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

Charles D. Schaper, Ph.D.*

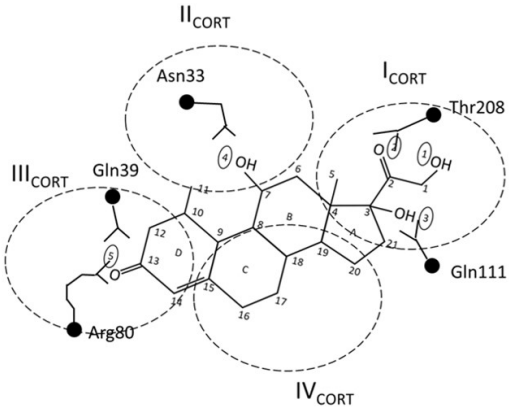
December 10, 2019

Abstract

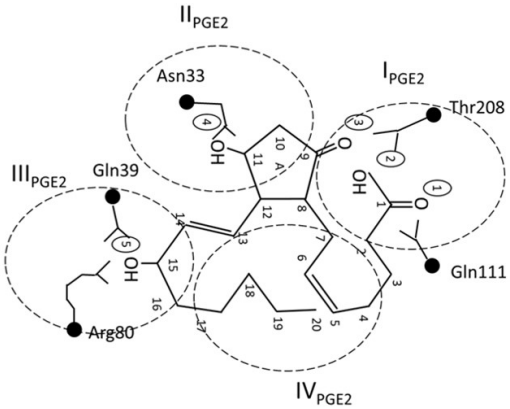
Here, competitive inhibition of cortisol by prostaglandins at the ligand binding domain of glucocorticoid receptors is shown for the first time. These biomaterials are widely distributed, as glucocorticoid receptors, cortisol and prostaglandins are found in almost every cell and tissue in the human body, and participate in a range of metabolic, neurologic, and immunity functions among others. Thus competitive inhibition preventing normative cellular processing of cortisol by the hormone associated with the inflammatory can explain the source of a significant number of ailments, signs, and symptoms of diseases. It is first noted that cortisol and prostaglandin E2 (PGE2) are of nearly the same molecular weight, and have nearly the same number of carbon, hydrogen and oxygen elements. Moreover, a conformational isomer of PGE2 positions the five oxygen-based functional groups at nearly the same position as the corresponding five groups of cortisol. Furthermore, the dimensional size and intermolecular spacing of the functional areas, all of which are located on the periphery of the molecules, is approximately the same. The core is hydrophobic for both molecules. And the double bonds positioned on the two branches of PGE2, combined with the five-carbon cyclic centering structure, enable PGE2 to position itself in the ligand binding domain equivalent to that of cortisol. The energy associated with PGE2 is at a lower energy state of cortisol, indicating preference in stability. A subsequent research article demonstrates that competitive inhibition of cortisol and PGE2 results in signs and symptoms of infectious agents, such as fever, chills, and fatigue.

*charlesschaper@comcast.net

Graphical Abstract



GR-Cort



GR-PGE2

1 Introduction

Cortisol is a very important hormone. It is released from the adrenal gland as directed by the hypothalamus, and results in increases in energy for use in metabolic consumption including muscular function and enhances the capability of the brain to utilize glucose. Further, cortisol is an integral part of the limbic system which is involved in the formation of thoughts that influence motivation, fear, and disposition. It is a so-called permissive hormone, in which it enhances the capability of the cells to utilize other hormones, such as epinephrine [1].

Because of its wide ranging physiological importance, dysfunction of the regulatory mechanisms of cortisol (CORT) can result in a significant ailments, including emotional factors such as depression. Cortisol, as the most abundant corticosteroid is generated via the HPA (hypothalamic-pituitary-adrenal) which has received significant attention in the research literature covering numerous functional relations [2, 3, 4, 5, 6, 7, 8]. The HPA axis involves the hypothalamic release of corticotropin-releasing factor (CRF) that binds to CRF receptors on the anterior pituitary gland, and in turn releases adrenocorticotropic hormone (ACTH). ACTH binds to receptors on the adrenal cortex and stimulates adrenal release of cortisol. To complete the feedback loop, the concentration of cortisol influences the hypothalamic release of CRF as well as the pituitary release of ACTH . The function of such corticosteroid action in the hypothalamus acts as a triggering mechanism [9] and synaptic potentiation have been noted [3]. In addition, cortisol has a significant influence not only on the hypothalamus but on most bodily functions at the organ level, such as renal function [10] and vasomotor properties.

