Journal of Steroid Biochemistry & Molecular Biology 128 (2012) 12–20

Contents lists available at SciVerse ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Variation in 3β-hydroxysteroid dehydrogenase activity and in pregnenolone supply rate can paradoxically alter androstenedione synthesis

Phuong T.T. Nguyen[a], Rita S.F. Lee[a,*], Alan J. Conley[b], James Sneyd[c], Tanya K. Soboleva[d]

[a] Agresearch Limited, Ruakura Research Centre, Private Bag 3123, Hamilton, New Zealand
[b] Department of Population Health and Reproduction, University of California, Davis, CA 95616, USA
[c] Department of Mathematics, University of Auckland, Private Bag 92019, Auckland, New Zealand
[d] Ministry of Agriculture and Forestry, PO Box 2526, Wellington, New Zealand

ARTICLE INFO

Article history:
Received 14 September 2011
Received in revised form
29 September 2011
Accepted 8 October 2011

Keywords:
Steroid synthesis
Mathematical model
Δ^5 pathway
Δ^4 pathway
Enzymatic control

ABSTRACT

The 3β-hydroxysteroid dehydrogenase/Δ^5–Δ^4 isomerase (3β-HSD) and 17α-hydroxylase/17,20-lyase cytochrome P450 (P450c17) enzymes are important in determining the balance of the synthesis of different steroids such as progesterone (P4), glucocorticoids, androgens, and estrogens. How this is achieved is not a simple matter because each of the two enzymes utilizes more than one substrate and some substrates are shared in common between the two enzymes. The two synthetic pathways, Δ^4 and Δ^5, are interlinked such that it is difficult to predict how the synthesis of each steroid changes when any of the enzyme activities is varied. In addition, the P450c17 enzyme exhibits different substrate specificities among species, particularly with respect to the 17,20-lyase activity. The mathematical model developed in this study simulates the network of reactions catalyzed by 3β-HSD and P450c17 that characterizes steroid synthesis in human, non-human primate, ovine, and bovine species. In these species, P450c17 has negligible 17,20-lyase activity with the Δ^4-steroid 17α-hydroxy-progesterone (17OH-P4); therefore androstenedione (A4) is synthesized efficiently only from dehydroepiandrosterone (DHEA) through the Δ^5 pathway. The model helps to understand the interplay between fluxes through the Δ^4 and Δ^5 pathways in this network, and how this determines the response of steroid synthesis to the variation in 3β-HSD activity or in the supply of the precursor substrate, pregnenolone (P5). The model simulations show that A4 synthesis can change paradoxically when 3β-HSD activity is varied. A decrease in 3β-HSD activity to a certain point can increase A4 synthesis by favouring metabolism through the Δ^5 pathway, though further decrease in 3β-HSD activity beyond that point eventually limits A4 synthesis. The model also showed that due to the competitive inhibition of the enzymes' activities by substrates and products, increasing the rate of P5 supply above a certain point can suppress the synthesis of A4, DHEA, and 17OH-P4, and consequently drive more P5 towards P4 synthesis.

1. Introduction

The primary crossroad in the synthesis of sex steroids and glucocorticoids involves two interconnected pathways (known as Δ^4 and Δ^5), both of which use pregnenolone (P5) as the precursor substrate, and two key enzymes, 3β-hydroxysteroid dehydrogenase/Δ^5–Δ^4 isomerase (3β-HSD) and 17α-hydroxylase/17,20-lyase cytochrome P450 (P450c17). In human, non-human primate, ovine, and bovine species, the 17,20-lyase activity of P450c17 is far more efficient in the conversion of the Δ^5-steroid 17α-hydroxy-pregnenolone (17OH-P5) into dehydroepiandrosterone (DHEA) than it is for the conversion of the Δ^4-steroid 17α-hydroxy-progesterone (17OH-P4) into androstenedione (A4) [1,2]. Consequently, in these species, A4, a substrate for estrogen formation, is synthesized efficiently only from DHEA through the Δ^5 pathway. The network involving the metabolism of P5, 17OH-P5, and DHEA by 3β-HSD activity, of P5 and progesterone (P4) by the 17α-hydroxylase activity, and of 17OH-P5 by the 17,20-lyase activity, as shown in Fig. 1, provides a simple starting point for studying the enzymatic control of steroid synthesis in these species. Although this network contains only five reactions and six metabolites (P5, P4, 17OH-P5, 17OH-P4, DHEA, and A4), the resulting steroidogenic profiles and fluxes are somewhat difficult to predict when enzyme or substrate availability is changed. This is because each of the two enzymes catalyzes the

Abbreviations: P450c17, 17α-hydroxylase/17,20-lyase cytochrome P450; 3β-HSD, 3β-hydroxysteroid dehydrogenase/Δ^5–Δ^4 isomerase; P5, pregnenolone; 17OH-P5, 17α-hydroxy-pregnenolone; P4, progesterone; 17OH-P4, 17α-hydroxy-progesterone; DHEA, dehydroepiandrosterone; A4, androstenedione; E2, 17β-estradiol.

* Corresponding author. Tel.: +64 7 838 5164; fax: +64 7 838 5628.
E-mail address: rita.lee@agresearch.co.nz (R.S.F. Lee).

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doi:10.1016/j.jsbmb.2011.10.003

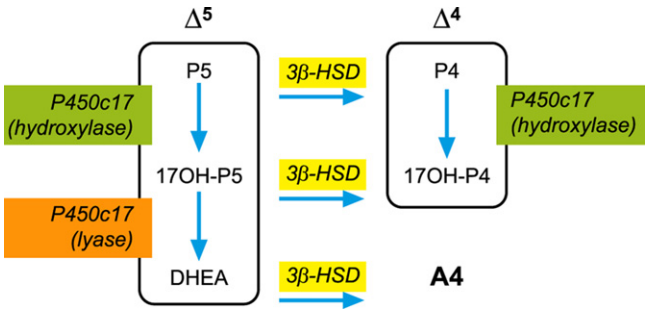

Fig. 1. Network of reactions catalyzed by 3β-HSD and P450c17 enzymes. This network is relevant for the human, non-human primate, ovine, and bovine species. The steroids involved are: P5, P4, 17OH-P5, 17OH-P4, DHEA A4. In these species, the 17,20-lyase activity of P450c17 with 17OH-P4 is negligible; hence, the conversion of 17OH-P4 into A4 is omitted.

metabolism of more than one substrate, and some of the substrates are shared in common by both enzymes. Consequently, several substrates compete for the common enzymes and the two enzymes also compete for common substrates. Additionally, 3β-HSD activity is inhibited by its own catalytic products [3,4].

