Competitive Inhibition of Cortisol by Prostaglandins at the Ligand Binding Domain of Glucocorticoid Receptors

Charles D. Schaper, Ph.D.*

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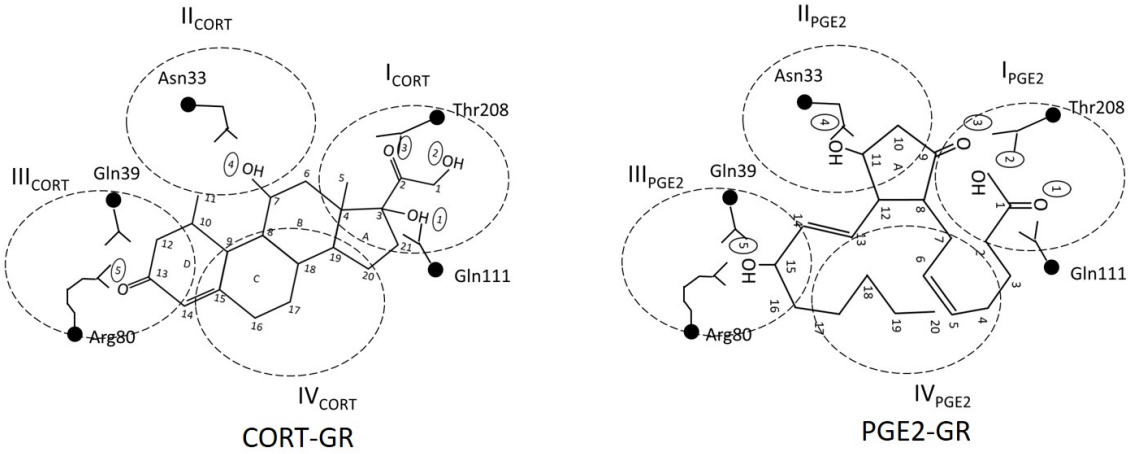
Abstract

Here, competitive inhibition of cortisol by prostaglandins at the ligand binding domain of glucocorticoid receptors is shown for the first time. Glucocorticoid receptors, cortisol, and prostaglandins are found in almost every cell and tissue in the human body, and participate in a range of metabolic, neurologic, and immunity functions, among others. Thus, competitive inhibition preventing normative cellular processing of cortisol, a stress hormone, by prostaglandins, which are associated with the inflammatory response, can explain the source of certain ailments including signs and symptoms of diseases. To assess molecular similarity that would enable competitive inhibition, it is first noted that cortisol (CORT, $C_{21}H_{30}O_5$) and prostaglandin E2 (PGE2, $C_{20}H_{32}O_5$) are of nearly the same molecular weight, and have nearly the same number of carbon, hydrogen and oxygen elements. The carbon-carbon double bonds on each of the two branches of PGE2, combined with the five-carbon cyclic centering structure, enable a conformational isomer of PGE2 to position the five oxygen-based functional groups at similar spatial positions as the corresponding five groups of CORT. Within the ligand binding domain (LBD), the average intermolecular spacing to the corresponding identical five functional groups is 1.877 Å for CORT versus 1.912 Å for PGE2. The conformational isomer of PGE2 within the LBD is at a lower energy state than CORT by 12.151%, indicating preference in stability. Both molecules exhibit a hydrophobic core. The dimensions of the LBD for PGE2 is slightly larger than CORT, by 1.857% corresponding on average of 0.266 Å at a spacing of 14.331 Å, resulting in an increase in volume of the LBD of 5.675%.

*cschaper@transferdevices.com

Graphical Abstract



Molecule	**O5-Arg80**[1]	**O4-Asn33**	**O3-Thr208**[2]	**O2-Thr208**	**O1-Gln111**
CORT-GR	1.826 Å	1.874 Å	1.741 Å	1.850 Å	2.096 Å
PGE2-GR	2.108 Å	2.118 Å	1.820 Å	1.726 Å	1.789 Å

1 Introduction

A recent case study presented curious data, as follows: the usual vaccine injections for pneumonia and the seasonal flu were administered to a healthy patient but several hours later, fever and chills developed in response to a routine of prolonged physical activity, which was reliably repeated over the next two days, albeit attenuated [1]. Mathematical modeling to characterize these observed dynamics of body temperature produced a predictive analysis, but the fundamental molecular pathways that were responsible for the initiation of a febrile response were left unknown and not specifically identified at the time, just the characteristics. However, further analysis associated with this investigative study determined that in order to explain the body temperature trajectory the likely causative molecules were cortisol, due to physical activity, and prostaglandins, due to the vaccine. But how can this be: we know about correlations, but how are cortisol and prostaglandins fundamentally related at a molecular level so as to produce symptomatic responses?

Setting aside this specific application of fever that motivated this research, here a much broader and quite fundamental concept is presented for the first time: it is the hypothesis presented in this article that there is a functional molecular binding site common to both cortisol, which is affiliated with stress, and prostaglandins, which are affiliated with inflammation and disease, that inhibits the normative processing of cortisol. If this is the case, then this research will close a gap in the knowledge base of cellular physiology, as it fundamentally links stress and disease at a molecular level. Moreover, this linkage will enable first principles reasoning and predictive modeling of the extent for certain signs and symptoms of disease as amplified by stress, both physical and cerebral, addressing conditions such as fever, fatigue, and potentially even emotional dysfunction associated with the limbic system, such as depression.

This research deals with a very important hormone, cortisol (CORT), and its target, the ligand binding domain (LBD) of glucocorticoid receptors (GR), which are found in high concentration and are widely distributed to nearly every cell, tissue, and organ in the human body, encoding for $3 - 10\%$ of the human genome [2]. Cortisol is released from the adrenal gland, directed by the hypothalamus, and is known as the stress hormone, resulting in enhancements to metabolic and cerebral capabilities [3]. Further, cortisol is an integral part of the limbic system which is involved in the formation of thoughts that influence motivation, fear, and mood. Moreover, cortisol is a permissive hormone, in which cellular capability utilizes other hormones to the fullest extent, and thus, in addition to its direct effect, cortisol indirectly influences many other body functions as it enables other hormones [4].

