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

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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 causative source of certain ailments and symptoms of diseases, and thus is of significant medical importance. 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 gene-regulating ligand binding domain (LBD), the average intermolecular spacing to the corresponding identical five functional groups is 1.877 Å for CORT versus 1.760 Å for PGE2. The conformational isomer of PGE2 within the LBD is at a lower energy state than CORT by 25.62%, indicating preference in stability. Both PGE2 and CORT exhibit a hydrophobic core within the LBD of GR. The distance of intermolecular binding sites of the LBD for lPGE2 is 1.760 Å and CORT is 1.877 Å. Due to enhanced hydrogen bonding of PGE2 within the LBD, a reduction of the spacing at the attachment sites is 0.648 Å at an average distance of 13.949 Å, resulting in a decrease in volume of the LBD of 13.135% for PGE2 relative to CORT. With the incorporation of two calcium ions into both PGE2 and CORT, the energy is further reduced by 79% and 94% respectively, with PGE2 with calcium ion stabilization lower by 16% the equivalent arrangement of CORT. With the introduction of calcium ions, the dimension of the LBD is reduced by 33.381% in PGE2 compared to CORT.

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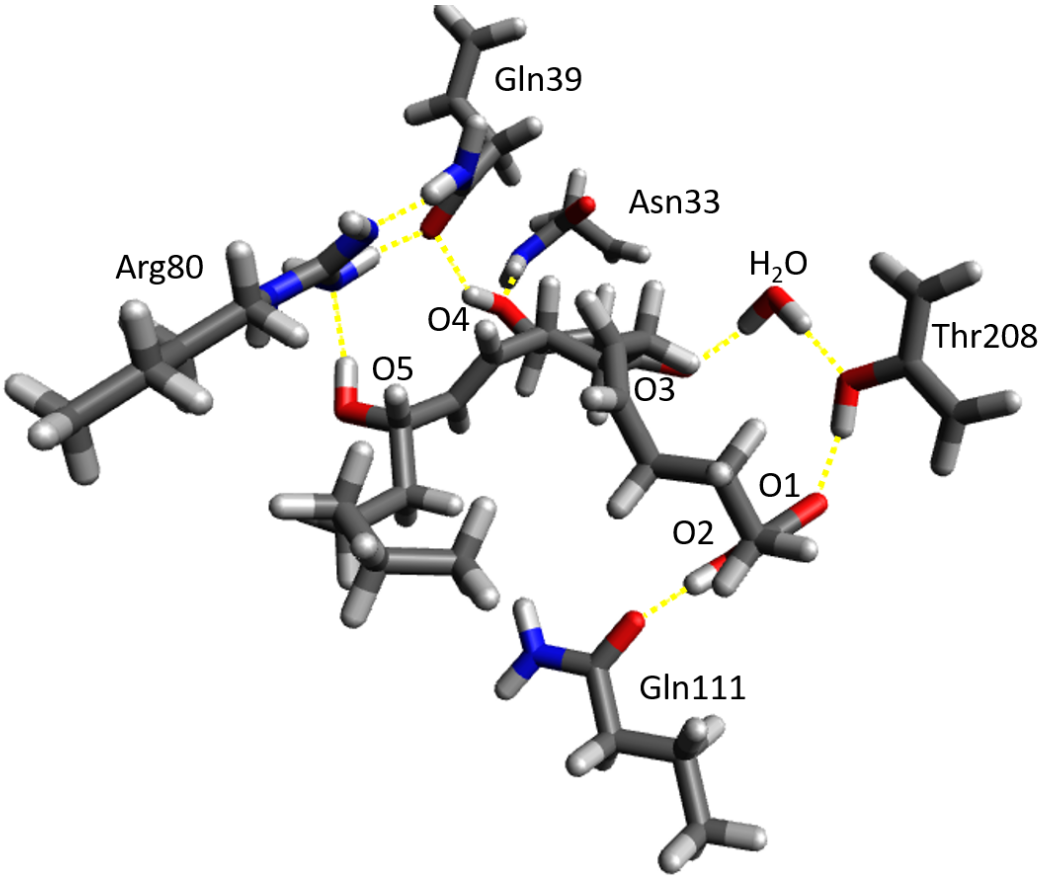
Graphical Abstract



Molecule-GR	**O5**	**O4**	**O3**	**O2**	**O1**	**Average**
CORT-GR	1.826 Å	1.874 Å	1.741 Å	1.850 Å	2.096 Å	1.877 Å
PGE2-GR	1.894 Å	1.791 Å	1.815 Å	1.573 Å	1.724 Å	1.760 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, signs of 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 of the observed dynamics of body temperature predicted the characteristics of the pathway that would cause such a response, but the actual identification of the molecules that were responsible for the initiation of a febrile response were left unknown and not specifically identified at the time, just the characteristics. 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 cardiovascular 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 responses associated with signs and symptoms of disease?

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 inducing cells to utilize other hormones to its fullest extent, and thus, in addition to a 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 and correlations, 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.