Mathematical modelling is an effective tool to complement laboratory experiments aimed at understanding the complex behaviour of biochemical networks. To date, few models of steroidogenesis have been developed, and these utilized only simplified kinetics of steroidogenic reactions [5,6]. Nevertheless, these models were able to predict the effects of endocrine disrupting chemicals on steroid production by the fish ovary and human H295R cultured cells, wherein the steroid concentrations were low. However, the application of simplified kinetics is not appropriate when steroid concentrations are sufficiently high that the competitive inhibition of the enzyme activities by both substrates and products becomes significant and can be physiologically relevant. For example, in the human placenta, the inhibition of 3β-HSD activity by its catalytic product P4 was suggested to be important in the regulation of P4 synthesis [7–9]. We, therefore, have developed a more complete model of the reactions catalyzed by 3β-HSD and P450c17, based on known catalytic mechanisms and substrate specificity of the human, non-human primates, ovine, and bovine enzymes, summarized as follows. 3β-HSD catalyzes two sequential reactions, dehydrogenation and isomerisation, without release of the intermediate product and co-enzyme [10,11]. Although purified 3β-HSD is capable of catalyzing the reverse reaction of the dehydrogenation, the many-fold higher rate of the subsequent irreversible isomerisation makes the whole sequence of reactions effectively unidirectional [3]. From these sequences of reactions, Δ^5-3β-hydroxysteroids (P5, 17OH-P5, DHEA) are converted into Δ^4-3-ketosteroids (P4, 17OH-P4, A4, respectively). In a mixed substrate environment, substrates competitively inhibit each other for access to the enzyme [7,12]. Furthermore, the activity of 3β-HSD is inhibited by its own catalytic products [3,4]. P450c17 also exhibits dual enzymatic activities: 17α-hydroxylase and 17,20-lyase [11]. However unlike in the case of 3β-HSD, the intermediate substrate 17OH-P5, synthesized in the 17α-hydroxylase reaction, is released from the enzyme, and then re-binds P450c17 to be converted into DHEA in the 17,20-lyase reaction [13]. In species such as human, non-human primates, ovine, and bovine, P450c17 also catalyzes the 17α-hydroxylation of P4 into 17OH-P4 but there is virtually no cleavage of 17OH-P4 into A4 [1].

With the aim of understanding why inhibition of 3β-HSD can increase circulating levels of 17β-estradiol (E2) in rhesus monkey and sheep [14–16], we developed a model to test the hypothesis that when the 17,20-lyase activity of P450c17 on 17OH-P4 is negligible in species like these, the synthesis of A4, a substrate for estrogen formation, can be increased by decreasing 3β-HSD activity. In steroidogenic cells, both 3β-HSD and P450c17 are membrane bound, and 3β-HSD can be present in both the mitochondrial and microsomal compartments while P450c17 is present only in the microsomal compartment [11,17]. Additionally, some human tissues have two kinetically distinct 3β-HSD isoforms [18]. In this model developed for understanding the enzymatic control of steroid synthesis, we assume that both 3β-HSD and P450c17 are active in the same compartment, and there is only one isoform of 3β-HSD in the system.

2. Method

2.1. Model development

2.1.1. Rates of change in steroid concentrations

The model (Fig. 2) includes: (i) supply of P5 at a constant rate; (ii) reactions (1)–(3), catalyzed by 3β-HSD, that convert P5 into P4, 17OH-P5 into 17OH-P4, DHEA into A4, respectively; (iii) reactions (4), (6) and (5), catalyzed by P450c17, that convert P5 into 17OH-P5, P4 into 17OH-P4, and 17OH-P5 into DHEA, respectively; (iv) secretion of every steroid in the network. The rates of change in concentration of steroids are described by the following set of equations:

$$\frac{d}{dt}[\mathrm{P5}] = R_{\mathrm{supplyP5}} - V_1 - V_4 - C\cdot[\mathrm{P5}]$$

$$\frac{d}{dt}[\mathrm{17OHP5}] = V_4 - V_2 - V_5 - C\cdot[\mathrm{17OHP5}]$$

$$\frac{d}{dt}[\mathrm{DHEA}] = V_5 - V_3 - C\cdot[\mathrm{DHEA}]$$

$$\frac{d}{dt}[\mathrm{P4}] = V_1 - V_6 - C\cdot[\mathrm{P4}]$$

$$\frac{d}{dt}[\mathrm{P4}] = V_1 - V_6 - C\cdot[\mathrm{P4}]$$

$$\frac{d}{dt}[\mathrm{17OHP4}] = V_2 + V_6 - C\cdot[\mathrm{17OHP4}]$$

$$\frac{d}{dt}[\mathrm{A4}] = V_3 - C\cdot[\mathrm{A4}]$$

In these equations, [P5], [17OHP5], [DHEA], [P4], [17OHP4], and [A4] are the concentrations of P5, 17OH-P5, DHEA, P4, 17OH-P4, and A4, respectively. R_{SupplyP5} is the P5 supply rate, and V_i, $i = 1$–6, are the rates of reactions (1)–(6), respectively. The expressions for V_i, $i = 1$–6, were derived as described below. $C\cdot[\mathrm{P5}]$, $C\cdot[\mathrm{P4}]$, $C\cdot[\mathrm{17OHP5}]$, $C\cdot[\mathrm{17OHP4}]$, $C\cdot[\mathrm{DHEA}]$, $C\cdot[A4]$ are the rates of secretion of P5, P4, 17OH-P5, 17OH-P4, DHEA, A4, respectively. We assume that the rate of secretion of a steroid is linearly dependent on the concentration of the steroid, with a coefficient C that is common for all the steroids in the network.