Furthermore, to note the broad scope of the work, not only does this study involve CORT and GR, but it also relates to prostaglandins, which are also found in high concentrations in the human body, and perform very important functions, most of which are related to signaling activities, especially those associated with disease. The most abundant prostaglandin is PGE2, which is derived from arachidonic acid, a common molecule to the

ever-present phospholipid bilayer of most cells, but also to many bacterial cell membranes that contribute to disease. Although on the surface it would seem that these hormones and proteins would be largely unrelated, we show for the first time that in actuality the molecular structures of CORT and an isomer of PGE2 are so similar that they interfere with each other at the GR. This interference is called competitive inhibition, and in this case the phenomena will suppress the normative functioning of CORT, precipitating responses resembling infection. Thus, by developing this hypothesis and providing quantifying relations, it is now possible to analyze the influence of disease on stress from a fundamental molecular basis and pathway.

Previously, the interaction of stress and disease was investigated mainly through neurological analysis, as opposed to the molecular arguments studied here. It is basic physiology that stressors, both physical and cerebral, stimulate the sympathetic nervous system, which in turn shuts down the immune system temporarily [5]. It is thus inferred that because the immune system became less responsive during stress, diseases had an opportunistic situation to multiply and thus invade healthy tissues, and cause illness. However, an analysis of neurological basis only does not indicate the molecular pathway to produce the signs and symptoms of such diseases; it will only indicate the source or absence of the materials that are associated with the presence of disease. Certainly there have been observations and correlations that the concentration of cortisol is altered during times of stress and disease, however the fundamental connection of how stress and disease directly interact at a molecular level have not been made: this connection is made in this article.

A brief search on the functions of cortisol indicate a wide list of active research, see for example [6, 7, 8, 9, 10, 11, 12]. It is also noted that in addition to gene transcription through the GR, synaptic potentiation via cortisol has been examined [13], which implies a non-genomic purpose as well. Furthermore, because of its wide ranging physiological importance, dysfunction of the regulatory mechanisms of CORT can result in illnesses: for example, levels that are too low due to a misfunctioning adrenal gland can result in a crisis of significant temperature variations, fatigue, and aches [14] that can be misdiagnosed as an infection [15]. Further correlations of symptoms of disease and cortisol are inferred from the undulation of fever characterizing disease such as brucella [16], which may be influenced by daily fluctuation [17, 18, 19].

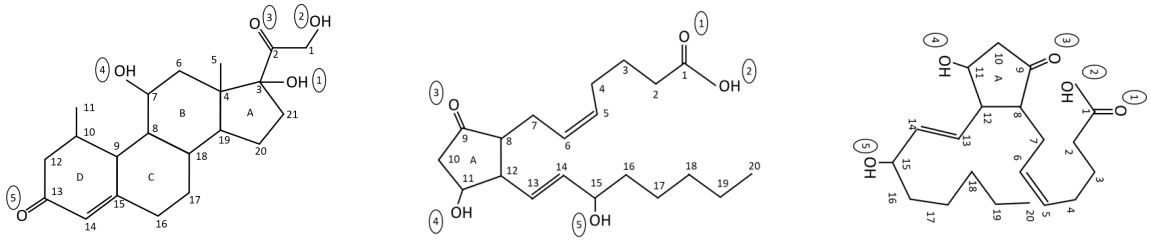
2 Results

The hypothesis of PGE2 and CORT competing for the LBD of GR is assessed initially by comparing the molecular weights, number of chemical elements, and positional distribution of functional groups. In a key step, conformational isomers, or conformers, of PGE2 are then evaluated through wire sketches to assess a feasible configuration that is representative of CORT. Detailed molecular models of the most promising conformer of PGE2 are then compared to CORT to determine the bond length, positioning, and spacing of the

intramolecular functional groups. Finally, the integration of the molecules and the functional groups at the LBD of GR are computed in terms of intermolecular spacing, hydrogen bonding, electrostatic potential, and intramolecular characteristics.

2.1 Comparing CORT and PGE2

In Figure 1, the chemical structure of CORT and PGE2 is presented in the standard arrangements. To make our case, we first note that CORT ($C_{21}H_{30}O_5$) and PGE2 ($C_{20}H_{32}O_5$) are of similar molecular weight, 362.46 g/mol versus 352.46 g/mol respectively, and have nearly the same number of chemical elements: 21 versus 20 carbon; 32 versus 30 hydrogen; and importantly the same number of oxygen elements, five, which can be arranged as five functional groups. When expressed in standard format the overall structure of PGE2 is dissimilar to that of the steroid structure of CORT; however, through single bond rotations at C4 and C12 of PGE2, a structure is formed resembling closer to CORT is formed. This structural expression maintains the same double bond arrangement of *cis* configuration at C5-C6 and *trans* configuration at C13-C14. The arrangement critically positions three oxygen groups together such that it is comparable in position to that of CORT at C1, C2 and C3. With further single-bond rotation at C15, a match of the functional groups is achieved, and with rotation at C16, C17, the dimensional size of CORT can be approximated by PGE2. Further, lateral stability is established through the interaction of C20 with the double bond of C5-C6, and a hydrophobic core is achieved, thus matching well the cyclic structure of CORT. In order for this alignment to occur, there needs to be a driving force, and as it will be shown, the ligand binding domain of the glucocorticoid receptor provides this driving force.



(a) Cortisol (b) PGE2 (c) PGE2 Conformer

Figure 1: A comparison of the molecular arrangements of (a) cortisol, $C_{21}H_{30}O_5$ *and (b) PGE2,* $C_{20}H_{32}O_5$ *in nominal configuration. In (c) a conformer of PGE2 is shown, that more closely resembles the cyclic structure of cortisol. Note that the cis and trans configurations of the carbon-carbon double bonds are conserved in the conformer of PGE2 relative to PGE2, and thus are the same molecule but just organized in a different positional arrangement.*

In Figure 2, the functional groups of CORT and the conformational isomer of PGE2

are compared to demonstrate approximate chemical equivalency within each region. Three oxygen groups are each present in groups I_{CORT} and I_{PGE2}, and one oxygen group each is present in II_{CORT} and III_{CORT} and in II_{PGE2} and III_{PGE2}. At the interior of the arrangement of both CORT and PGE2, a hydrophobic center exists, comprised of approximately six carbons each. The two carbon-carbon double bonds of PGE2 are well-positioned to approximate the rigidity and dimensional restrictions associated with the cyclic structure of CORT. The greater potential mobility of the carboxylic acid group of I_{PGE2} is available to offset the carbon intermediate to the carbonyl and hydroxyl group of the equivalent structure within I_{CORT}. The intramolecular spacing of the three functional group regions is similar to a first approximation, and are all located about the periphery of the molecules.

