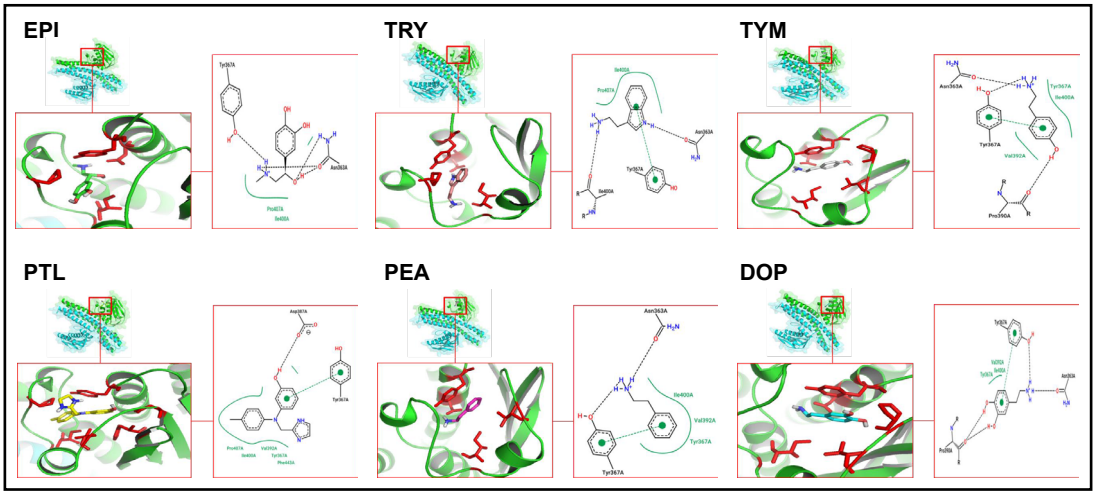

Fig. 2. Visualization of the predicted chemical interaction between ligands and QseC. The 3D structure of the protein was modeled using SWISS MODEL. The ligand and receptor interactions were analyzed using PyMol and PoseView. The green dotted lines represent hydrophobic interactions, the black dotted lines represent hydrogen interactions and the green lines with green font for amino acids represents the π interactions. The visualization was drawn using ChemSketch.

viability experiments using LNCaP treated with TAs and DOP (each 50 µg/mL) with and without EPI (50 µg/mL) showed no difference compared to the control, with one exception; LNCaP treated only with EPI showed higher cell viability (Fig. 3B). The higher cell viability was probably due to the increased cell growth induced by the oscillation of intracellular Ca++ level [29-31].

TAs and DOP do not inhibit the QseBC kinase activity

In order to confirm the *in silico* prediction of TA interaction with QseC, we carried out *in vitro* kinase assays of QseC and QseB with additions of TAs and DOP respectively in the presence and absence of EPI. We also included PTL as negative control because PTL has antagonistic effects against QseC [21]. Surprisingly, the *in vitro* experiments showed that the TA and DOP do not have a significant effect on the kinase activity of QseBC, irrespective of the presence and absence of EPI (Fig. 4).