Dysfunction of cortisol release results in many significant ailments: for example, too low cortisol levels due to misfunctioning adrenal gland can result in a crisis called adrenal insufficiency with symptoms that include fever, chills, fatigue, loss of appetite, stomach pain, body aches [11]. It is noted that these symptoms of adrenal insufficiency are similar to that of an infection, which is even at times misdiagnosed [12] as such. Thus, it indicates a potential relation between cortisol levels and symptoms due to infectious disease. Further, there may even be a connection with Alzheimer disease, in which it has been noted that elevated levels of glucocorticoids and HPA axis dysfunction are consistently observed in patients [13]. Further correlations can be inferred from the undulation of fever characterizing disease such as brucella [14] and the rise and fall of cortisol during the day and night [15] as well as respiratory systems during sleep [16, 17].

The glucocorticoid receptor (GR) is the receptor for cortisol, and this receptor is expressed in most every cell, tissue and organ in the body, including neural stem cells. It regulates genes that controls metabolism, development and the immune response. The glucocorticoid receptor resides in the cytosol and after the glucocorticoid receptor binds to

cortisol, the complex is activated. The GR thus functions as a hormone-regulated transcription regulatory factor involved in the expression of responsive genes, which likely represent up to ten percent of the genome that can be affected by the ligand-activated GR [18]. It has been noted to be involved in both pro-inflammatory and anti-inflammatory responses, and is involved in both basal and stress-related homeostasis.

Relevant to immune function, the prostaglandins are found in most tissues and organs, and are produced by almost all nucleated cells. They are autocrine and paracrine lipid mediators that act upon platelets, endothelium, uterine and mast cells. They are synthesized in the cellular response from the fatty acid arachidonic acid, which can be produced from phospholipids via phospholipase-A2, then brought to either the cyclooxygenase (COX) pathway or the lipoxygenase pathway.. Prostaglandins (PG) are a family of molecules that has received significant attention, but its association with cortisol has not been examined prior to this study, in which we show thus that the association with prostaglandins thereby provides a way in which cortisol can exhibit localized control even though it is uniformly distributed throughout the systems. Because of the wide distribution throughout cells and tissues of the three bioactive materials: cortisol, prostaglandin, and glucocorticoid receptors; the work developed in this paper is important as it demonstrates a link and controlling association amongst these group of materials that may be crucial to determining the integrative pathways of physiological function during normative and diseased states, and provides a pathway showing the link with stress. It is shown for the first time that configurations of the prostaglandins have a similar chemical affinity for the ligand binding domain of glucocorticoid receptors, which are prevalent in nearly every human cell.

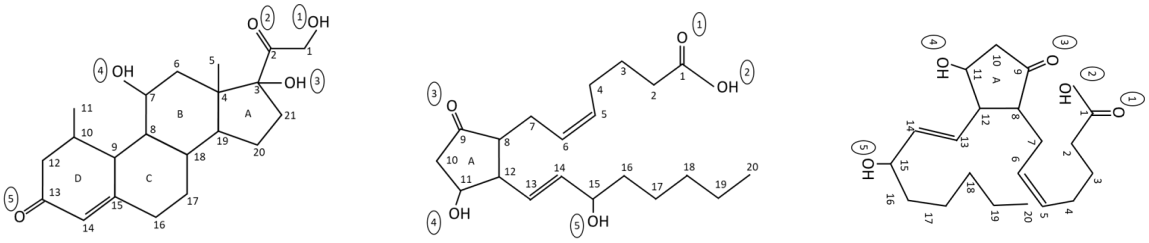
In this research article, the approach taken to show the association of PG with CORT and GR, is to first establish a molecular model comparing CORT and PGE2 in terms of molecular weight, chemical elements, functional groups, electrostatic metrics, hydrophobic interactions, hydrogen bonding capability, and topology. Additional analysis is performed by comparing with molecules closely related to CORT and PGE2, including dexamethasone and prednisone, as well as other types of prostaglandins.

2 Results

The association of PGE2 at GR is analyzed first through molecular models to evaluate equivalent chemical affinity with cortisol; thus competitive inhibition is established. Additional comparisons are drawn to closely related molecules of CORT and PGE2. Cellular response is then studied comparing the output signals from exogenous pulses of CORT and PG, and closed-loop analysis is then evaluated for systemic response.