2.1.2. Rates of reactions catalyzed by 3β-HSD

Conversion of a Δ^5-3β-hydroxysteroid into a Δ^4-3-ketosteroid by 3β-HSD is modelled as follows: a substrate (S) associates with the enzyme (E) to form an enzyme–substrate complex (E_S); subsequently the substrate is irreversibly converted into a product, to form an enzyme–product complex (E_P). As 3β-HSD is known to be competitively inhibited by its own products, we assume that after being formed, products reversibly dissociate from the active site of

enzyme and can therefore competitively inhibit substrates entering the enzyme's active site. The enzymatic steps are:

$$E + S \underset{k_{-s}}{\overset{k_s}{\rightleftharpoons}} E_S \xrightarrow{k'} E_P \underset{k_p}{\overset{k_{-p}}{\rightleftharpoons}} E + P$$

where k_s, k_{-s}, k_p and k_{-p} are association and dissociation rate constants and k' is the catalytic constant of the enzyme for the substrate. Maximum reaction rate is $V_m = k' \cdot e_0$, where e_0 is the enzyme concentration. Note that the maximum reaction rate in here is not exactly the same as the V_{max} normally reported in kinetic studies such as [18,19], which represents the catalytic activity of one milligram of enzyme. $V_{max} = k_{cat} \cdot e$, and has the unit of nmol/min/mg enzyme. In this case, e is the number of moles of the enzyme in one milligram of that enzyme. Like k', k_{cat} is the catalytic constant of the enzyme that is expressed as moles product per min per mole enzyme. Thus, similar to the catalytic constant, the V_{max} value characterizes the catalytic capacity of the enzyme. The maximum reaction rate in here, $V_m = k' \cdot e_0$, on the other hand, represents the total activity of the enzymes, and depends on the enzyme concentration (e_0) which is expressed as moles enzyme per liter (L). In this study, V_m is expressed as micromoles product per second per L, i.e. μmol/s/L or μM/s.

In a mixed substrate environment, reactions (1)–(3) in Fig. 2 compete with each other for 3β-HSD. The respective maximum reaction rates of 3β-HSD in these reactions are $V_{mi} = k'_i \cdot e_0$, $i = 1,2,3$. Based on the above arguments, it could be deduced that although the maximum reaction rates are not strictly V_{max}, the ratio of V_{mi} (V_{m1}:V_{m2}:V_{m3}) is equal to the ratio of the catalytic constants, k'_i because the concentration of the enzyme (e_0) is the same for each of these reactions, and this in turn, is equal to the ratio of V_{max} values of 3β-HSD for P5, 17OH-P5, and DHEA, respectively.

The maximum reaction rates of 3β-HSD in the reactions (1)–(3) will be the same, i.e. $V_{m1} = V_{m2} = V_{m3} = V_{m3\beta\text{-HSD}}$, when the V_{max} values for the three substrates are similar, as in the case of human 3β-HSD type 1, wherein the V_{max} values for P5, 17OH-P5, DHEA are 45.7, 42.5, 43.3 nmol/min/mg, respectively [18]. This reduces the number of parameters required, as $V_{m3\beta\text{-HSD}}$ alone can represent V_{mi}, $i = 1,2,3$, and the change in 3β-HSD activity can be described by variation in the value of the $V_{m3\beta\text{-HSD}}$ parameter. Applying the equilibrium approximation (Appendix A), the rates of reactions (1)–(3) are:

$$V_1 = \frac{V_{m3\beta\text{-HSD}} \cdot [P5]}{K_{m1} \cdot \left(1 + ([17OHP5]/K_{m2}) + ([DHEA]/K_{m3}) + ([P4]/K_{p1}) + ([17OHP4]/K_{p2}) + ([P4]/K_{p3})\right) + [P5]}$$

$$V_2 = \frac{V_{m3\beta\text{-HSD}} \cdot [17OHP5]}{K_{m2} \cdot \left(1 + ([P5]/K_{m1}) + ([DHEA]/K_{m3}) + ([P4]/K_{p1}) + ([17OHP4]/K_{p2}) + ([A4]/K_{p3})\right) + [17OHP5]}$$

$$V_3 = \frac{V_{m3\beta\text{-HSD}} \cdot [DHEA]}{K_{m3} \cdot \left(1 + ([17OHP5]/K_{m2}) + ([P5]/K_{m1}) + ([P4]/K_{p1}) + ([17OHP4]/K_{p2}) + ([A4]/K_{p3})\right) + [DHEA]}$$

K_{mi}, $i = 1,2,3$ and K_{pi}, $i = 1,2,3$ are affinities of 3β-HSD for substrates P5, 17OH-P5, DHEA, and products P4, 17OH-P4, A4, respectively. These reaction rates illustrate that the metabolism of a substrate is competitively inhibited by the other substrates and by all the products. Furthermore, the inhibition parameter K_i of a substrate to the metabolism of the other substrates is equal to the affinity of 3β-HSD for it (K_m). Thus, this model of the 3β-HSD reactions is consistent with the experimental results in [7,12] which showed that the inhibition parameter K_i of a substrate to the metabolism of the other substrates is not significantly different from the affinity (K_m) of 3β-HSD for that substrate. Similarly, the inhibition parameter (K_i) of a product to the reactions