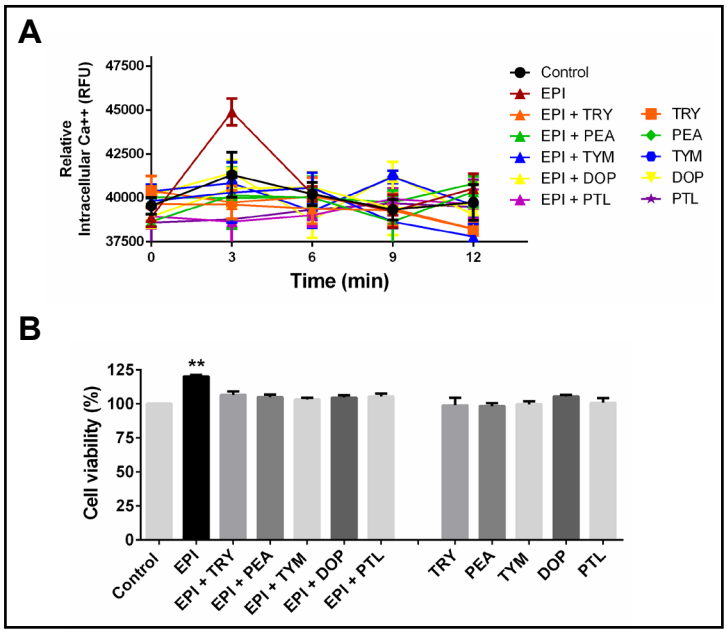

Fig. 3. TA and DOP negate the effect of EPI in increasing the intracellular Ca++ level. (A) The effects of TA and DOP on α_1-AR were examined in LNCAP cells. The cells were treated with the Fluo-8 NW and incubated for 1 h. TA, DOP and PTL (positive control) were added separately to the LNCAP cells at concentration 50 µg/ml for 5 min. Then, EPI (50 µg/ml) was added and incubated further for 12 min and the intracellular Ca++ level of LNCAP cells was measured every 3 min. Treatment with EPI alone caused an oscillation of intracellular Ca++ level of LNCAP cells while in the presence of TA, DOP and PTL, the intracellular Ca++ levels were similar to the control (H_2O). The addition of TA, DOP and PTL without EPI did not show any significant effect on intracellular Ca++ level. The data are shown in relative fluorescence unit (RFU). (B) The treatment of TA, DOP and PTL (50 µg/ml) with and without EPI (50 µg/ml) did not show significant effect on viability of the LNCAP cells. The addition of EPI (50 µg/ml) alone increased the LNCAP cells viability. Each data point is the mean value ± SEM from minimum 3 independent replications, *p<0.05; **p<0.01, data were analyzed using Students t-test.

Discussion

Trace amines (TA) are reported to be present endogenously in the human body at very low concentrations [32]. However, TA are also produced by microorganisms and many of them are part of the human microbiota as either gut [19] or skin microbiota [20]. TA can interact not only with TA associated receptors (TAARs) but also with some adrenergic receptors (ARs), such as α2-AR and ß-AR [15-17]. These receptors are also reported to be able to interact with dopamine (DOP), an endogenous biogenic neurotransmitter [33, 34].

On α2-AR, TA and DOP act as agonist similar to EPI and NEPI [15, 17, 19]. TA and DOP interact with α2-AR at the orthosteric site similar to EPI and NEPI. As for α2-AR, TA and DOP have been reported to interact with the allosteric site as partial antagonist [16]. Our *in silico* experiments showed that TA and DOP have similar binding affinity towards α1-AR compared to EPI (Table 1). This suggests that as competitive (TRY and TYM) and allosteric (PEA and DOP) ligands, both have a similar high probability to bind to the receptor as EPI (Table 2 and Fig. 1). This could be the reason why TA and DOP antagonize the effect of EPI at the Ca++ level as well as LNCAP cell proliferation (Fig. 3).

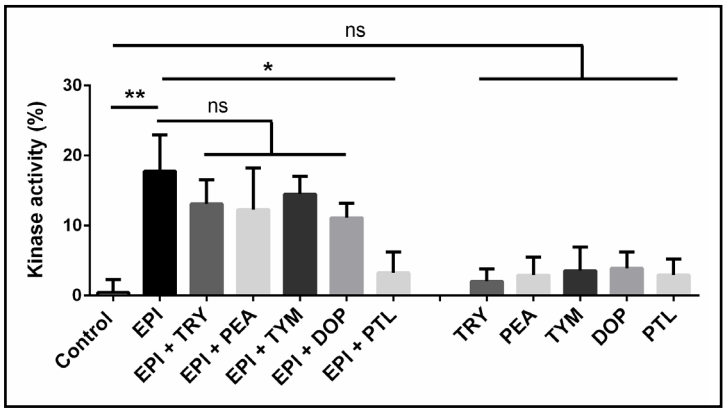

Fig. 4. TA and DOP showed no effect on QseC kinase activity. The QseC enriched membrane and QseB isolated from E. coli BL21 were used for in vitro kinase activity assay in the presence EPI together with TAs, DOP and PTL. After 30 min of incubation at room temperature, the kinase activity was determined by measuring the remaining ATP in the reaction and it showed that the addition of EPI increased the kinase activity of QseBC significantly. The addition of TAs and DOP did not show significant effect while PTL showed significant inhibition of kinase activity. (For all graphs, each data point is the mean value ± SEM (n = 3), *p<0.05; **p<0.01; ***p<0.001. Student t test was used for statistical calculations.

The activation of α1-AR is followed by an activation of Ca++ transporter and an increase of cytoplasmic Ca++. The presence of TA and DOP inhibit the activation of α1-AR by EPI either via competitive or allosteric antagonist interaction and subsequently the increase of Ca++ is also abrogated as shown in Fig. 3A. TA and DOP inhibit cell proliferation as effective as PTL (Fig. 3B). This is due to the antagonistic effect of TA and DOP on α1-AR, which causes the inhibition of the activity of the Ca++ transporter. As a result, Ca++ levels became lower and consequently inhibiting the cell proliferation, which is regulated by the Ca++ level [29-31]. Another effect related to the Ca++ level is smooth muscle contraction [35]. As α1-AR is expressed in many organs and tissues, such as in urinary bladder [36], prostate [37], urethra [36], heart [38] and adipocytes [39], the presence of TA and DOP will have an effect to the function of these organs or tissues due to the decrease of smooth muscle contraction or proliferation.