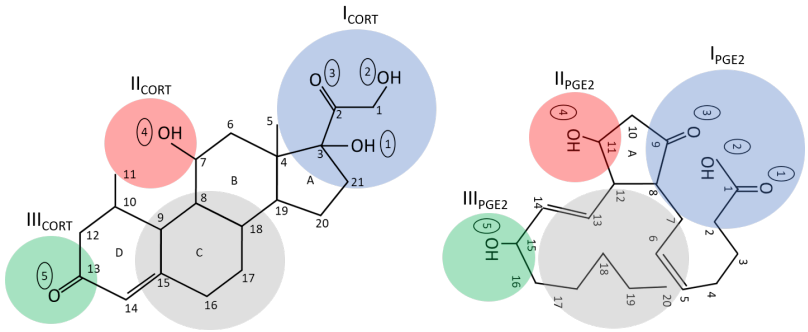
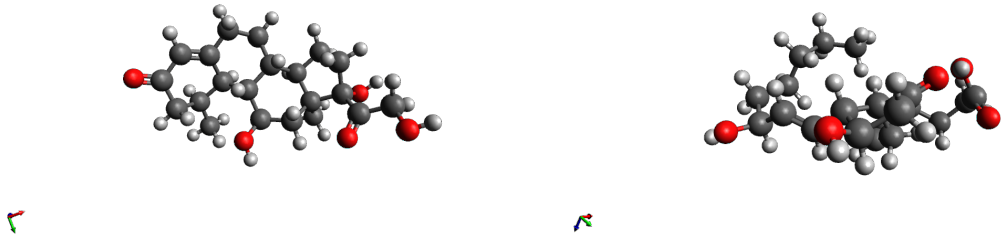


Figure 2: With the reconfiguration of PGE2 and with a rotation of approximately 45 degrees, the cortisol and PGE2 molecules are compared for the functional groups and relative positioning. It is noted that the three sites are correlated with I_{cort} and I_{cort} having three oxygen groups, with one each on the other two groups. The positioning of the functional groups is on the exterior of the PGE2 molecule, equivalent to cortisol, and the interior is composed solely of carbon and hydrogen groups. In addition, the spacing of the groups is approximately consistent, with the width approximately 5 carbon bonds wide for each. The spacing in between functional groups and angular configuration is also approximately the same.

2.2 Structural Analysis

To evaluate the positional relations and dimensional similarity of the functional groups comprising CORT and PGE2, the sketches are developed as three dimensional models, as indicated in Figure 3 with the bond lengths and intramolecular spacing determined through the software simulation routines assuming a universal force field. The projected dimension is approximately 11.4Å × 5.0Å for CORT and 10.6Å × 4.7Å for PGE2. Thus, both CORT and PGE2 conformer have the same aspect ratio of 2.3.

In Table 1, the intramolecular distances are compared between the five oxygen-based functional groups of CORT and those corresponding to the PGE2 conformer of prostaglandin



(a) CORT (b) PGE2

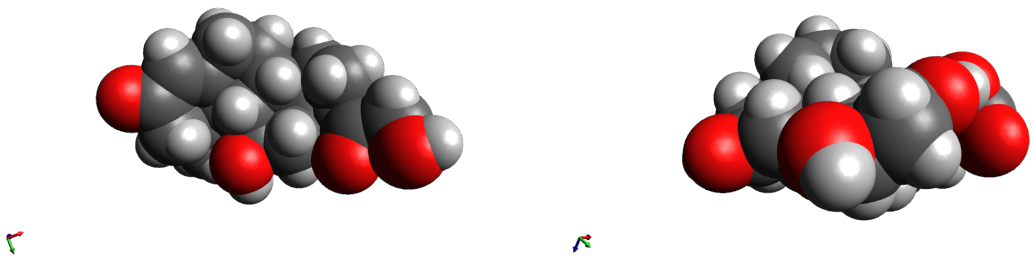
Figure 3: The three dimensional configuration of cortisol and prostaglandin E2. The spacing of the two functional groups of the five membered ring of PGE2 from C9-C11 is 4.681 Å *which is close to the corresponding position of CORT at C2-C7 of 4.657* Å*. This is crucial because this dimension can not be changed easily with PGE2, whereas the other dimensions can be optimized by alternative conformational isomers of PGE2 in association with the force field of the LBD.*

E2. In general good agreement is indicated between CORT and PGE2, within the three functional group regions. Especially important, is that for the five membered carbon ring of PGE2, which corresponds to the spacing of O4-O3, it is noted that it is in good agreement with O4-O3 of CORT. This is critical not only because it connects the I and II functional groups, but also because the distance for O4-O3 PGE2 is fixed due to the cyclic structure. Whereas, the O5-O4 and the O3-O2 groups of PGE2 can be adjusted to optimal distances because of the freedom of motion due to the two branches. We note however that the trans and cis configurations of PGE2 aid in the positioning of the functional groups of the structure. The distance of O3-O2 is different between CORT and PGE2. However, one intermediary water molecule will be introduced to CORT and to PGE2, but at different locations, that will establish an equivalent configuration for hydrogen bonding; moreover, both the PGE2 groups corresponding to the carboxylic acid group on an unconstrained branch will enable optimal positioning.

In Figure 4 the volume of space occupied by the molecules are compared by depicting the van der Waals hard shell radius for CORT and PGE2 conformer. It is seen that the volume and positional arrangement of the volume is similar. The periphery space occupied by the three functional regions have similar presentation, and are accessible to intermolecular forces which will be important for PGE2 conformer to associate with the LBD. The relative dimensional profile is similar and the hydrophobic nature within the core is similar.