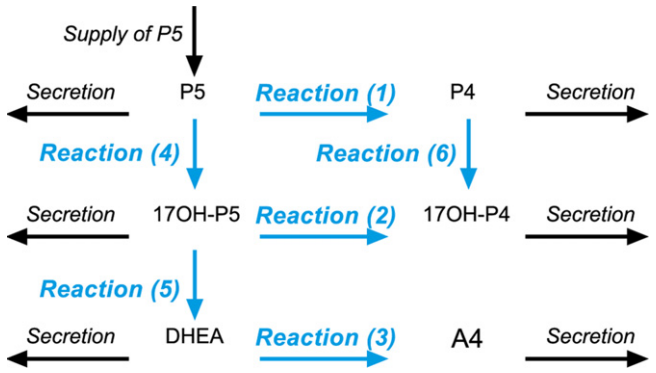

Fig. 2. A model of steroid synthesis for human, non-human primate, ovine, and bovine species. The model includes: supply of P5 at a constant rate; reactions (1)–(3) catalyzed by 3β-HSD, reactions (4)–(6) catalyzed by P450c17, secretion of P5, P4, 17OH-P5, 17OH-P4, DHEA, A4.

catalyzed by 3β-HSD is equal to the affinity of the enzyme for the product.

When the V_{max} values for P5, 17OH-P5, DHEA are not similar, the values of α and β in the formulas $V_{m1} = \alpha \cdot V_{m3\beta\text{-HSD}}$, $V_{m2} = \beta \cdot V_{m3\beta\text{-HSD}}$, $V_{m3} = V_{m3\beta\text{-HSD}}$ could be deduced from the ratio of these V_{max} values. For example, when V_{max} values for P5, 17OH-P5, and DHEA, respectively are 82.9, 60.6, 81.7 nmol/min/mg, i.e. similar to those of the human 3β-HSD type 2 reported in [18], $\alpha = 1.01$, $\beta = 0.74$. In this case, rates of reactions (1)–(3) are αV_1, $\beta \cdot V_2$, V_3, respectively, where V_1, V_2, V_3 are the same as above. In the case of human 3β-HSD type 1, $\alpha \approx \beta \approx 1$. Thus, changes in the concentration of 3β-HSD or changes that equally affect the catalytic capacity of 3β-HSD for the three substrates are still described by change in the value of $V_{m3\beta\text{-HSD}}$ parameter.

2.1.3. Rates of reactions catalyzed by P450c17

The transformation of P5 into DHEA by P450c17 through two reactions, in which DHEA is essentially produced from the released intermediate 17OH-P5 [13], is modelled as follows. Firstly, P5 associates with P450c17 (E^1) to form an enzyme–substrate complex (C_4), then the 17α-hydroxylase activity irreversibly converts P5 into 17OH-P5, and 17OH-P5 is irreversibly released from the enzyme. Combining the two irreversible elemental steps, the reaction (4) becomes:

$$E^1 + P5 \underset{k_{-s_4}}{\overset{k_{s_4}}{\rightleftharpoons}} C_4 \xrightarrow{k'_4} E^1 + 17OHP5$$

The released 17OH-P5 subsequently re-associates reversibly with the enzyme, forming another enzyme–substrate complex. 17OH-P5 is then converted into DHEA by the 17,20-lyase activity. The reaction (5) is:

$$E^1 + 17OHP5 \underset{k_{-s_5}}{\overset{k_{s_5}}{\rightleftharpoons}} C_5 \xrightarrow{k'_5} E^1 + DHEA$$

The reaction (6) is:

$$E^1 + P4 \underset{k_{-s_6}}{\overset{k_{s_6}}{\rightleftharpoons}} C_6 \xrightarrow{k'_6} E^1 + 17OHP4$$

In this model, 17OH-P4 is the end product of P450c17 in the Δ^4 pathway. The catalytic constant, and consequently the maximum reaction rates of 17α-hydroxylase activity for both P5 and P4 are the same when applying the model to the human-type enzymes, as the reported V_{max} of P450c17 for P5 and P4 are similar [21]. Applying the equilibrium approximation (Appendix B), the rates of reactions (4)–(6) are:

$$V_4 = \frac{V_{mHyd} \cdot [P5]}{K_{m4} \cdot \left(1 + ([P4]/K_{m6}) + ([17OHP5]/K_{m5})\right) + [P5]}$$

$$V_5 = \frac{V_{mLyase} \cdot [17OHP5]}{K_{m5} \cdot \left(1 + ([P4]/K_{m6}) + ([P5]/K_{m4})\right) + [17OHP5]}$$

$$V_6 = \frac{V_{mHyd} \cdot [P4]}{K_{m6} \cdot \left(1 + ([P5]/K_{m4}) + ([17OHP5]/K_{m5})\right) + [P4]}$$

K_{mi}, i = 4,5,6 are the affinities of P450c17 for P5, 17OH-P5 and P4, respectively. V_{mHyd}, V_{mLyase} are maximum reaction rates of 17α-hydroxylase and 17,20-lyase activities, respectively. Thus, the rates of reactions (4)–(6) do not contain inhibition by the catalytic products. Note that if the mechanism of the transformation from P5 into DHEA was similar to that reported in [22], i.e. DHEA is formed from the subsequent 17,20-lyase reaction of a fraction of 17OH-P5 that does not dissociate from the active site of P450c17 after being formed, the expressions of V_4, V_5, V_6 would still be the same as above.

2.2. Dynamic simulations, steady-state analysis, and sensitivity analysis

The model's variables are: [P5], [17OHP5], [DHEA], [P4], [17OHP4], and [A4]—concentration of the steroids P5, 17OH-P5, DHEA, P4, 17OH-P4, and A4, respectively. The model parameters are: K_{m1}, K_{m2}, K_{m3}, K_{p1}, K_{p2}, and K_{p3}—affinities of 3β-HSD for P5, 17OH-P5, DHEA, P4, 17OH-P4, and A4, respectively, K_{m4}, K_{m5}, and K_{m6}—affinities of P450c17 for P5, 17OH-P5, and P4, respectively, $V_{m3β\text{-}HSD}$, V_{mHyd}, and V_{mLyase}—maximum reaction rates of 3β-HSD, 17α-hydroxylase, and 17,20-lyase, respectively, $R_{supplyP5}$—P5 supply rate, and C—secretion rate coefficient. As affinities are often reported in μM (μmol/L), steroid concentrations and affinities in this model are expressed in μM. As a consequence, the maximum reaction rates and the rate of P5 supply have units of μM/s (μmol/L/s or μmol/s/L), and the secretion rate coefficient, units of 1/s.