As TA and DOP showed an interaction with mammalian AR, we wondered whether TA and DOP might also interact with bacterial AR known as QseC. QseC plays an important role in the regulation of many virulence factors of pathogenic Gram-negative bacteria such as *E. coli* [40, 41], *Salmonella enterica* [42, 43], *Aggregatibacter actinomycetemcomitans* [44], or *Actinobacillus pleuropneumoniae* [45]. Our *in silico* prediction studies showed that TA and DOP have a similar high binding affinity towards QseC as with the known agonist (EPI) and antagonist (PTL); all of these compounds also interact at orthosteric sites. On the basis of these results, we postulate that TA and DOP should have an effect on the activity of QseC. However, TA and DOP showed no significant effect on QseC kinase activity (Fig. 4). The discrepancy between *in silico* and *in vitro* experiments is not uncommon, since a certain inaccuracy is inherent in the *in silico* prediction. Computational predictions, however, are not always accurate [22]; hence one has to verify the *in silico* results experimentally. Furthermore, TA, DOP, EPI and PTL share only one interaction site with AI-3, namely the orthosteric Tyr367 of QseC (Table 2). A comparison of the binding affinities shows that the binding affinity of EPI and PTL to QseC is stronger than that of AI-3 (Table 1). This suggests that the mammalian ligands are more efficient than the bacteria's own ligand AI-3, which is formed by threonine dehydrogenase and "abortive" tRNA synthetase [27].

The importance of this work is that we show that TA interact not only with α2-AR and ß-AR but also with α1-AR. This is a new finding demonstrating the versatility of TA in its interaction with mammalian AR. When studying the interaction of the human α1-AR with the ligands, our *in silico* and experimental data agree very well (Table 3). As shown in Fig. 5A, EPI acts on α1-AR as an agonist. It triggers a cascade of reactions starting with Gq (G alpha q) signaling, the activation of phospholipase C followed by activation of Ca++ channel. In LNCAP cells, diacylglycerol (DAG)-directed TRPC1 and TRPC3 Ca-channels are present. It is

Table 3. Summary of TA and DOP interaction on α_1-AR and QseC in silico and in vitro. ns: no significant effect observed; nd: not determined

Compound	α1-AR			QseC		
	Interaction site (in silico)	Ca++ uptake (in vitro)	Role	Interaction site (in silico)	Kinase activity (in vitro)	Role
EPI	orthosteric	increased	agonist	orthosteric	increased	agonist
PTL	orthosteric	control level	antagonist	orthosteric	control level	antagonist
TRY	orthosteric	control level	antagonist	orthosteric	ns	-
PEA	allosteric	control level	antagonist	orthosteric	ns	-
TYM	orthosteric	control level	antagonist	orthosteric	ns	-
DOP	allosteric	control level	antagonist	orthosteric	ns	-
AI-3	nd	nd	nd	orthosteric	nd	agonist