This study is developed based on molecular models comparing PGE2 and CORT, and its basis stems from a mathematical model characterizing an experimental measurement of body temperature variations. To relate this work with existing experimental data, it is useful to assess the literature experimentally relating cortisol and disease, of which wide reports of linkage has been noted, for example in viral infection [6], neuroimmunity[7], renal cancer [8], depression [9], and somatization disorder[10]. It is also noted that in addition to gene transcription through the LBD of GR, synaptic potentiation via cortisol has been examined [11, 12], which implies a non-genomic purpose as well. Furthermore, because of its wide ranging physiological importance, dysfunction of the regulatory mechanisms of CORT can be directly seen: for example, levels of cortisol that are too low due to a misfunctioning adrenal gland can result in adrenal crisis with significant temperature variations, fatigue, and aches [13] that can be misdiagnosed as an infection [14]. Further correlations of symptoms of disease and cortisol are inferred from the undulation of fever characterizing disease such as brucella [15]. In this paper, we will describe a fundamental causative link of cortisol and prostaglandins, in particular PGE2, which effectively induce signs and symptoms of disease.

2 Results

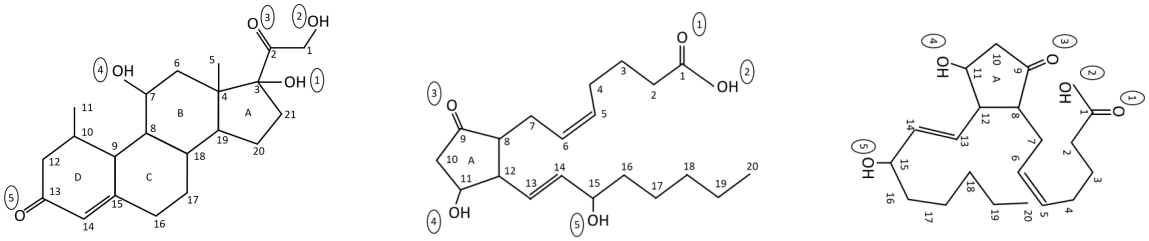
The hypothesis of PGE2 and CORT competing for the LBD of GR is assessed initially by comparing the molecular weights, number of chemical elements, and positional distribution of functional groups. In a key step, conformational isomers, or conformers, of PGE2 are then evaluated through wire sketches to 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, molecular energy, 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 structure of CORT; however, through single bond rotations at C4 and C12 of PGE2, a structure is formed resembling closer to that of CORT. This structural expression maintains the same double bond arrangement of *cis* configuration at C5-C6 and *trans* configuration at C13-C14, and thus is the same molecule. 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. Further, it is noted that the five functional groups of the conformer of PGE2 are all positioned on the periphery, as in CORT, and the core is hydrophobic, as is CORT. There are groups of three functional groups located on one side of the molecule for each, that is O1, O2, and O3 are approximately equivalent, and there is one oxygen group in the middle for each, and one oxygen group on the opposite side. Along one edge for both molecules, no functional groups are present.

2.2 Structural Analysis

To evaluate the positional relations and dimensional similarity of the functional groups comprising CORT and PGE2, three dimensional models are developed using the molecular simulator, employing a standard force field of MMFF94. In Table 1, the intramolecular distances are compared between the five oxygen-based functional groups of CORT and those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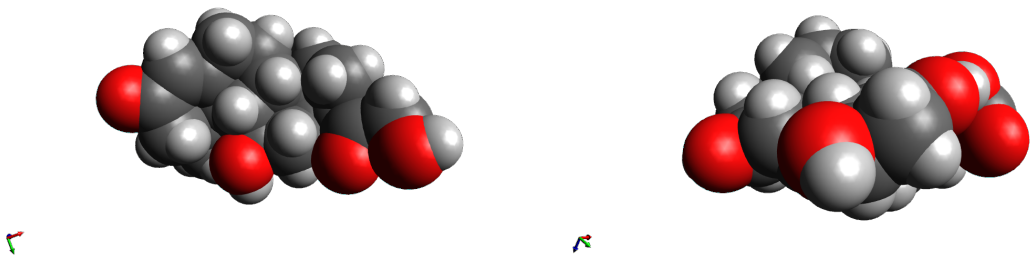
Molecule	O5-O4	O4-O3	O3-O2	O2-O1
CORT	5.634 Å	4.657 Å	4.274 Å	2.592 Å
PGE2	5.175 Å	4.681 Å	2.634 Å	2.244 Å