Molecule	O5-O4	O4-O3	O3-O2	O2-O1
CORT	5.634 Å	4.657 Å	4.274 Å	2.592 Å
PGE2	5.175 Å	4.681 Å	2.634 Å	2.244 Å

Table 1: The distance between the five functional groups of cortisol and prostaglandin E2 is compared. The five membered ring of PGE2 corresponds to the critical O4-O3 group, and it is noted that it is in agreement with that of CORT. This is critical because it connects the I and II functional groups, and the distance for O4-O3 PGE2 is fixed due to the ring structure; whereas the O5-O4 and O3-O2 groups of PGE2 can be adjusted to optimal distances by motion of the branches. The distance of O3-O2 is different between CORT and PGE2. However, the PGE2 group corresponds to the carboxylic acid group and because of its electrostatic potential may provide equivalent binding capability as the hydroxyl group of CORT despite the longer difference to the corresponding attachment site at GR.



(a) CORT Van der Waals spheres (b) PGE2 Van der Waals spheres

Figure 4: For cortisol and prostaglandin E2, the model with the van der Waals hard shell configurations indicates the peripheral positioning of the functional groups, which are thus accessible for binding to corresponding areas of the LBD.

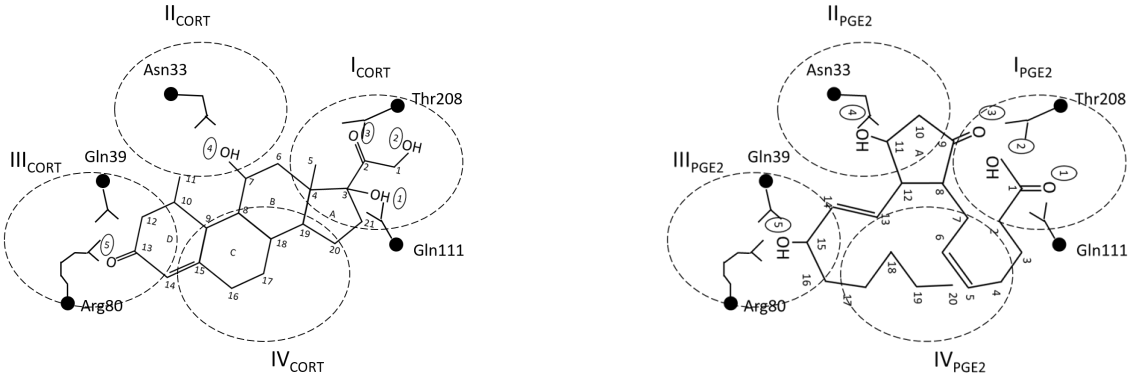
2.3 Ligand Binding Domain of Glucocorticoid Receptors

Recently, high resolution crystal structures of the ligand binding domain of glucocorticoid receptors have identified the amino acids responsible for the positioning of cortisol [20]. In this section, the approximate spatial relationship of PGE2 conformer is developed based upon the amino acids identified in these crystal structures. We first analyze simple wire sketches, which are illustrative, and then apply the molecular modeling software programs for spatial analysis.

In Figure 5, the approximate spatial positional relationship is presented of CORT and PGE2 within the LBD of GR. It indicates similarity between the molecular association of GR-PGE2 and GR-CORT. It is noted that the approximate dimensional arrangement of the functional groups GR-PGE2 is appropriate as placed in three regions, and may even

be preferred in comparison to GR-CORT as III_{CORT} requires the presence of water (not shown), whereas with III_{PGE2} the hydroxyl group is available to interact directly with Gln39 and Arg80. In I_{PGE2} the single hydroxyl group is available for interaction with Gln111 and Thr208.

Further examination of Figure 5 also indicates the importance of the five-membered carbon ring, as it positions the two branches of PGE2 to associate with the hydrogen bonding centers of LBD of GR. This establishes the hydrophobic core including the potential multi-ring structure, and the functional groups surrounding the core. Moreover, the ring structure of PGE2 sets-up the functional groups on the exterior of the molecular and an internal hydrophobic core, which includes a pair of carbon-carbon double bonds at C5-C6 and C13-C14 interacts with groups of GR including Leu77, Met73, Trp69, Met70, Leu32, Phe92, Cys205, Phe204 (not shown in figure but positioned above and below the internal core of PGE2). The internal hydrophobic core for each of CORT and PGE2 is comprised of six carbons.



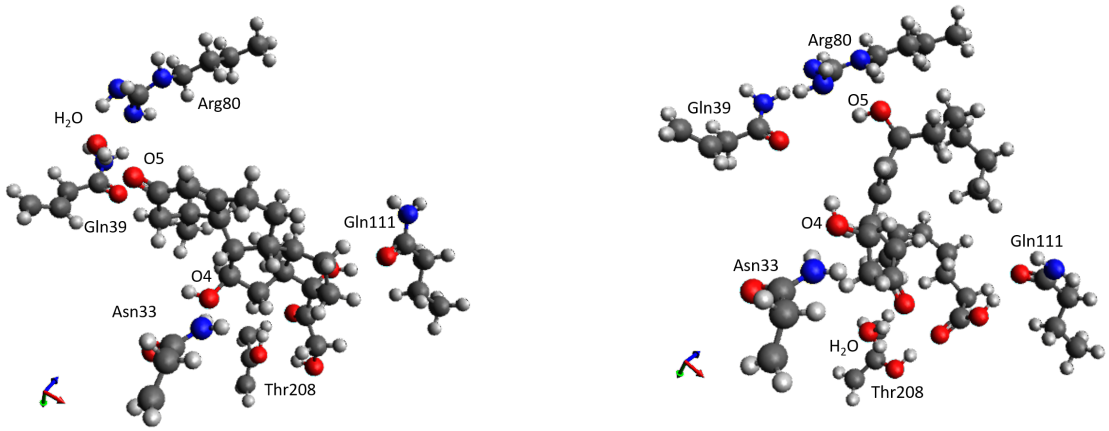
(a) CORT-GR

(b) PGE2-GR

Figure 5: For the ancestral glucocorticoid protein receptor (AncGR2), which is a stable GR useful for crystal studies, at the ligand binding domain, a fit of a conformer of PGE2 very similar is noted to that which is achieved with CORT. There is substantial equivalent hydrogen bonding at the three functional groups, and at the interior there is substantial hydrophobic interaction and similarity. There may be an improved interaction with PGE2 relative to cortisol with Gln39 and Arg80 since CORT requires an external water molecule since only a carbonyl group is available, whereas PGE2 already has a hydroxyl group. The three membered oxygen group of PGE2 contains enough free motion as it is unconstrained by rings to achieve optimal alignment. The double bond alignment of PGE2 at the hydrophobic core is noted. It is also noted that the ring structure of PGE2 formed via the C8-C12 bond is critical to achieve the proper configuration of the two branches and subsequent fit within the hydrophobic core, which incorporates two carbon-carbon double bonds.