2.1 Equivalent Affinity of CORT and PGE2

In Figure 1, the chemical structure of CORT and PGE2 is presented in the standard low-energy configurations. To make our case, we first note that CORT and PGE2 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 in five functional groups. When expressed in standard format outside of solution the structure of PGE2 is dissimilar to the ring structure of CORT; however through a rotation at C4 and C13 of PGE2, a structure resembling a set of rings is formed as indicated in Figure **??**. This structural expression maintains the same double bond arrangement of *cis* configuration on C5-C6 and *trans* configuration on C13-C14. It is also noted that 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 rotation and alignment, including out-of-plane alignment, at C6, C13, C18, the four ring structures of CORT can be approximated by PGE2, as within the core there is charge delocalization that will be favorable and through C20 lateral stability can be established. We note that in order for this alignment to occur, there needs to be a driving force, and as we will show, the ligand binding domain of the glucocorticoid receptor provides this driving force.


OH
OH
OH

in the relative positioning of the oxygen-related functional groups of the two molecules. The four ring structure of CORT is similar to a four ring structure approximated in PGE2 through resonance stabilization.

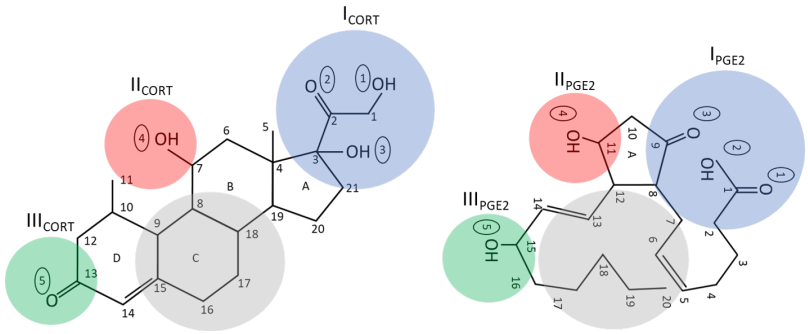


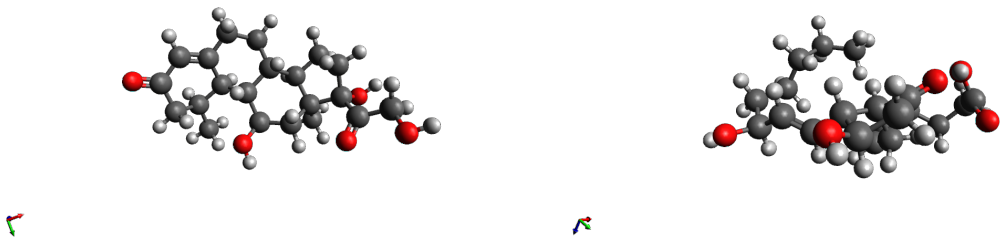
Figure 2: With the reconfiguration of PGE2 and with a rotation of approximately 45 degrees, the cortisol and PGE2 molecules are compared for the functional groups and relative positioning. It is noted that the three sites are correlated with I_{cort} and I_{cort} having three oxygen groups, with one 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.

To coordinate this new research on linking CORT and PGE2 at GR, we analyze the literature that involved detailed studies with molecules similar to CORT, namely dexamethasone (DEX) and prednisone (PRED). An analysis is instructive in terms of chemical affinity of DEX and PRED to relate their structural differences also to that of PGE2 to determine the functional groups where the association with the GR is strongest. From this information, a comparative analysis can be performed to estimate the chemical affinity of PGE2 at GR in comparison to CORT at GR. For example, DEX and PRED are generally more active over a longer period of time than CORT, and thus it can be inferred which functional group may be critical to the association with GR relative to the other functional groups. Using this comparative analysis, it can be inferred that the double bond at C10 and C12 is important to improving the hydrogen bonding capability at C13. This would indicate that PGE2 shows a higher affiliation for GR at C15 due to the hydroxyl group instead of the carbonyl corresponding to CORT.

2.2 Structural Comparison

To evaluate the positional relation and similarity of the functional groups between PGE2 and CORT, the wireframe sketches are developed as three dimensional models, as indicated

in Figure 3. The overall dimension of these structures is approximately $11.4\mathring{A} \times 5.0\mathring{A}$ for cortisol and $10.6\mathring{A} \times 4.7\mathring{A}$ for PGE2. The dimension for PGE2 can be adjusted by slightly positioning the two branches. Both cortisol and PGE2 have the same aspect ratio of 2.3.