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

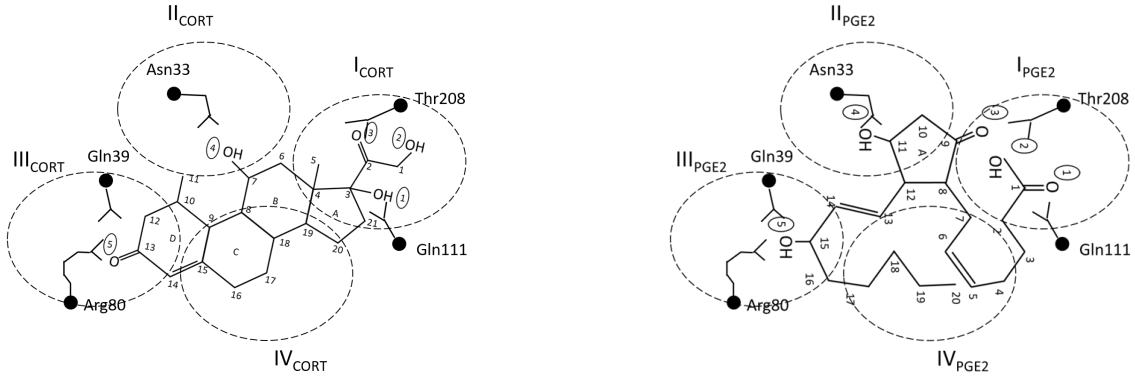
Figure 2: For cortisol and prostaglandin E2, the model with the van der Waals hard shell configurations indicates the peripheral positioning of the functional groups, which are thus accessible for binding to corresponding areas of the LBD.

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 acid groups responsible for the positioning of cortisol [16]. 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 3, the approximate spatial positional relationship is presented of CORT and PGE2 within the LBD of GR, indicating similarity. It is noted that the approximate dimensional arrangement of the functional groups PGE2-GR is appropriate as placed in three regions, and may even be preferred in comparison to CORT-GR as $\mathrm{III}_{\mathrm{CORT}}$ 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 3 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).

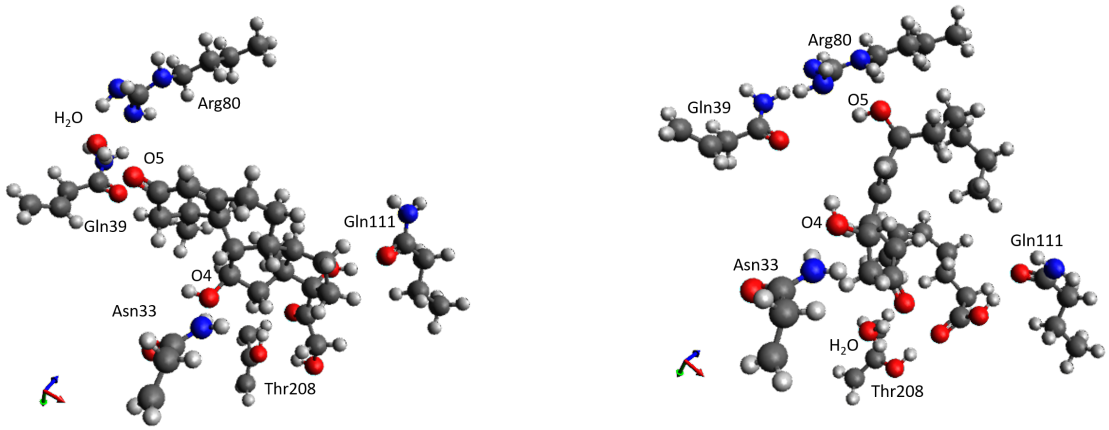


(a) CORT-GR (b) PGE2-GR

Figure 3: 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 simulation is conducted in the following manner: first the side groups of the amino acids comprising the LBD 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 4. 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 the force field of MMFF94 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 $-1{,}044$ kJ/mol while that of cortisol was nominally $-$ 580 kJ/mol, with best case configurations depending upon the initial arrangement running to a lower state -917 kJ/mol, which indicates that the PGE2 conformer is a more stable configuration, requiring more energy to separate and capable of remaining associated with the LBD for greater periods.



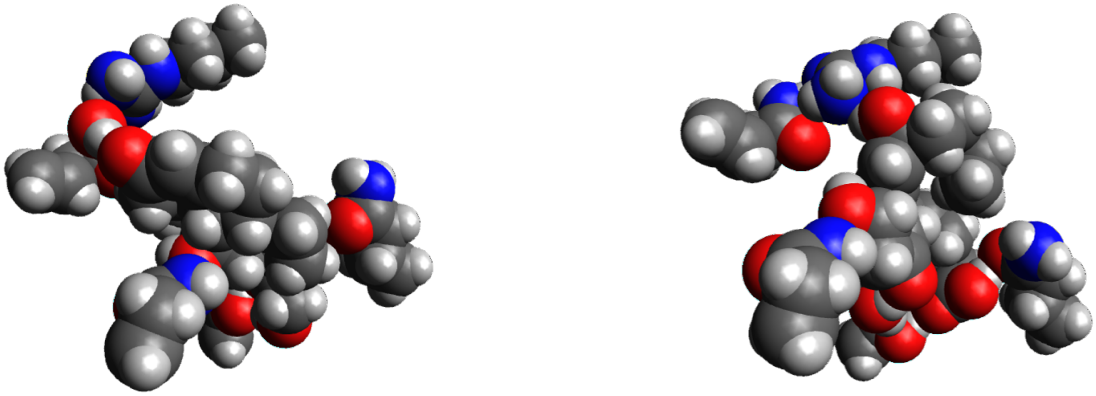
(a) CORT-GR

(b) PGE2-GR

Figure 4: 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.