To assess the three dimensional nature of the arrangements, molecular modeling soft-

ware is used in the following manner: first the side groups of the amino acids are positioned relative to CORT, and then CORT is replaced with the conformer of PGE2 to characterize the intermolecular association. The molecular modeling program is used to optimize bond lengths, elemental positioning, and intermolecular spacing. The three dimensional positioning of cortisol and prostaglandin E2 as a conformer is exhibited in Figure 6. As indicated earlier, the water molecule for CORT is positioned between O5 and Arg80, while for PGE2, the water molecule is positioned between O3 and Thr208. Both show similar interaction with the binding regions of the GR protein, in terms of intermolecular spacing. To establish the positioning a uniform force field is applied to the simulation, and the geometry optimized to minimize the energy level of the molecule. The program converges to a local minimum as the positioning initially of the molecules influences the ultimate convergence, including with respect to the position of the hydrogen groups. However, the overall energy of the PGE2 molecule was calculated as -1044 kJ/mol while that of cortisol was -917 kJ/mol, which indicates that the PGE2 conformer is more stable configuration, requiring more energy to separate.



(a) CORT-GR (b) PGE2-GR

Figure 6: Configuration of (a) CORT and (b) PGE2 within the LBD of GR, using the positioning of the side chains with polar groups relative to those of the hormones. It is noted that there is an association that is relative comparably in terms of spacing between CORT and PGE2, and that there is similarity in overall dimensional profile.

In terms of intermolecular spacing of the five oxygen-based functional groups from the corresponding amino acid side chains, Table 2 provides a comparison of CORT and PGE2 within the LBD of GR. The range of spacing for CORT is from 1.741 Å to 2.096 Å, and for PGE2 the range is from 1.726 Å to 2.118 Å; the average spacing for CORT is 1.877 Å and for PGE2, the average spacing is 1.912 Å. Without the water molecule placed in the vicinity of O3 and Thr208, the spacing of the five oxygen-based groups would have

Molecule	O5-Arg80[1]	O4-Asn33	O3-Thr208[2]	O2-Thr208	O1-Gln111
CORT-GR	1.826 Å	1.874 Å	1.741 Å	1.850 Å	2.096 Å
PGE2-GR	2.108 Å	2.118 Å	1.820 Å	1.726 Å	1.789 Å
Difference	−0.282 Å	−0.244 Å	−0.079 Å	0.124 Å	0.307 Å

Table 2: The intermolecular spacing of the oxygen-based functional groups to amino acid side group elements comprising the LBD. For CORT, the O5 group space is to a water molecule which is then associated with Arg80 at 2.026 Å*, and for PGE2 to O3 group space is to a water molecule which is then associated with Thr208 at 2.349* Å*. The average spacing for CORT is calculated at 1.877* Å *and for PGE2, the average spacing is 1.912* Å*. The average difference in spacing is thus −0.174* AA*, which is 9.268% greater for PGE2 than CORT.*

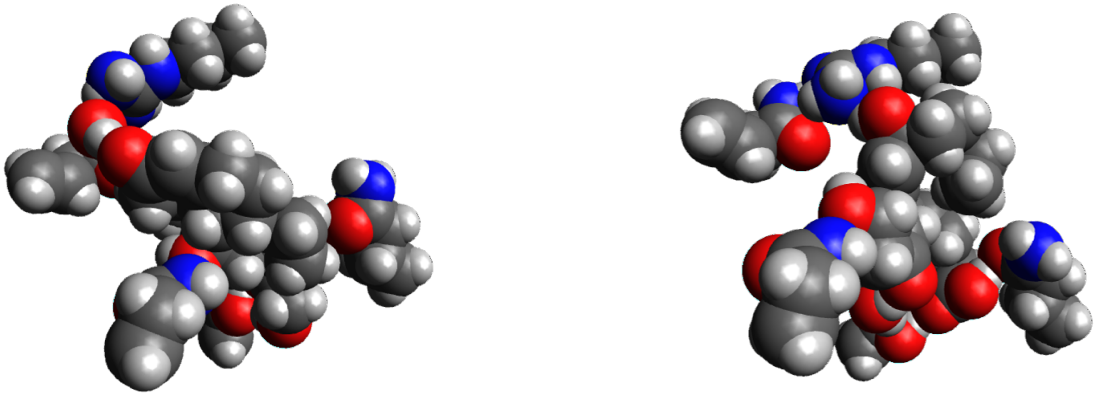
been respectively 2.213 Å, 2.140 Å, 4.588 Å, 1.773 Å, and 2.806 Å, respectively, and the energy level is − 1002 kJ/mol, indicating that the water molecule is slightly stabilizing. The spacing from the inserted water molecule of PGE2 at O3 to Thr208 is 2.349 Å; for CORT, the spacing from the water molecule to Arg80 at O5 is 2.026 Å.

The Van der Waals space filling model representations are presented in Figure 7 for CORT and PGE2 to show that both molecules occupy similar volume within the LBD, which helps in making the case that PGE2 exhibits competitive inhibition with PGE2. The arrangement of the functional groups along the periphery show similar distance spacing. One of the branches of PGE2 does show out of plane positioning compared to the tighter profile of CORT.