(a) CORT (b) PGE2

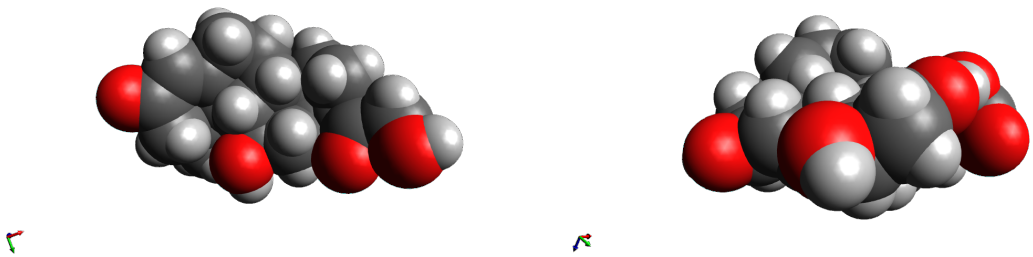
Figure 3: The three dimensional configuration of cortisol and prostaglandin E2. The spacing of the two functional groups of the five membered ring of PGE2 from C9-C11 is 4.681 Å *which is close to the corresponding position of CORT at C2-C7 of 4.657* Å*. This is crucial because this dimension can not 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.*

In Table 1, the distances between the five functional groups of cortisol and a conformation of prostaglandin E2 are compared. In general good agreement is indicated. Especially important, is the five membered ring of PGE2 corresponds to the critical 4-3 group, and it is noted that it is in agreement with that of CORT. This is critical not only because it connects the I and II functional groups, but also because the distance for 4-3 PGE2 is fixed due to the ring structure. The 5-4 and 3-2 groups of PGE2 can be adjusted to optimal distances because of the freedom of motion due to the braches. We note however that the trans and cis configurations of PGE2 aid in the positioning of the structure. The distance of 2-1 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.

In Figure 4 the van der Waals hard shell radius is applied to indicate the space filling notion of the cortisol and PGE2 conformer arrangements. It is noted that the periphery functional groups have similar presentation, which will be important as the PGE2 molecule assocates with the LBD. The relative size is similar and the hydrophobic nature of the elements is similar.

Molecule	5-4	4-3	3-2	2-1
CORT	5.634	4.657	2.592	4.274
PGE2	5.175	4.681	2.634	2.244

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 4-3 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 4-3 PGE2 is fixed due to the ring structure; whereas the 5-4 and 3-2 groups of PGE2 can be adjusted to optimal distances. The distance of 2-1 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

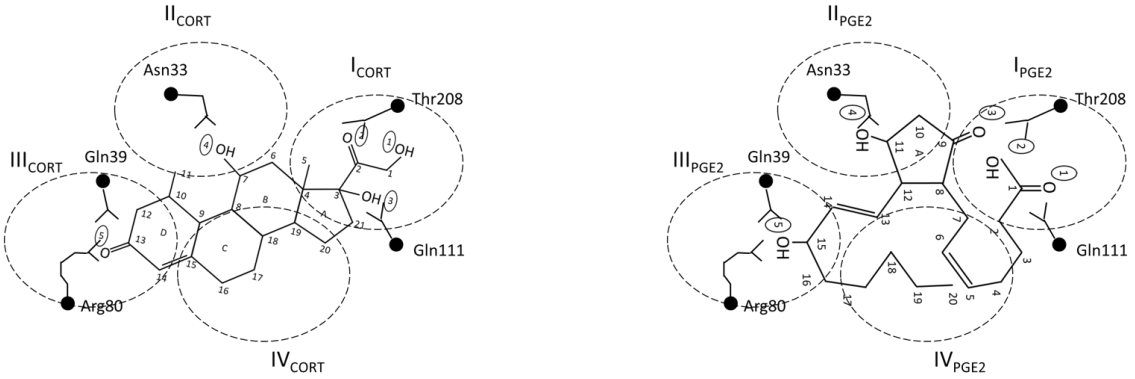
Recently, high resolution crystal structures of the ligand binding domain of glucocorticoid receptors have identified the amino acids responsible for the positioning of cortisol [19]. In this section, the approximate spatial relationship of PGE2 is developed based on the amino acids identified in the crystal structures.