The van der Waals space filling model representations are presented in Figure 5 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



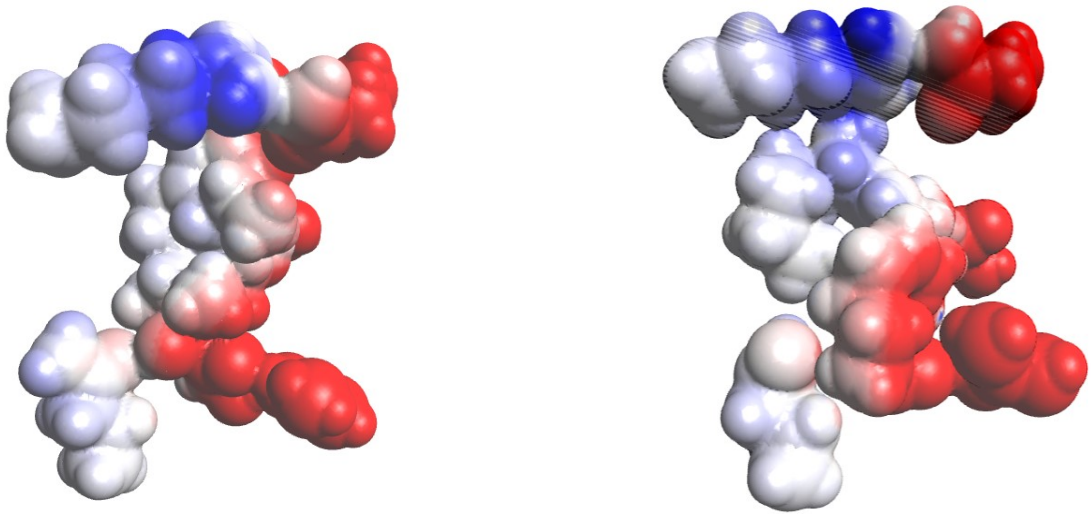
(a) CORT-GR (b) PGE2-GR

Figure 5: 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.

presented in Figure 6. Similarity is noted, with the periphery groups possessing the electrostatic charge, with the central areas neutral, including one side of the structure. The areas of greatest electrostatic concentration are maintained in a comparison of the two molecules.

The wire frame for the configuration of the conformer of PGE2 is depicted in Figure 7. It indicates a close association with the predicted sketches of the earlier section, which were done based largely on dimensional arguments. The force field of the simulation program positions the C20 group towards the double bond of the alpha chain of PGE2, 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.

Related to the molecular dynamics of introducing the PGE2 molecule into the slot at the LBD of GR which nominally is designated for CORT, the dimensions were calculated of the PGE2 molecule with rotation at C12 but prior to the final conformational arrangement involving the positioning of the carboxylic acid group. The linear structure of PGE2 positions the three leading functional groups with a 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, and enabling its entry. 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



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

Figure 6: 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.

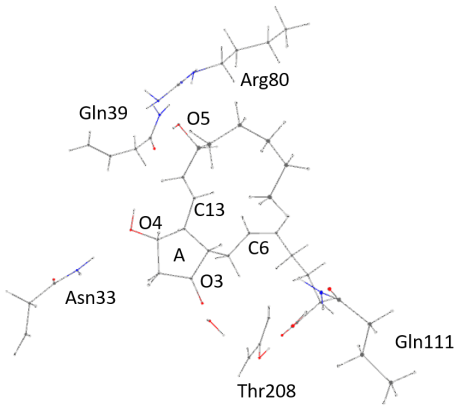


Figure 7: A wire frame model is used to indicate the relative positioning of the PGE2 conformer within the LBD. It is noted that the cis and trans configurations of the double bonds are maintained, and that the molecular model is similar to the approximate planar sketches presented in an earlier section.

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 I_{PGE2} and II_{PGE2}. The hydrophobic core then drives the hydroxyl group on the second (β) branch to III_{PGE2} and then the carboxylic acid group of the first (α) branch to bind to the associated LBD corresponding to the functional groups of I_{PGE2}. The C20 carbon is then positioned out-of-plane with respect to the double bond groups of C5 and C6 to produce a lower energy state.

To evaluate a more energetically favorable configuration of the PGE2 molecule, a slight deviation was made to Arg80-Gln39 in order to position C5 at its intersection. After optimization in the chosen force field using the molecular modeling software, the result produced an energy of -1,152 kJ/mol which is 10.345 % lower than the result using the initial conditions associated with the LBD of CORT. The arrangement of PGE2 within the LBD for this low energy configuration is shown in Figure 8, along with the lines of hydrogen bonding. It is seen that extensive hydrogen bonding is achieved throughout the molecule. Even though the shift was performed in the other side of the molecule at Arg80-Gln39, the carboxylic acid group actually realigned, with O1 actually interacting with Thr208.