The electrostatic potential of the surfaces of CORT and PGE2 within the LBD is presented in Figure 8. Similarity is noted, with the periphery groups possessing the electrostatic charge, with the central areas neutral. The areas of greatest electrostatic concentration are maintained in a comparison of the two molecules

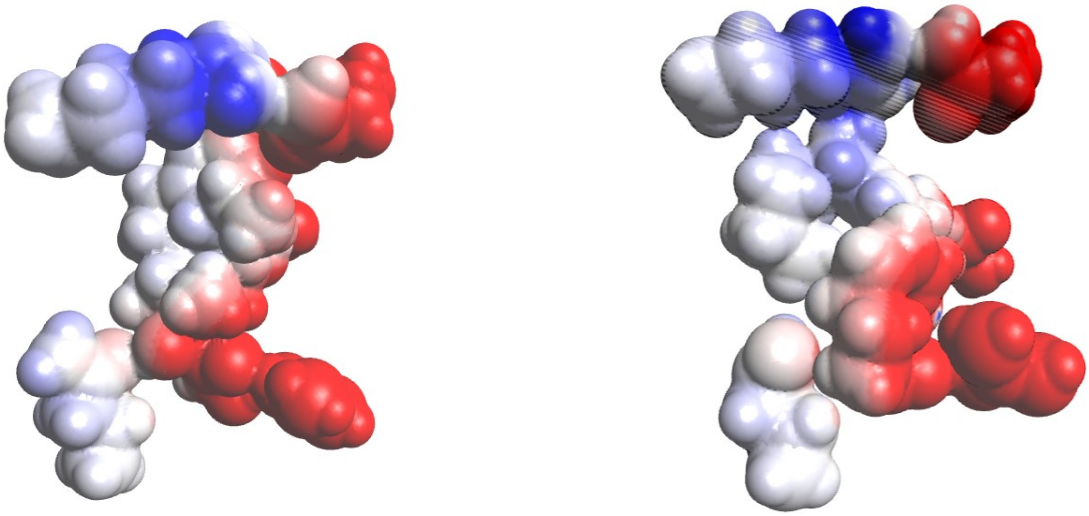
In Figure 9, the force generated at the side chains of the polar groups associated with GR, and with the functional groups of PGE2 is indicated. The largest forces are generated at the Arg80 group which shows a relation to the O5 group attached to C15. This indicates the potential for motion of Arg80 and Gln39, and thus conformational variation of the GR relative to that associated with CORT. The original positioning of cortisol was used in setting the position of Arg80 in the simulation. The distortion will cause potentially the GR to become non-expressive when connected with PGE2. It is also noted that the forces at the carboxylic group of PGE2 position the branch and C1 in the middle of Thr208 and Gln111.

The wire frame for the configuration of the conformer of PGE2 is depicted in Figure 10. It indicates a close association with the predicted sketches of the earlier section, which were



(a) CORT-GR (b) PGE2-GR

Figure 7: For the hard shell configuration of van der Waals radius, the arrangements within the LBD of (a) cortisol and (b) PGE2 conformer indicates that there is an appropriate level of spacing.



(a) GR-Cortisol (b) GR-PGE2

Figure 8: The electrostatic potential surfaces of cortisol and PGE2 within the LBD indicates similarity within the molecular structure demonstrating neutral core and acidic periphery structures from the five oxygen based functional groups.

done based largely on dimensional arguments. The universal force field of the simulation program positions the C20 group towards the double bond of the alpha chain of PGE2,

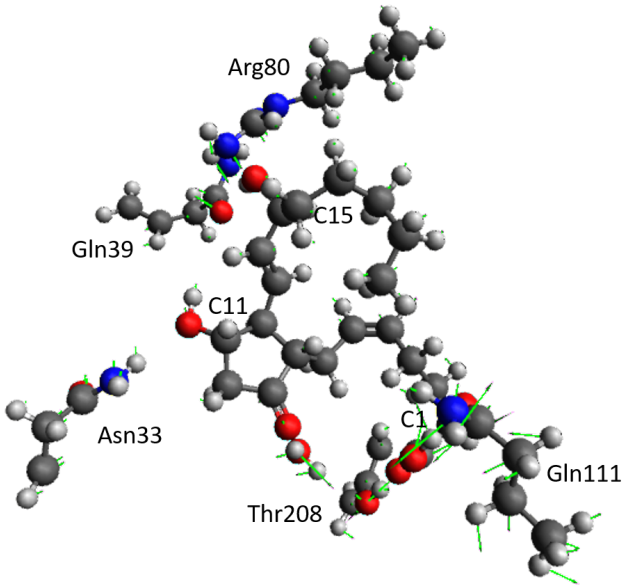


Figure 9: The force generated in this positional arrangement of PGE2 indicates potential motion in Arg80 towards O5 and with the carboxylic acid group towards Thr208. Thus it would be expected that the conformational arrangement of GR would be altered with the insertion of PGE2 at the LBD, instead of CORT.

but out-of plane, which ultimately became its position when the optimization routine was utilized the minimize the energy levels. The *cis* and *trans* configurations of the molecule are also retained.

We believe that these modeling results are the first indicating the interaction of PGE2 with GR, occupying the space that CORT would normally occupy. Importantly, it indicates the potential for competitive inhibition at the ligand binding domain of GR. It can be inferred that other structures where CORT interacts, such as membrane bound receptors that produce non-genomic responses, that there would be similar competitive inhibitory influence of conformers of PGE2 on CORT.

2.4 Other Prostaglandins

In the family of prostaglandins, other configurations besides PGE2 show similarity in chemical affinity in comparison to CORT, and thus a capability for intermolecular activity within the LBD of GR and therefore competitive inhibition with CORT. Table 3 lists several other prostaglandins, and indicates the main difference with respect to PGE2, and its possible difference in association with the LBD of GR under study. The result indicates that all prostaglandin have potential for competitive association with CORT, and thus may be

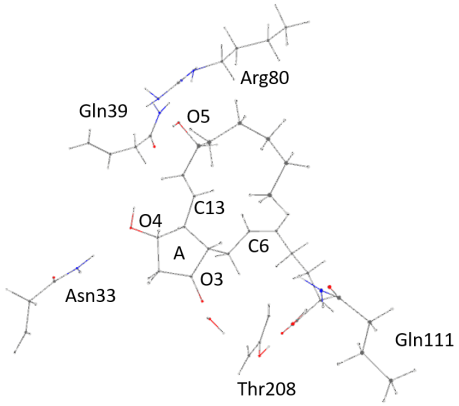


Figure 10: A wire frame model is used to indicate the relative positioning of the PGE2 conformer within the LBD. It is noted that the cis and trans configurations of the double bonds are maintained, and that the molecular model is similar to the approximate planar sketches presented in an earlier section.