In a dynamic simulation, all parameters are held constant while variables (steroid concentrations) change with time, from an initial state towards the steady-state. In our steady-state analysis, the impact of variation in 3β-HSD activity (which is simulated by the variation in the value of the $V_{m3β\text{-}HSD}$ parameter) or in the rate of P5 supply (which is simulated by the variation in the value of the $R_{supplyP5}$ parameter) on the steady-state steroid concentrations is analysed. The robustness of the qualitative results obtained in the steady-state analysis is tested in sensitivity analysis. When changes in the absolute values of parameters do not alter a qualitative result, the qualitative result is robust against parameter changes.

The computation of this model was implemented on Matlab (version 7.0, The Mathworks), using the ode23s solver.

Table 1
Values of the enzyme affinity parameters.

Affinity	Enzyme	Substrate or product	Value (μM)
K_{m1}	3β-HSD	P5	2.8
K_{m2}		17OH-P5	3.5
K_{m3}		DHEA	3.7
K_{p1}		P4	3.0
K_{p2}		17OH-P4	3.0
K_{p3}		A4	1.0
K_{m4}	P450c17	P5	0.79
K_{m5}		17OH-P5	0.83
K_{m6}		P4	0.73

3. Results

3.1. Setting parameter values

Analysis shows that the time to reach steady-state from any initial state is most sensitive to the secretion rate coefficient (parameter C), and is generally insensitive to the absolute values of the other parameters. As the time required for biochemical systems to reach steady-state is often in the range of seconds to minutes, we deduce that reasonable values of the parameter C are between 0.01 s^{-1} and 1 s^{-1}. In this study, simulations are run with the parameter C set at 0.1 s^{-1}, i.e. the time for reaching steady-state is about 100 s.

In our steady-state analysis, either $V_{m3β\text{-}HSD}$ or $R_{supplyP5}$ is varied within a range that should generally cover the likely physiological range of 3β-HSD activity and P5 supply rate, respectively. However, it is not a simple matter to deduce the feasible range of $V_{m3β\text{-}HSD}$ in the units μM/s (rate/L) from reported 3β-HSD activity measured in tissues extracts, which is normally expressed as rate/mg protein [23,24]. Similarly, there are no reliable data available for estimating the feasible range of the P5 supply rate. Therefore, $V_{m3β\text{-}HSD}$ and $R_{supplyP5}$ were varied within sufficiently wide ranges that allow for the capture of all qualitative behaviours.

As shown in Sections 3.3 and 3.4, all the qualitative results obtained in the steady-state analysis conducted in this study are robust against changes in the absolute values of the parameters V_{mHyd}, V_{mLyase}, K_{mi}, i = 1–6 and K_{pi}, i = 1,2,3. Thus, although the ratio of 17α-hydroxylase to 17,20-lyase activity (which is described by the ratio V_{mHyd}:V_{mLyase}) can vary among species, tissues, and depend on the availability of co-factors [25], any difference in this ratio do not affect the qualitative results obtained in this study. Based on published data [21,25], we set V_{mHyd}:V_{mLyase} = 3:1. Similarly, the affinities of enzymes for substrates vary among enzyme isoforms or species. However, the robustness of the qualitative results against changes in the K_{mi}, i = 1–6 and K_{pi}, i = 1,2,3 means that any differences or uncertainties in the enzymes' affinities have no bearing on the qualitative results obtained in this study. Therefore, the values of the affinity parameters were chosen from some published values of the human, non-human primates, ovine, or bovine enzymes, as shown in Table 1. K_{m1}, K_{m2}, K_{m3} are similar to the affinities of the human 3β-HSD type 1 for substrates P5, 17OH-P5, and DHEA, as published in [18]. K_{p1}, K_{p2}, K_{p3} were chosen based on following assumptions and evidence: (a) the affinities of 3β-HSD for its catalytic products are in the same range as that for its substrates; (b) the human placental 3β-HSD has about 3-fold higher affinity for A4 than for P4 [3]; and (c) the affinity of 3β-HSD for 17OH-P4 is similar to that for P4. K_{m4}, K_{m5}, K_{m6}, are similar to the affinities of the human P450c17 enzyme for substrates P5, 17OH-P5, and P4, as published in [21].

3.2. A dynamic simulation

Fig. 3 shows the dynamics of steroid concentrations in a simulation example. In this example, steroids have zero