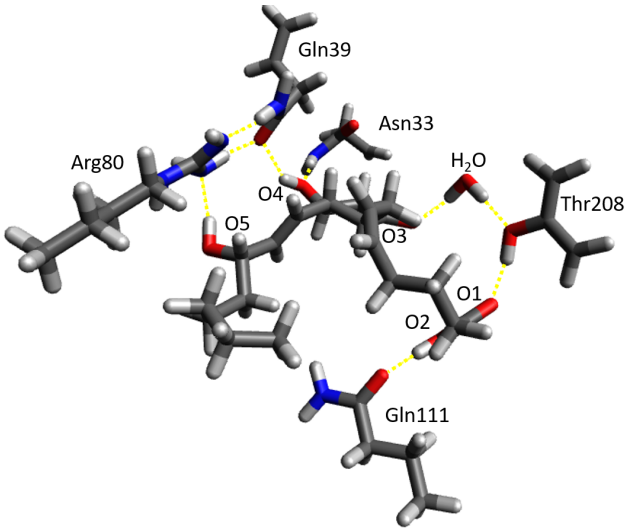


Figure 8: For the low energy configuration, extensive hydrogen bonding is seen for each of the five oxygen-based functional groups. The carboxylic group is positioned to form a chain of hydrogen bonding that includes a water molecule, organizing O3, O2, and O1. At the other end a chain of hydrogen bonding is also seen through O5 and O4.

To evaluate the positional arrangement of the PGE2, the electrostatic potential is indicated in Figure 12. The shape with the side chains of the LBD can be seen to be different than the earlier one at a higher energy level. At the Arg80-Gln39 position, it now angles down towards the O5 position of PGE2. This may indicate additional dysfunction

of the LBD due to PGE2 instead of CORT.

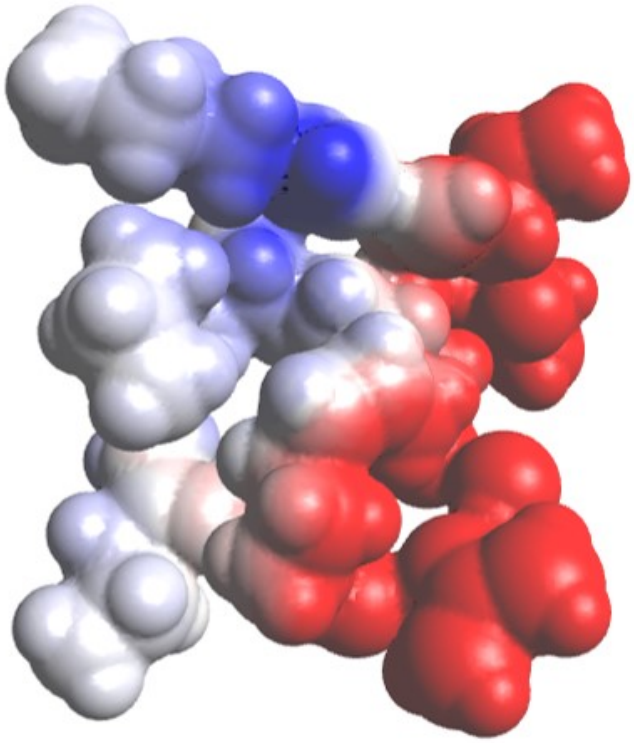


Figure 9: The electrostatic potential, space filling model of the low energy PGE2 configuration. It is noted that the Arg80-Gln39 complex is positioned in a different angular configuration, although the carbons attached to the protein are approximately the same, while the Asn33 group is repositioned relative to CORT.

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 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. For the low energy configuration of PGE2, the average bond distance for the nearest neighbor is 1.760 Å, which is tighter than CORT by 6.232%. In fact, each oxygen group has hydrogen bonding

Molecule-GR	**O5**	**O4**	**O3**	**O2**	**O1**	**Average**
CORT-GR	1.826 Å	1.874 Å	1.741 Å	1.850 Å	2.096 Å	1.877 Å
PGE2-GR	2.108 Å	2.118 Å	1.820 Å	1.726 Å	1.789 Å	1.912 Å
$PGE2^{LE}$-GR	1.894 Å	1.791 Å	1.815 Å	1.573 Å	1.724 Å	1.760 Å
CORT−PGE2	−0.282 Å	−0.244 Å	−0.079 Å	+0.124 Å	+0.307 Å	-0.0348 Å
CORT−$PGE2^{LE}$	−0.068 Å	+0.083 Å	−0.074 Å	+0.277 Å	+0.367 Å	0.117 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*, corresponding to an average difference of 1.854% greater for PGE2 than CORT. However, for the low energy configuration, denoted by* $PGE2^{LE}$*, the average bond distance is 1.760* AA *which is tighter configuration than CORT, by 6.232%.*

attraction to a side chain group or the inserted water group. For all, the carboxylic acid area has the shortest intermolecular distance at 1.573 Å.