In Figure 5, the approximate spatial positional relationship of PGE2 with GR, instead of CORT, is presented to indicate similarity between the molecular association of GR-PGE2 and GR-CORT. It is noted that the approximate dimensional arrangement of GR-PGE2 is appropriate, and may even be preferred in comparison to GR-CORT as $\mathrm{III}_{\mathrm{CORT}}$ requires the presence of water, whereas with $\mathrm{III}_{\mathrm{PGE2}}$ the hydroxyl group is available to interact directly with Gln39 and Arg80. In $\mathrm{I}_{\mathrm{PGE2}}$ 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 hydrogen bonding surrounding the core. As 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 carboxylic acid group of the first branch to bind to the associated GR functional groups of I_{PGE2} and the hydroxyl group on the second branch is driven to III_{PGE2} The C20 carbon is then positioned with the double bond groups of C5 and C6 of to produce the lowest energy state.

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 below the internal core of PGE2). The internal hydrophobic core for each of CORT and PGE2 includes six carbons.



(a) GR-Cortisol

(b) GR-PGE2

Figure 5: For the ancestral glucocorticoid protein receptor (AncGR2), which is a stable GR useful for crystal studies, at the ligand binding domain (LBD), a fit of PGE2 very similar is noted to that which is achieved with CORT as indicated in the inset. 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 (green shaded region) 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 (blue shaded region). 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 (gray shaded region), which incorporates two carbon-carbon double bonds. Thus the effectiveness of NSAIDs, which disrupts the formation of the C8-C12 bond, is apparent

The three dimensional positioning of cortisol and prostaglandin E2 as a conformer is exhibited in Figure 6. Both show similar interaction with the binding regions of the GR protein, in terms of spacing, which ranges from 1.5 Åto 4 Å, depending upon the particular positioning of the molecules. To establish the positioning a uniform force field was applied to the simulation, and the geometry optimized to minimize the energy level of the molecule. It converges to a local minimum as the positioning initially of the molecules influenced the ultimate convergence, particularly with respect to the position of the hydrogen groups. The overall energy of the PGE2 molecule was -1002 kJ/mol while that of cortisol was -917 kJ/mol, which indicates that the PGE2 is more stable, requiring more energy to separate.

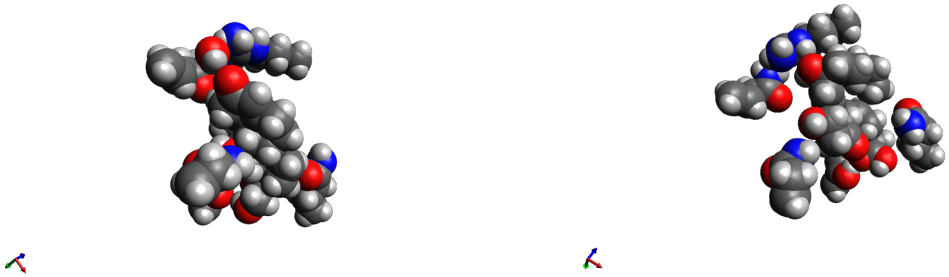
(a) GR-Cortisol (b) GR-PGE2

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.

The space filling models is presented in Figure 7 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 shows similar distance spacing.

In Figure 8, 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 motion towards the O5 group attached to C15. This indicates the potential for motion, and thus conformational variation of the GR relative to that associated with cortisol. The original positioning of cortisol was used in setting the dimension of Arg80 in the simulation. The distortion will cause potentially the GR to become non-expressive when connected with PGE2.

The wire frame for the configuration of PGE2 is depicted in Figure 9. It indicates a close association with the predicted sketches of the earlier section, which were done based on purely dimensional arguments. The universal force field 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 utlilized the minimize the energy levels. The



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

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 that enables interactive electrostatic positioning ranging from 1.5 Åto 4.5 Å.