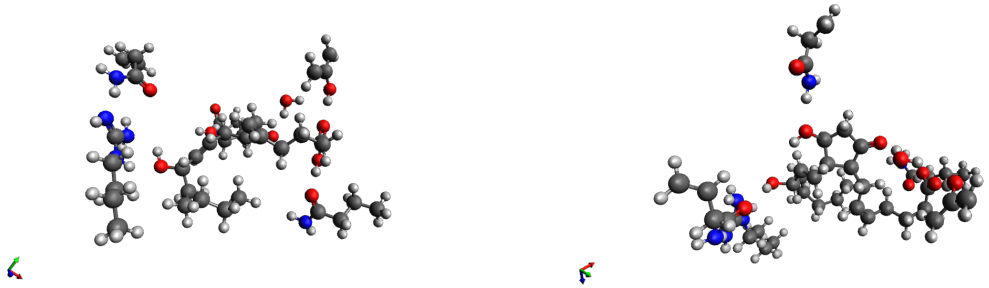
capable of altering the intended rate of gene expression of CORT.

Related to the molecular dynamics of introducing the PGE2 molecule into the slot at the LBD of GR which nominally is designated for CORT, Figure 11(a) presents the PGE2 molecule with rotation at C12 but prior to the final configurational arrangement involving the positioning of the carboxylic acid group to establish the hydrophobic region. The linear structure of PGE2 indicates the three head functional groups which has spacing of 4.627 Å (O5 to O4) and 4.640 Å (O4 to O3) prior to the final configuration. This indicates a positional arrangement that is nearly the same in dimension, thereby providing the possibility of the orientation of PGE2 being reversed when presenting to the LBD of GR; that is, for example, O3 may associate with Gln39 and ARG80. Also, it is noted that the five-membered ring and the double bond carbon achieves a planar structure consistent with the nominal corticosteroid structure of CORT. Further it is noted that the spacing to the carboxylic group from the lead functional groups to the double bond on the alpha group is 6.569 Å, which is greater than the width of the LBD structure in the final configuration. Hence, there is sufficient size for PGE2 to enter without the possibility for out-of-plane steric hinderance from the upper and lower portions of the slot opening of LBD of GR. Thus for the molecular dynamics of positioning of PGE2, it seems likely that the five carbon ring is first positioned at $\mathrm{I}_{\mathrm{PGE2}}$ and $\mathrm{II}_{\mathrm{PGE2}}$. The hydrophobic core as noted in Figure 11(b) then drives the hydroxyl group on the second branch to $\mathrm{III}_{\mathrm{PGE2}}$ and then the carboxylic acid group of the first branch to bind to the associated LBD corresponding to the functional groups of $\mathrm{I}_{\mathrm{PGE2}}$. The C20 is then positioned out-of-plane with respect to the double bond groups of C5 and C6 to produce the lowest energy state.

The elemental positioning of the structures was performed without constraints on the

Molecule	Formula	Difference from PGE2	Difference in Affinity at GR
PGE1	$C_{20}H_{34}O_5$	C5-C6 single bond	$\Downarrow$ hydrophobic core
PGE3	$C_{20}H_{30}O_5$	double bond at C17-C18	$\Downarrow$ C20 positioning
PGD2	$C_{20}H_{32}O_5$	OH at C9; O at C11	$\Uparrow$ I_{PGE2} hydrogen bonding $\Downarrow$ II_{PGE2} hydrogen bonding
PGFα	$C_{20}H_{34}O_5$	OH at C9	$\Uparrow$ II_{PGE2} hydrogen bonding

Table 3: Analysis of the chemical affinity of other prostaglandins relative to PGE2 at GR, which to a first approximation would indicate that PGE1 and PGE3 would be slightly less stable within GR than PGE2, but both would should effective competitive inhibition. PGD2 shows improvements in PGE2 in hydrogen bonding as does PGFα, which may result in slightly different kinetic effects than PGE2.



(a) Entry Point (b) Hydrophobic Core

Figure 11: For PGE2, (a) the entry point within the GR for a linear conformer of PGE2 at the LBD of GR. In (b) the top down view of PGE2 indicating the hydrophobic core, and the periphery of the functional groups for association with polar groups associated with GR.

base carbon of the amino acid, which is the attachment point of the side chain to the protein structure of the GR. Hence, it is important to determine how much motion of the base carbon was needed in order to position the functional heads of the side chains for interaction with either CORT or PGE2. This measurement will also relate to the conformational adjustment of the protein in order to accommodate PGE2 instead of the usual CORT. In Table 4, the distances between the base carbon of each of the functional groups is calculated, along with the difference, indicating excellent agreement between the intermolecular spacing of the groups comprising the GR protein. However, although the difference is slight, only 1.9% difference on average, with a worst-case spacing difference of

Groups C0 - C0	CORT Distance	PGE2 Distance	CORT − PGE2 Difference
Thr208-Gln111	10.545 Å	10.774 Å	−0.229 Å
Thr208-Arg80	17.005 Å	17.207 Å	−0.202 Å
Thr208-Gln39	14.355 Å	14.453 Å	−0.098 Å
Thr208-Asn33	11.701 Å	12.116 Å	−0.415 Å
Gln111-Arg80	15.214 Å	15.609 Å	−0.395 Å
Gln111-Gln39	19.836 Å	20.162 Å	−0.326 Å
Gln111-Asn33	15.461 Å	15.689 Å	−0.228 Å
Gln39-Arg80	10.545 Å	10.774 Å	−0.229 Å
Gln39-Asn33	11.201 Å	11.517 Å	−0.316 Å
Arg80-Asn33	17.456 Å	17.680 Å	−0.224 Å

Table 4: Difference in position of the base carbon of the amino acid side chains comprising the functional groups of the LBD of GR. It indicates that while the functional groups comprising the attachment points to either CORT and PGE2 are positioned almost identical, the base carbons comprising the protein structure are relatively stable. However, the LBD for PGE2 is slightly larger, just 1.9%, than the LBD for CORT.

0.415 Å, the directions are all the same, indicating that the PGE2 LBD is slightly larger than that of CORT. The standard deviation of the difference is 0.096 Å and the average distance is −0.266 AA compared to an average spacing for CORT of 14.319 Å.