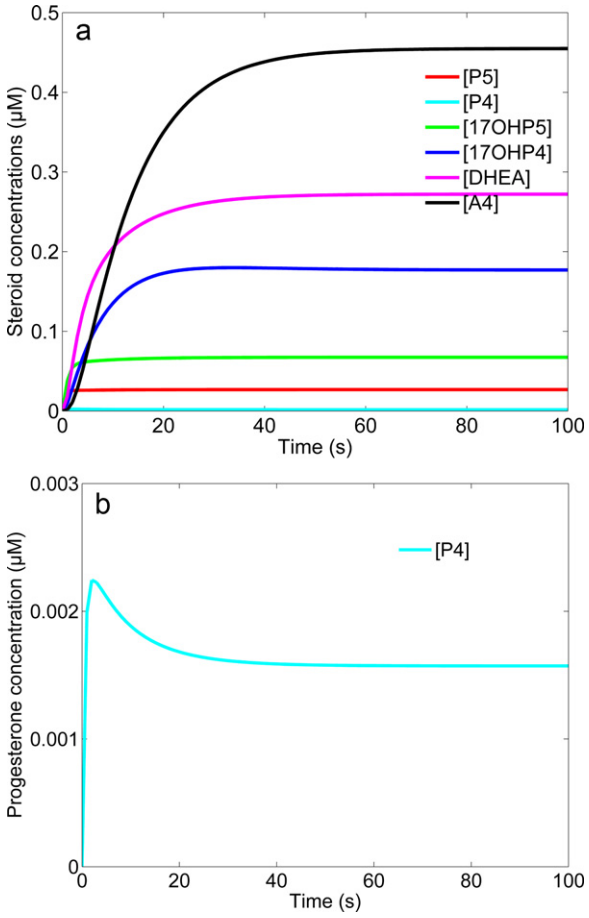

Fig. 3. A dynamic simulation of steroid concentrations, from an initial state towards steady-state. (a) Dynamics of [P5], [P4], [17OHP5], [17OHP4], [DHEA], and [A4]. (b) Dynamics of only [P4]. The model parameter values used in this example are: $V_{m3\beta\text{-}HSD} = 1\ \mu M/s$, $V_{mHyd} = 3 \cdot V_{mLyase} = 3\ \mu M/s$, $R_{supplyP5} = 0.1\ \mu M/s$, $C = 0.1\ s^{-1}$, the affinity parameters (K_{mi}, $i = 1$–6, K_{pi}, $i = 1$–3) are as in Table 1. At the initial state, all the steroids have zero-concentration.

concentration at initial state. The secretion rate coefficient and affinity parameters are as described in Section 3.1, and $V_{m3\beta\text{-}HSD} = 1\ \mu M/s$, $V_{mHyd} = 3 \cdot V_{mLyase} = 3\ \mu M/s$, $R_{supplyP5} = 0.1\ \mu M/s$. Under this parameter setting, where $K_{m4} < 1/3.K_{m1}$ (the affinity of P450c17 for P5 is several times higher than the affinity of 3β-HSD for P5), and $V_{mHyd} > V_{m3\beta\text{-}HSD}$ (the activity of P450c17 is higher than that of 3β-HSD), P450c17 out-competes 3β-HSD for P5, thereby the flux down the Δ^5 pathway is favoured. As a consequence, the concentration of P4 throughout the dynamics course is much lower than that of the other steroids. Therefore, the details in the dynamics of P4 concentration are not discernable when the dynamics of all six steroids are shown together in the same graph (Fig. 3a). Because of this, the dynamics of P4 concentration is shown separately (Fig. 3b).

The figures illustrate that concentrations of all steroids, except P4, increase with time until steady-state values are reached at about 100 s. The concentration of P4 initially increases to a peak value and then decreases until the steady-state value is reached (Fig. 3b). Note that the dynamics will be different if the simulation is run from another initial state; however, the steady-state concentrations attained for all steroids remain the same. Thus, the concentrations of steroids attained at the steady-state are determined by the parameter settings of the model system, such as the rate of P5 supply, the levels of enzymes' activities, the enzymes' affinities for steroids, and the secretion rate coefficient, but are independent of the initial state.