The elemental positioning of the structures was performed without constraints on the 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 3, 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 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. However, for the low energy state position, there is actually a reduction in size. The average spacing is 13.949 Å, which is 4.438 % smaller. This corresponds to an approximate volume shrinkage of 12.732% of the LBD for the low energy configuration of PGE2. The main difference is in the positioning Asn33 in the low energy configuration, which has hydrogen bonding

Groups C0 - C0	CORT Distance	PGE2 Distance	PGE2^{LE} Distance	PGE2 Difference	PGE2^{LE} Difference
Thr208-Gln111	10.545 Å	10.774 Å	9.785 Å	−0.229 Å	+ 0.760Å
Thr208-Arg80	17.005 Å	17.207 Å	16.658 Å	−0.202 Å	+ 0.347Å
Thr208-Gln39	14.355 Å	14.453 Å	13.904 Å	−0.098 Å	+ 0.451Å
Thr208-Asn33	11.701 Å	12.116 Å	12.643 Å	−0.415 Å	− 0.942Å
Gln111-Arg80	15.214 Å	15.609 Å	14.080 Å	−0.395 Å	+ 1.134 Å
Gln111-Gln39	19.836 Å	20.162 Å	18.172 Å	−0.326 Å	+ 1.664 Å
Gln111-Asn33	15.461 Å	15.689 Å	16.503 Å	−0.228 Å	− 1.042Å
Gln39-Arg80	13.203 Å	13.190 Å	13.129 Å	+0.013 Å	+ 0.074 Å
Gln39-Asn33	11.201 Å	11.517 Å	7.638 Å	−0.316 Å	+ 3.563 Å
Arg80-Asn33	17.456 Å	17.680 Å	16.986 Å	−0.224 Å	+ 0.470 Å

Table 3: 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. For the low energy configuration of PGE2, denoted as PGE2^{LE} the LBD is actually smaller than CORT on average, by 4.438%.

tapped into Gln39 and Arg80 arrangement, whereas the higher configuration, Asn33 only interacts with O4. The spacing between Arg80 and Gln39 is approximately the same for all three configurations, indicating stability of the LBD configuration.

2.4 Incorporating Calcium Ions

It has been indicated that there is an association of two calcium ions with cortisol into the cytosol [17]. In this section, the role of calcium is explored for both CORT and PGE2 at the LBD of GR to show that both molecular structures have two sites each where calcium ions can be incorporated, resulting in a reduction of the energy of the overall structure. The molecular organization and a comparison is indicated in Figure 10 for CORT and in Figure 12 for PGE2, which includes an electrostatic potential mapping to show distribution of the electron density across the configurations. Similarity is noted in terms of the incorporation of the calcium ions at two ends of the molecular arrangements, which disrupts the hydrogen bonding, and positions the calcium ion within the central area for electron sharing with three or four of the oxygen based functional groups. Additional calcium ions are not possible to be incorporated at other regions of the overall structure.

To determine the position of the calcium ions, estimates were used to position the calcium ions at locations of high electrostatic potential, and then the calculations were performed using molecular modeling software to determine the lowest energy positions. With

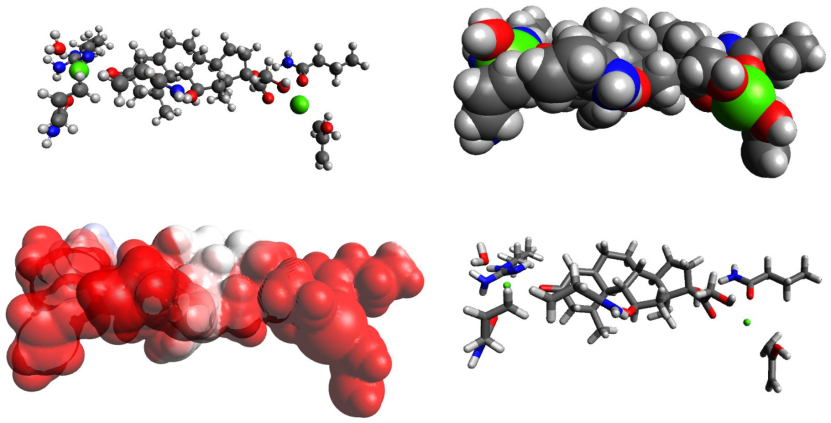


Figure 10: With two calcium ions optimal positioned within the CORT-GR complex, it is noted that the oxygen based functional groups of CORT come into association with Calcium ions at both ends of the molecular arrangement to stabilize the domain at a lower energy state.