3 Discussion

This research has indicated that there is similarity in the molecular configurations of CORT and a conformer of PGE2 which would enable binding at the LBD of GR. Favorable agreement is achieved across multiple areas of comparison including molecular weight, number of chemical elements and functional groups, relative positioning of functional groups, hydrogen bonding, hydrophobic centering, topology, dimensional size, and intramolecular and intermolecular arrangement of structures. Moreover, the conformational isomer of PGE2 at the LBD of GR appears to be relatively stable, that is energetically lower by 12.151%, in comparison to CORT, which would make the comparison relevant as PGE2 would occupy the position for significant periods. Other structures related to PGE2 including PGE1, PGE3, PGD2, and PGFα also show compatibility with the LBD of GR.

The intermolecular spacing of CORT to the LBD structures is nearly equivalent to that of PGE2, both with respect to the functional groups of the amino acid side chains, and to the relative positioning of the base carbon attached to the GR protein. The average intermolecular spacing of the functional group is nearly equivalent of 1.877 Å for CORT, which differs by 0.174 Å on average. The average distance of the functional groups is

1.8% in agreement, and the worst-case distance perturbation of the base carbon is 0.415 Å corresponding to a difference of 3.5%. The electrostatic potential for each molecule appears similar, and the net dipole is roughly the same. Both exhibit strong hydrogen bonding.

However, there are differences, albeit ever so slight, which may make the difference in whether there is gene expression or not. The LBD for PGE2 was 1.9% larger, and each of the ten intermolecular spacings of the base carbons of the amino acid side chains of GR were expanded an average of 0.266 Å from their corresponding position had CORT been in the LBD. This corresponds to a swelling of 5.675% in the volume of the PGE2 LBD as compared to CORT. The positioning of the intermolecular functional group spacing of CORT and PGE2 is slightly different of 9.268 % greater for PGE2, which may account for the slightly larger size of the LBD for PGE2. Another difference was that although one water molecule was each needed for CORT and for PGE2 to achieve the hydrogen bonding interaction, it was situated at different positions within the LBD, with the water molecule associating with Arg80 for CORT and associating with Thr208 for PGE2. Apparently, these differences, while small in magnitude, are significant enough to alter the capability for gene expression of GR.

Because of molecular similarity, competitive inhibition by PGE2 within the LBD will thereby influence the rate of cellular processing of CORT, which can be the source of dysfunctional cellular, and hence systemic responses since GR is widely distributed. Moreover as cortisol is also a permissive hormone, which itself impacts the processing rates of other hormones, competitive inhibition of cortisol would also alter the functional response of other homeostatic control processes. While this research has focused on the ligand binding domain of the glucocorticoid receptor, other receptors where cortisol acts could also be influenced by prostaglandins. This would include membrane bound receptors upon which cortisol may act, which would influence the potentiation of the cell.

Based upon the correlations of disease and stress, the results of this study would seem more than coincidental that a conformer of PGE2, a biomarker of inflammation, and CORT, a biomarker of stress, would exhibit molecular similarity. With higher concentrations of PGE2 that are prevalent during states of disease, the signs and symptoms of disease would thus be amplified as the normative processing of cortisol would be altered due to competition for the GR. Moreover, it is therefore not surprising that crisis such as those of cortisol insufficiency would result in flu-like symptoms, as the low rates of cortisol processing would be similar to that exhibited by competitive inhibition by PGE2. PGE2 is most notably arisen due to an infection, such as from gram-negative bacteria which possess lipopolysaccharides, and thus it would appear that many signs and symptoms of disease are due to the misprocessing of cortisol. Thus, the chronic nature of stress increases the likelihood that a form of illness will manifest, and then the dysfunctional processing of cortisol will amplify the signs and symptoms associated with the infection.

4 Methods

The methods used in this analysis were as follows:

- Molecular Modeling: A study on experimental data of a fever in response to physical activity and infectious agents involved the development of a mathematical model and a sketch of a pathway, but not the molecules responsible for the pathway [1]. In surveying the possibilities, cortisol and prostaglandins became likely as the causative molecules in relation to the pathway for fever. In an assessment of reactivity of PGE2 and CORT, similarity became evident through a simple repositioning of two single-bonds of PGE2 to produce a conformer that resembled cortisol, which led to the potential association of each with the glucocorticoid receptor. Fortunately, the ligand binding domain of the glucocorticoid receptor for cortisol was just measured and published in [20] from crystal studies. Using this arrangement as a basis for the positioning of the binding structures of the LBD, PGE2 was organized to match CORT as closely as possible in terms of hydrogen bonding, core cyclic structures and hydrophobic characteristics, electrostatic interaction, intramolecular interactions, and topography, while retaining the *cis* and *trans* positioning structure of its double bonds to achieve the approximate comparative structure of Table 5. The simulation program Avogadro was used for the three-dimensional molecular models, which included a bond length and element positioning program based on a universal force field. The supplemental information provides videos of the three-dimensional arrangement of CORT and PGE2 conformer at the side chains of the amino acids comprising the functional LBD of GR. One matter of interest to note is that the mathematics of the pathway was developed first, independent of the chemistry, and then afterwards the chemistry was discovered assessed that was capable of meeting the constraints as defined by the requirements of the model. In general, this type of protocol may be useful in identifying new pathways for other areas. Moreover, this role of cortisol and competitive inhibition at GR would seem to be significant in explaining and modeling the fundamental sources of the signs and symptoms of disease. The capability to model and predict the trajectories of such responses will lead to the development of molecular solutions to counteract interference at the LBD.

Item	Cortisol	Prostaglandin E2 Conformer
Molecular Weight	362.46 g/mol	352.46 g/mol
C elements	21	20
H elements	32	30
O elements	5	5
core	hydrophobic	hydrophobic
core C	6	6
periphery binding	hydrogen bonding	hydrogen bonding
periphery groups	three O/OH	three O/OH
O at (I/II/III) groups	3/1/1	3/1/1
O5 functional group	O + H_2O	OH
O3 functional group	OH	O + H_2O
Approximate Size C-C	8 x 5	8 x 5

Table 5: Initial comparison of the chemical affinity of Cortisol and a conformer of Prostaglandin E2 at the ligand binding domain of glucocorticoid receptors, before molecular modeling.

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