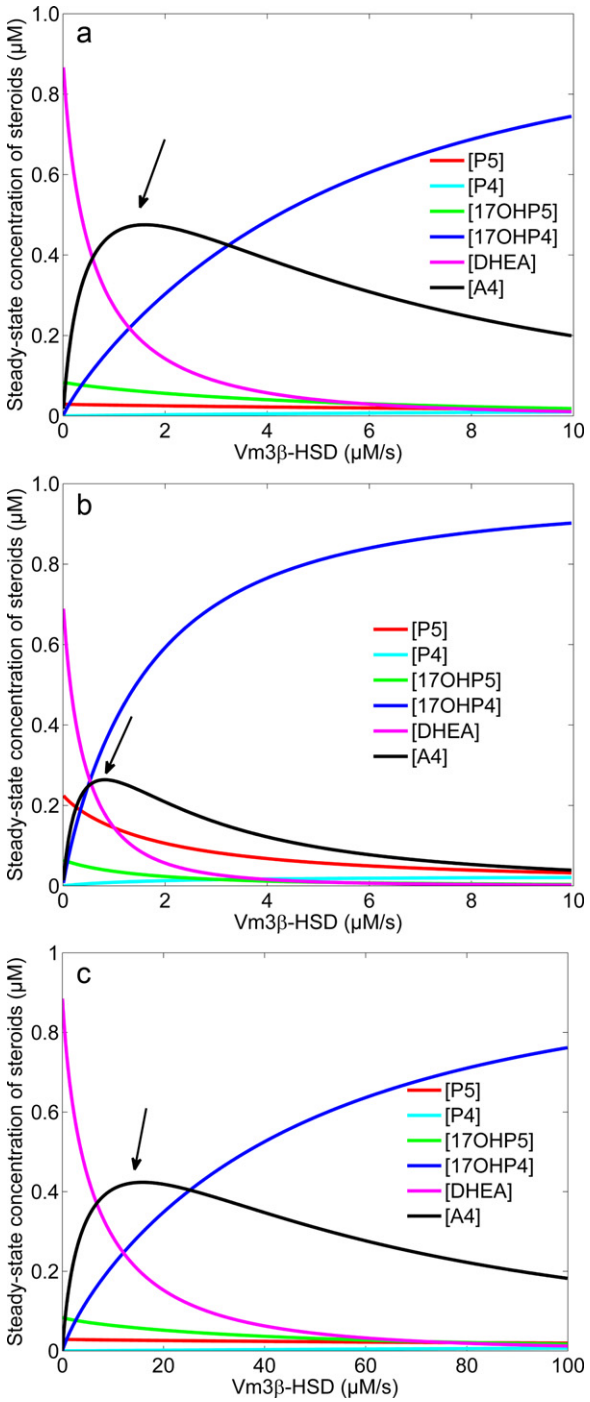

Fig. 4. Changes in steady-state concentrations of steroids with varying 3β-HSD activity. In (a), $V_{m3\beta\text{-}HSD}$ is varied from 0 μM/s to 10 μM/s, and values of the parameters, other than $V_{m3\beta\text{-}HSD}$, are the same as those under Fig. 3. At the turning point of A4 synthesis: $V_{m3\beta\text{-}HSD} = 1.62\ \mu M/s$; [A4] = 0.48 μM. In (b), K_{m4} value (7.9 μM) is 10 times higher than that in (a). At the turning point of A4 synthesis, $V_{m3\beta\text{-}HSD} = 0.83\ \mu M/s$, [A4] = 0.26 μM. In (c), the modifications and rates of reactions (1)–(3) characterize the human 3β-HSD type 2 enzyme (see Section 2.1.2); $K_{m1} = 49.5\ \mu M$, $K_{m2} = 17.8$, $K_{m3} = 47.3\ \mu M$; and $V_{m3\beta\text{-}HSD}$ is varied in the range from 0 μM/s to 100 μM/s. At the turning point of A4 synthesis: $V_{m3\beta\text{-}HSD} = 16.01\ \mu M/s$, [A4] = 0.42 μM. The turning points of A4 synthesis in (a–c) are indicated by the arrows.

3.3. The impact of varying 3β-HSD activity

Results of the steady-state analysis in which the parameter $V_{m3\beta\text{-}HSD}$ is varied from 0.01 μM/s to 10 μM/s, a range wide enough to capture all qualitative behaviours, are shown in Fig. 4a. The

Table 2
$V_{m3\beta\text{-HSD}}$ value of the turning point in models with different human 3β-HSD types.

Enzyme	K_{m1} (μM)	K_{m2} (μM)	K_{m3} (μM)	Ratio V_{m1}:V_{m2}:V_{m3}	$V_{m3\beta\text{-HSD}}$ of the turning point (μM/s)
Type 1	2.8	3.5	3.7	45.7:42.5:43.3, i.e., ~ 1:1:1	1.62
Type 2	49.5	17.8	47.3	82.9:60.6:81.7, i.e., 1.01:0.74:1	16.01

The respective values of K_{m1}, K_{m2}, K_{m3}, of human 3β-HSD type 1 and type 2 enzymes are from [18]. The ratio V_{m1}:V_{m2}:V_{m3}: is deduced from the ratio reported in [18] for the V_{max} values of the enzyme types for substrates P5, 17OH-P5, and DHEA, respectively.

values of the parameters, other than $V_{m3\beta\text{-HSD}}$ are the same as in the simulation example in Section 3.2. The curves illustrate that when 3β-HSD activity is decreased, the synthesis of P4 and 17OH-P4 both decrease as expected, while that of A4 varies paradoxically. A decrease in 3β-HSD activity up to a certain point increases A4 synthesis, but a further decrease in 3β-HSD activity beyond that point rapidly decreases A4 synthesis.

A sensitivity analysis shows that these qualitative results are robust against changes in the absolute values of the model's parameters, other than $V_{m3\beta\text{-HSD}}$. For example, when increasing K_{m4} value by 10-fold, from 0.79 μM to 7.9 μM, i.e. decreasing the affinity of P450c17 for P5 to one tenth, all the qualitative results described above remain unchanged (Fig. 4b). A decrease in 3β-HSD activity still results in a decrease in P4 and 17OH-P4 synthesis while A4 synthesis can either increase or decrease, depending on whether the 3β-HSD activity is higher or lower than a certain level (Fig. 4b), which we call the turning point of A4 synthesis. Quantitatively, the $V_{m3\beta\text{-HSD}}$ value at the turning point, which describes the 3β-HSD activity at that point, depends on the absolute values of the other parameters. For instance, when increasing K_{m4} from 0.79 μM to 7.9 μM, the $V_{m3\beta\text{-HSD}}$ value at the turning point is decreased nearly two-fold, from 1.62 μM/s to 0.83 μM/s, (Fig. 4a,b). This 10-fold increase in the value of K_{m4} also lowered the steady-state concentration of A4 at the turning point from 0.48 μM to 0.26 μM (Fig. 4a,b). Similarly, all the above described qualitative results remain unchanged when K_{m1}, K_{m2}, K_{m3} are changed from those resembling the affinities of human 3β-HSD type 1 enzyme to that resembling the affinities of human 3β-HSD type 2 (Table 2), and the rates of reactions (1)–(3) are written in the form that characterizes the human 3β-HSD type 2, which are $\alpha \cdot V_1$, $\beta \cdot V_2$, V_3, where $\alpha = 1.01$, $\beta = 0.74$ (see Section 2.1.2), (Fig. 4c). However, the $V_{m3\beta\text{-HSD}}$ value at the turning point of A4 synthesis is increased by about 10-fold (Table 2). Because of this, the range of $V_{m3\beta\text{-HSD}}$ values on the horizontal axis in Fig. 4c is expanded, compared with Fig. 4a and b, so that all the qualitative behaviours can be captured. Similarly, the turning point of A4 synthesis, when $V_{m3\beta\text{-HSD}}$ values are varied, is still present when $R_{supplyP5}$, V_{mHyd}, or V_{mLyase} is increased; however the absolute value of $V_{m3\beta\text{-HSD}}$ at this point increases.

3.4. The impact of varying P5 supply rate

Results of the steady-state analysis in which $R_{supplyP5}$ is varied between 0.01 and 10 μM/s, a sufficiently wide range to capture of all qualitative results, are shown in Fig. 5. Parameters other than $R_{supplyP5}$ are the same as in the simulation example in Section 3.2. The curves illustrate that increasing the P5 supply rate initially increases the synthesis of all the steroids; however, when the P5 supply rate reaches a certain level, further increasing this rate will firstly suppress the synthesis of A4, then DHEA, and lastly, 17OH-P4. In contrast, increasing P5 supply rate resulted in a continual increase in P4 production.

A sensitivity analysis also shows that changing the values of the parameters, other than $R_{supplyP5}$, do not change the qualitative results described above, i.e. these qualitative results are robust against parameter changes.