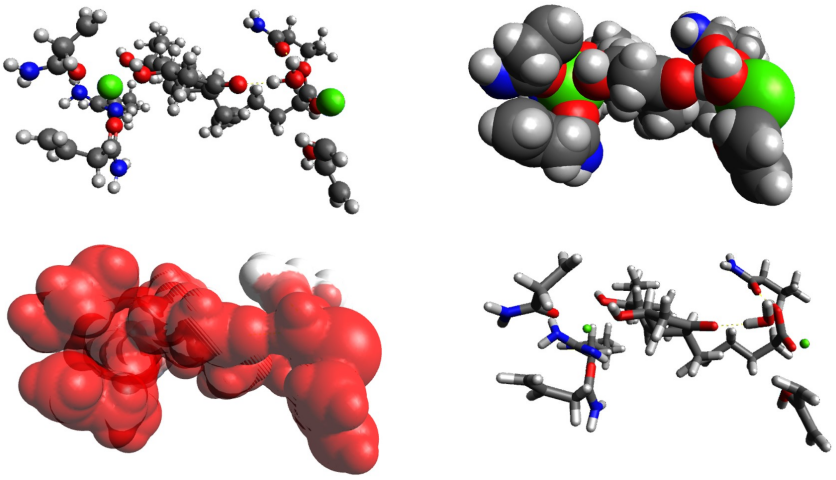


Figure 11: Several representations of the optimal position of two calcium ions at regions of high electrostatic potential for PGE2. It is seen that the Ca^{2+} ions stabilize the overall structure by distributing further the electron potential across the presenting area of the molecule when comes into contact with the representative GR molecular fits.

the calcium ions, there is a significant reduction in energy for both molecules, corresponding to −1,781 kJ/mol for CORT and −2,064 kJ/mol for PGE2. Thus PGE2 arrangement is more stable. A calculation for PGE2 without the presence of water molecule was evatuated, which was −1,961 kJ/mol, thus favoring the addition of the water molecule. Moreover, the

Groups C0 - C0	**CORT/Ca^{2+} Distance**	**PGE2/Ca^{2+} Distance**	**CORT − PGE2 Difference**
Thr208-Gln111	7.891 Å	12.094 Å	− 4.203 Å
Thr208-Arg80	22.683 Å	17.568Å	+ 5.115 Å
Thr208-Gln39	17.357 Å	13.980 Å	+ 3.377 Å
Thr208-Asn33	15.252 Å	13.390 Å	+ 1.862 Å
Gln111-Arg80	22.821 Å	13.453 Å	+ 9.278 Å
Gln111-Gln39	19.858 Å	15.656 Å	+ 4.202 Å
Gln111-Asn33	17.646 Å	18.115 Å	− 0.469Å
Gln39-Arg80	12.514 Å	12.621 Å	− 0.107 Å
Gln39-Asn33	3.906 Å	5.453 Å	− 1.547 Å
Arg80-Asn33	15.441 Å	13.275 Å	+ 2.166 Å

Table 4: . Comparison of the base carbon distribution for the LBD with two calcium ions positioned within the optimal locations, as determined through a force field of MMFF94. The difference is calculated and indicates that there is significant shrinkage in the volume of the GR LBD for PGE2 relative to CORT of 33.381%.

addition of the calcium ions was a significant stabilizing force, producing a reduction in energy levels by 79.160% for PGE2 and 94.202% for CORT, with PGE2 lower than CORT by 15.889%. In addition, the incorporation of a potassium ion was evaluated, and it was possible to incorporate two potassium ions at approximately the same location as calcium for both CORT and PGE2. However, the energy of the system was lower for calcium in comparison to potassium by approximately 15% indicating a preference for calcium.

There was a significant difference in overall size in this case. The spacing of the calcium ions was 16.010 Å for CORT while it was 11.521 Å for PGE2. In Table 4, the distances between the base LBD carbons is indicated. The average distance is 15.537 Å for CORT and 13.570 Å for PGE2, indicating a reduction in the size of the LBD for PGE2 by 1.967 Å. This corresponds to an estimated 33.381 % reduction in the volume for PGE2 from CORT, which visually is noticeable in the molecular models.

2.5 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 5 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 are capable of altering the intended rate of gene expression of CORT.

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 5: 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.

2.6 Pathway of Competitive Inhibition

In Figure 12, a basic relation describing the sequence from the introduction of active biomaterials to the resultant output of the glucocorticoid receptor, designated as U, is indicated. It begins with the Arachidonic acid, A, pathways to generate PGE2, P, via the cyclooxygenase enzyme, E. After PGE2 associates with the glucocorticoid receptor, G, it is in competition with CORT, C. The rates of the process will thus be influenced by the stability of PGE2 and CORT with GR, and based on the modeling results, would slightly favor PGE2. Additional analysis can be performed using a similar flow can be used for other prostaglandins, and for related materials to CORT, including synthetic materials, such as dexamethasone and hydrocortosone. Using this relation, mathematical models can be developed of the kinetics, which can be incoporated within overall material balances to predict the concentration variations and expressions over time.

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 competitive inhibition at the ligand binding domain of GR, and altered activity of CORT in the presence of PGE2. 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 interactive influence of PGE2 on CORT, and vice versa.