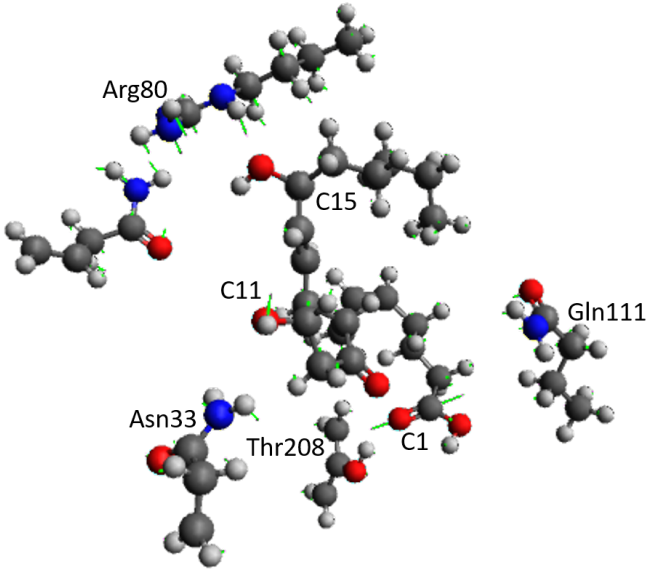


Figure 8: The force generated in this positional arrangement of PGE2 indicates potential motion in Arg80 towards O5 and with the carboxylic acid group towards Thr208. Thus it would be expected that the conformational arrangement of GR would be altered with the insertion of PGE2 at the LBD, instead of CORT.

cis and trans configurations of the molecule is 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

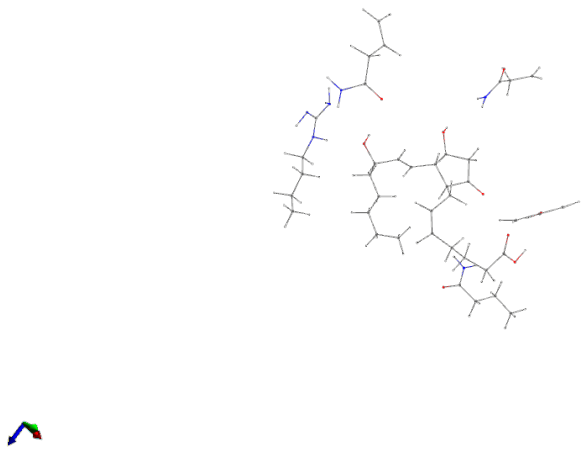


Figure 9: A wire frame model is used to indicate the relative positioning of the PGE2 conformer within the LBD. It is noted that the cis and trans configurations of the double bonds are maintained, and that the molecular model is similar to the approximate planar sketches presented in an earlier section.

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 PGE2 on CORT.

2.4 Chemical Affinity of Other Prostaglandins at LBD of GR

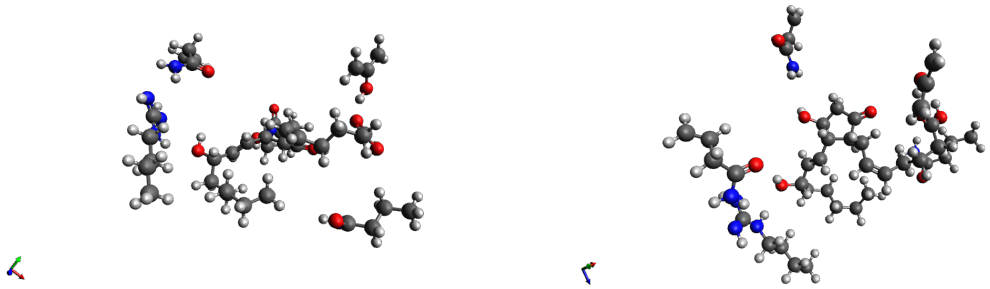
In the family of prostaglandins, other configurations besides PGE2 show similarity in chemical affinity in comparison to CORT, and thus a capability for intermolecular activity with the LBD of GR and therefore competitive inhibition with CORT. Table 2 lists several other prostaglandins, and indicates 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 are capable of altering the expression of CORT if PG was not present.

Related to the dynamics of introducing the PGE2 molecule into the slot of GR designated for CORT, Figure 10 is presented prior to the final configurational arrangement of the PGE2 conformer involving the positioning of the carboxylic acid group and the closure of the ring structure in the hydrophobic region. The linear structure of PGE2 indicates the three head functional groups which has spacing of 4.627 Å(OH to OH) and 4.640 Å(OH to O). 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 GR. Moreover, it is noted that the five-membered ring and the double bond carbon

Molecule	Formula	Difference from PGE2	Difference in Affinity at GR
PGE1	C20H34O5	C5-C6 single bond	$\Downarrow$ hydrophobic core
PGE3	C20H30O5	double bond at C17-C18	$\Downarrow$ C20 positioning
PGD2	C20H32O5	OH at C9; O at C11	$\Uparrow$ $\mathrm{I}_{\mathrm{PGE2}}$ hydrogen bonding $\Downarrow$ $\mathrm{II}_{\mathrm{PGE2}}$ hydrogen bonding
PGFα	C20H34O5	OH at C9	$\Uparrow$ $\mathrm{II}_{\mathrm{PGE2}}$ hydrogen bonding

Table 2: 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 improvments in PGE2 in hydrogen bonding as does PGFα, which may result in slightly different kinetic effects than PGE2.