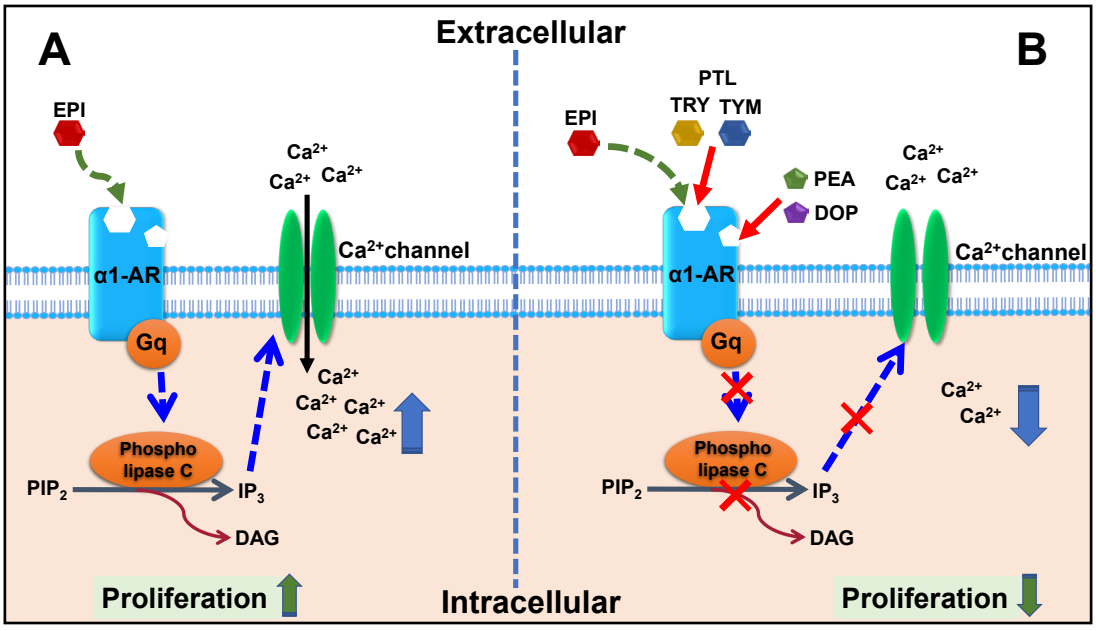

Fig. 5. Illustration of the agonistic effect of EPI on α1-AR and antagonizing effect of TA. (A) EPI interacts with α1-AR at the orthosteric site and activates the Gq protein. The activation of Gq protein is followed by the phospholipase C in a cascade reaction and produces inositol triphosphate (IP3). IP3 then activates the Ca++ channel. The accumulation of Ca++ eventually leads to an increase in cell proliferation. (B) PTL, TRY, TYM, PEA and DOP act as 1-AR antagonists. While PTL, TRY and TYM bind to α1-AR orthosteric site and thus acting as competitive inhibitors, PEA and DOP bind to the allosteric site of α1-AR, thus acting as allosteric inhibitors.

assumed that in prostate cancer epithelial cells, α1-ARs is functionally coupled to the Ca++ permeable TRPC1 and TRPC3 Ca-channels [46].

Accumulation of intracellular Ca++ eventually leads to an increase in cell proliferation. However, in the presence of PTL, TRY, TYM, PEA and DOP, the activation of α1-AR is antagonized (Fig. 5B). Interestingly, PTL, TRY and TYM are binding to the same binding site as EPI (orthosteric) thus acting as competitive inhibitors. On the other hand, PEA and DOP are binding to different binding site thus acting as allosteric inhibitors. It is noteworthy to mention that the three TA differ with respect to the binding site. While TRY and TYM bind to orthosteric sites, PEA is binding allosterically. This observation indicates that in future, we will have to analyze each of the TA molecules individually. The finding that TA act as α1-AR antagonists is important because they may have a negative effect on cardioprotection, as it has recently reported that Gq coupling is required for cardioprotection by an alpha-1A-AR agonist [47].

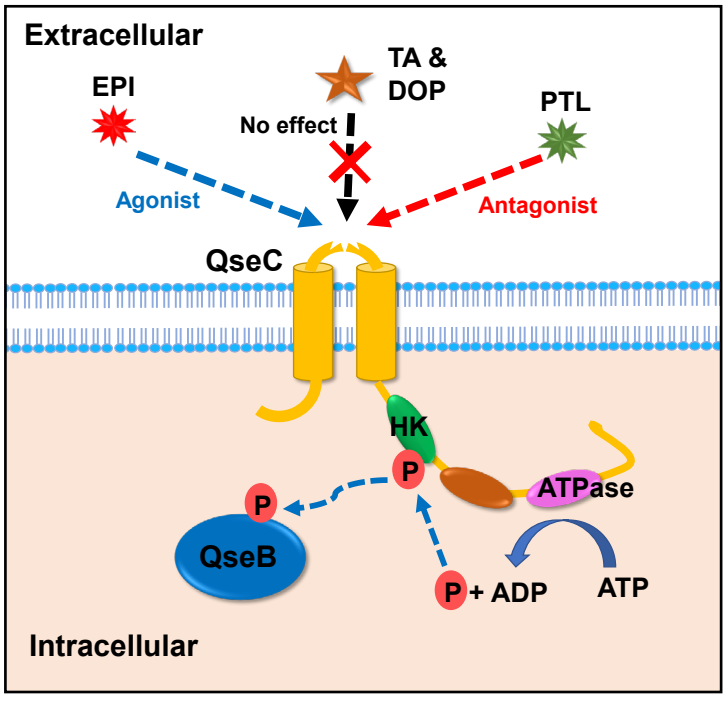

Fig. 6. Summary of the interaction and activity of neurotransmitters on QseC of *E. coli*. QseC is the quorum-sensing sensor kinase which is part of the quorum sensing regulation in various Gram-negative bacteria. Interestingly, EPI (adrenalin) triggers an adrenergic signaling cascade by interacting with the bacterial QseC involving dephosphorylation of QseB; whereas PTL acts as antagonist. Our in silico modeling showed that TA and DOP can bind to QseC but they have no the effect on kinase activity of QseB.

While the *in silico* binding studies of TA and DOP with the human α1-AR correspond very well with their activity, this was not the case with the bacterial QseC. Our *in silico* study demonstrated that the binding of TA and DOP with QseC was similar to that for EPI and PTL. However, TA and DOP had no effect on the phosphorylation activity. A summary of these results is shown in Fig. 6.

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Author contributions

FG and AL designed the study. AL, VDK and RAR designed and performed the experiments. FG, AL and VDK wrote the manuscript.

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Statement of Ethics

The authors have no ethical conflicts to disclose.

Disclosure Statement

The authors have no conflicts of interest to declare.

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