3 Discussion

The hypothesis of this research paper is that cortisol and prostaglandins, most notably PGE2, compete for the same functional binding site of the GR. This is important because

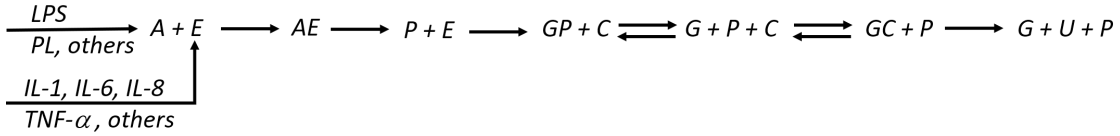


Figure 12: Relationship indicating the source of biomaterials, conversion to PGE2, and subsequent competitive inhibition with Cortisol at the GR. The source starts from Arachidonic Acid, which can come from the phospholipid bilayer or infectious agents, and the enzyme cyclooxygenase, which can be generated from the cytokines, for example. Either one in excess will produce PGE2, which then competes with CORT for the LBD. After association with CORT for sufficient time, the GR produces a controlled output U, such as mRNA in synthesizing new protein structures.

it thereby addresses a common point to multiple problems in physiology involving disease and stress, both physical and cerebral. Going forward, the results of this work enable a point of intersection for sources of the symptomology for a number of diseases as now the causative reason is known: cortisol is not fully expressed as intended, since PGE2 occupies some of the LBD sites of GR, and prevents the full and normative expression of the GR at both the decision center, such as the hypothalamus, as well as its target sites, effector cells.

There are many experiments in the research literature for which the hypothesis of this paper can be evaluated. For example, the study of [18] notes that chronic fatigue syndrome is correlated with low cortisol levels and HPA axis dysfunction, which is also cited in [19], noting that symptoms are enhanced with higher levels of stress. As indicated by this theory, hypocortisolism would be the result if PGE2 molecules occupying many LBD sites of the GR where cortisol nominally would reside, since the response of the hypothalamus will produce diminished levels of corticotropin releasing hormone because only a fraction of the GR responsible for its secretion are active, whereas the rest are ineffective due to interference of the competing PGE2 molecule. In addition, at the target site, the effects of diminshed actionable cortisol-GR associations will also produce sub-optimal effects.

Furthermore, it is of additional confirming evidence to note that our mathematical models, as defined in the supplemental material [1] predicted that body temperature variations can be explained by association of chemicals associated with cardiovascular and a viral components, in which the cardiovascular component was actively involved with the CNS control action in response to temperature variation. This led to the analysis of cortisol and PGE2, and therefore it would some too coincidental that the prediction based on an independent mathematical model would result in such a high level of molecular match between cortisol and PGE2. Furthermore, as these materials are biomarkers of immune and endocrine function with known association, there is a fundamental reason for a correlation, and this paper has uncovered it.

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 25.62%, 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 0.093 Å on average, and the average distance of the functional groups is closer for a low energy configuration of PGE2, achieving 1.760 Å, which on average corresponds to a difference of 6.232% . 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, most notably with the addition of calcium ions, which may make the difference in whether there is gene expression. The intermolecular spacings of the base carbons for the low energy LBD configuration were shifted by an average of 0.647 Å from their corresponding position had CORT been in the LBD. This corresponds to a reduction of 4.443% in the volume of the PGE2 LBD as compared to CORT. 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. There is more hydrogen bonding in the PGE2 configuration than that of CORT, especially at the carboxylic acid group. With the addition of calcium ions, a significant reduction in size of the PGE2 was 33.381% smaller, and the positioning of the carbon connections points of the LBD within the GR were greater, including 9.278 Å for one spacing, which may be signficant to prevent normative functioning of the LBD.

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 response, 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, therefore it is consistent that adrenal crisis due to 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; the dysfunctional processing of cortisol will amplify the signs and symptoms associated with prostaglandin concentrations due to disease. We believe these result to be of significant medical importance.

While it has been expressed as competitive influence of PGE2 on CORT, it is noted that there may be situations in which it is desirable for PGE2 to locally temper the response of CORT. For example, for situations in which there is local damage and inflammatory action is needed, it can be useful for PGE2 to occupy the GR site and locally reduce the effects of CORT by tempering its response. Thus, there is the potential that PGE2 and CORT achieve a symbiotic relation through regulation via the LBD of GR, dependent upon the localized concentrations of PGE2 to reduce the endocrine systemic signal of CORT.

As competitive inhibition of PGE2 and CORT has now been identified in this paper, what would be the next step - that is, what can be done about it. The first order of business would be to mathematically model the characteristics, and develop predictive techniques. With an analytic representation, treatment options can be developed by inverting the model to minimize deviations from normative function even in the presence of PGE2 generating factors, such as infections and disease[20].

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 [16] 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

Item	Cortisol	Prostaglandin E2 Conformer
Molecular Weight	362.46 g/mol	352.46 g/mol
C elements	21	20
H elements	30	32
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 6: 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.

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 6. 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 of the association was discovered in meeting the constraints as defined by the requirements of the mathematical model. In general, this type of protocol, developing the mathematics and then analyzing the chemistry, which will preclude the downfalls of curve-fitting, 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.

5 Competing Interests

There are neither competing interests nor funding sources to declare.

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