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 structure in the final configuration. Hence, there is sufficient size for PGE2 to enter without the possiblity for out-of-plane hinderence from the upper and lower portions of the GR slot LBD.



(a) Entry Point (b) Hydrophobic Core

Figure 10: For PGE2, (a) Entry point within the GR and (b) top down view of PGE2 indicating the hydrophobic core at the ligand binding domain of the GR.

3 Discussion

This research has indicated for this first time, that at the GR there is competitive inhibition between CORT and PGE2. We believe that this relationship is important not only during normative physiological homeostasis in maintaining optimal localized control, but also the source of significant instances of dysfunction during diseased states. Because of the significance and distribution of the bioactive materials: specifically, a connection between GR, CORT, and PG is critical to the understanding of human physiology and internal control systems.

In this article, a significant relation is shown between configurations of molecular positioning of PGE2 within the ligand binding domain of GR that is normally occupied by CORT. Favorable agreement is achieved across multiple areas of comparison including molecular weight, functional groups, hydrogen bonding, hydrophobic positioning, and topology including overall dimensional and ring arrangement. The proposed PGE2 configuration at GR appears stable and comparable to CORT and its analogues including DEX and PRED. Other structures of PG including PGE1, PGE3, PGD2, and PGFα also show compatibility, and thus have the potential to exhibit similiar dysfunction at levels of the stress hormone CORT.

Thus, the situation of competitive inhibition is established, and the rate of hormone processing of CORT at GR will be altered by PG. 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, which due to its autocrine and paracrine activity, occupy both the intracellular and extracellular medium. This would influence neurotransmission function, as cortisol acts on synaptic potentiation of membrane bound receptors altering ion channel flow, thereby depolarizing the cell, and thus decreasing the threshold for action potential.

Stress is well known to play a major role in health, and here we have shown competitive inhibition of a hormone associated with the inflammatory response; therefore this provides a molecular basis for that concern. This produces competitive inhibition at nearly every cell, tissue and organ in the human body, preventing normative processing of the stress response when in a state of inflammation. Thus, there is high sensitivity to the presence of infectious agents when processing high levels of stress, and the likelihood for signs and symptoms of disease to manifest is amplified. A follow-on research articile will indicate that the competitive inhibition of PGE2 and cortisol results in flu-like symptoms including fever [20].

4 Methods

The methods used in this analysis were as follows:

- Molecular Modeling: The ligand binding domain (LBD) of the glucocorticoid recep-

Item	Cortisol	Prostaglandin E2
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
III functional group	O + H_2O	OH
Approximate Size C-C	8 x 5	8 x 5
Cyclical Stuctures	4	4

Table 3: Comparison of the chemical affinity of Cortisol and Prostaglandin E2 in a preferred configuration at the ligand binding domain of the glucocorticoid receptor.

tor for cortisol was identified in [19] from crystal studies, which also indicated the relative positioning of dexamethasone within the LBD. Using these arrangements as a basis for the positioning of the binding structures of the LBD, we analyzed PGE2 and separated the periphery of the structure into three areas of functional groups, and the core hydrophobic structure. We then organized the PGE2 molecule to match CORT as closely as possible in terms of hydrogen bonding, core ring structures and hydrophobic nature, electrostatic interaction, intramolecular interactions, and topography, while retaining the cis and trans positioning structure of its double bonds to achieve the following approximate comparative structure of Table 3. The simulation program Avogadro was used for the three dimensional molecular models. The supplemental information provides videos of CORT and PGE2 at the LBD of GR.

Bibliography

[1] J. E. Hall, *Guyton and Hall textbook of medical physiology.* Elsevier Health Sciences, 2015.

[2] M. N. Silverman, B. D. Pearce, C. A. Biron, and A. H. Miller, "Immune modulation of the hypothalamic-pituitary-adrenal (hpa) axis during viral infection," *Viral immunology*, vol. 18, no. 1, pp. 41–78, 2005.

[3] L. Groc, D. Choquet, and F. Chaouloff, "The stress hormone corticosterone conditions ampar surface trafficking and synaptic potentiation," *Nature neuroscience*, vol. 11, no. 8, p. 868, 2008.

[4] F. L. Groeneweg, H. Karst, E. R. de Kloet, and M. Joëls, "Mineralocorticoid and glucocorticoid receptors at the neuronal membrane, regulators of nongenomic corticosteroid signalling," *Molecular and cellular endocrinology*, vol. 350, no. 2, pp. 299–309, 2012.

[5] V. A. de Vrind, A. Rozeboom, I. G. Wolterink-Donselaar, M. C. Luijendijk-Berg, and R. A. Adan, "Effects of gaba and leptin receptor-expressing neurons in the lateral hypothalamus on feeding, locomotion, and thermogenesis," *Obesity*, 2019.

[6] D. J. Walker and K. A. Spencer, "Glucocorticoid programming of neuroimmune function," *General and comparative endocrinology*, vol. 256, pp. 80–88, 2018.

[7] C. Klein, W. Jonas, P. Wiedmer, S. Schreyer, L. Akyüz, J. Spranger, R. Hellweg, and B. Steiner, "High-fat diet and physical exercise differentially modulate adult neurogenesis in the mouse hypothalamus," *Neuroscience*, vol. 400, pp. 146–156, 2019.

[8] S. L. Padilla, J. G. Perez, M. Ben-Hamo, C. W. Johnson, R. E. Sanchez, I. L. Bussi, R. D. Palmiter, and O. Horacio, "Kisspeptin neurons in the arcuate nucleus of the hypothalamus orchestrate circadian rhythms and metabolism," *Current Biology*, vol. 29, no. 4, pp. 592–604, 2019.

[9] E. M. Viho, J. C. Buurstede, A. Mahfouz, L. L. Koorneef, L. T. van Weert, R. Houtman, H. J. Hunt, J. Kroon, and O. C. Meijer, "Corticosteroid action in the brain: the potential of selective receptor modulation," *Neuroendocrinology*, 2019.

[10] N. Shimizu, M. Hasumi, T. Hamano, M. Iijima, T. Yoshioka, Y. Yamazaki, and H. Sasano, "Renal primitive neuroectodermal tumor with elevated plasma adrenocorticotropic hormone levels: A case report," *IJU Case Reports*, vol. 2, no. 3, pp. 128–131, 2019.

[11] R. L. Rushworth, D. J. Torpy, and H. Falhammar, "Adrenal crisis," *New England Journal of Medicine*, vol. 381, no. 9, pp. 852–861, 2019.

[12] R. Dineen, C. J. Thompson, and M. Sherlock, "Adrenal crisis: prevention and management in adult patients," *Therapeutic advances in endocrinology and metabolism*, vol. 10, p. 2042018819848218, 2019.

[13] G. Canet, N. Chevallier, V. Perrier, C. Desrumaux, and L. Givalois, "Targeting glucocorticoid receptors: A new avenue for alzheimer's disease therapy," in *Pathology, Prevention and Therapeutics of Neurodegenerative Disease*. Springer, 2019, pp. 173–183.

[14] C. G. Ephrem, M. J. Matar, G. C. Chalhoub, and W. G. Greige, "Brucella endocarditis: diagnostic challenges." *Journal of Infection in Developing Countries*, vol. 12, 2018.

[15] S. S. Simons, R. Beijers, A. H. Cillessen, and C. de Weerth, "Development of the cortisol circadian rhythm in the light of stress early in life," *Psychoneuroendocrinology*, vol. 62, pp. 292–300, 2015.

[16] W. D. Todd, H. Fenselau, J. L. Wang, R. Zhang, N. L. Machado, A. Venner, R. Y. Broadhurst, S. Kaur, T. Lynagh, D. P. Olson *et al.*, "A hypothalamic circuit for the circadian control of aggression," *Nature neuroscience*, vol. 21, no. 5, p. 717, 2018.

[17] E. E. Benarroch, "Control of the cardiovascular and respiratory systems during sleep," *Autonomic Neuroscience*, 2019.

[18] T. Kino, "Glucocorticoid receptor," in *Endotext [Internet]*. MDText. com, Inc., 2017.

[19] X. Liu, Y. Wang, and E. A. Ortlund, "First high-resolution crystal structures of the glucocorticoid receptor ligand-binding domain–peroxisome proliferator-activated γ coactivator 1-α complex with endogenous and synthetic glucocorticoids," *Molecular Pharmacology*, vol. 96, no. 4, pp. 408–417, 2019.

[20] C. Schaper, "Identifying integrative molecular pathways for predictive modeling of infectious disease," 2019, *BioRxiv*: https://doi.org/10.1101/835546.