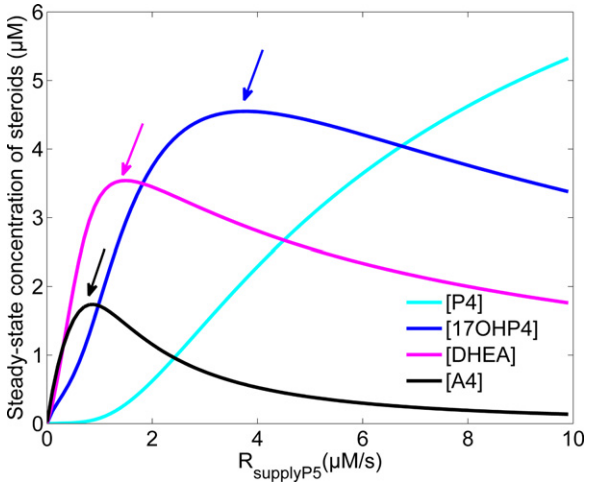

Fig. 5. Changes in steady-state concentrations of the steroids with varying P5 supply rate. $R_{supplyP5}$ is varied from 0 μM/s to 10 μM/s, and values of the parameters, other than $R_{supplyP5}$, are the same as those under Fig. 3. The points beyond which increasing P5 supply rate suppresses the synthesis of A4, DHEA, 17OH-P4, respectively, are indicated by the arrows. The curves for [P5] and [17OH-P5] are not shown because these are rapidly increased when $R_{supplyP5}$ increases, making the changes in [A4], [DHEA], [17OH-P4], and [P4] indiscernible when shown together.

4. Discussion

The overall goal of this study was to better understand the complexities of enzymatic control of steroid synthesis in species such as human, non-human primates, ovine and bovine wherein the P450c17 enzyme exhibits minimal 17,20-lyase activity with 17OH-P4 as a substrate. To this end, we have developed a mathematical model in which the reactions catalyzed by the two key enzymes are described, based on the known catalytic mechanisms and substrate specificity of the enzymes in these species. While this model is only relevant to steroid synthesis in the above mentioned species, it should be also possible to develop an alternative model wherein P450c17 is more active for the conversion of 17OH-P4 into A4 than it is for the conversion of 17OH-P5 into DHEA, such as in the rat and pig.

The model shows that A4 synthesis can either increase or decrease with decreasing 3β-HSD activity, depending on the extent to which 3β-HSD activity is decreased: before or after the turning point. The paradoxical response of A4 synthesis to variation in 3β-HSD is robust against changes in the model system's characteristics, such as P5 supply rate, the level of P450c17 activity, the affinity of P450c17 for P5, P4, and 17OH-P5, or the kinetic properties of the 3β-HSD enzyme (type 1 versus type 2). Therefore, the principle determinant of the response when 3β-HSD activity is varied is the nature of the reactions and the way they are connected in the steroidogenic network. The model predicts that in species where the 17,20-lyase activity with 17OH-P4 is negligible, it is possible that A4 synthesis increases when 3β-HSD activity is decreased (or is inhibited), up to a certain point. Such a response is not intuitive because 3β-HSD is directly required for the conversion of DHEA into A4, the only efficient route through which A4 is synthesized

in these species. Nonetheless, this behaviour has been observed *in vitro* in insect cells expressing human recombinant P450c17 with either 3β-HSD type 1 or type 2 [26] and in reconstituted human enzyme systems [27]. A similar response in A4 synthesis was also reported in *in vivo* systems, such as in rhesus monkeys during the mid-luteal phase of the menstrual cycle, and in lutectomised and ovarectomized 90-day pregnant ewes, where inhibition of 3β-HSD by trilostane increased the circulating levels of E2 [14–16]. These results were predicted [1] and can be explained in the following way: initial decrease in 3β-HSD activity decreases P4 and 17OH-P4 synthesis, allowing 17OH-P5 to accumulate, and thereby shift the flux down the Δ^5 pathway towards DHEA production. Increased DHEA availability is able to overcome partial inhibition of 3β-HSD, resulting in more A4 synthesis, and consequently more E2 produced, assuming that 17β-hydroxysteroid dehydrogenase and P450 aromatase enzymes are not limiting. It is likely that the amount of trilostane administered in these experiments was not sufficient to inhibit the 3β-HSD to the level necessary to limit A4 and, therefore, E2 synthesis.

Thus, depending on the extent to which 3β-HSD activity is decreased in an experiment, it is possible that only one type of response for A4 synthesis (either increased or decreased) will be observed. Unless 3β-HSD activity is varied over a sufficiently wide range, it is unlikely that both responses, predicted by the model, will be seen. Simulations using this model may be helpful in predicting the level of change in 3β-HSD activity required to get a desired response in steroid synthesis.

The absolute value of the 3β-HSD activity at the turning point of A4 synthesis, on the other hand, depends on other characteristics of the model system, such as kinetic properties of the enzymes, the level of P450c17 activity, and the rate of P5 supply. The higher the value of the 3β-HSD activity is at the turning point, the lesser the degree of inhibition of 3β-HSD activity that is required to achieve reduction of A4 synthesis. The steady-state concentration of A4 at the turning point is also sensitive to changes in the other characteristics of the model system. Because the A4 steady-state concentration at this point is maximal, it reflects the capacity of the system for A4 synthesis. When the affinity of P450c17 for P5 is decreased, the flux through the Δ^5 pathway decreases and therefore, the capacity of the system for A4 synthesis in general, is also reduced (Fig. 4a and b).

By taking into consideration competitive inhibition of the enzymes by substrates and products, the model shows that when the rate of P5 supply reaches a certain level, further increases in P5 supply rate will suppress the synthesis of A4, DHEA, and 17OH-P4, but not the synthesis of P4. Obviously, at low rates of P5 supply, the concentrations of all steroids are low and inhibition of P450c17 by substrates and 3β-HSD by both substrates and products is negligible. Increasing the P5 supply rate progressively increases the synthesis of all the steroids to a point when the steroid concentrations reach levels that are sufficient to start inhibiting the activities of the both enzymes. The more reaction steps there are between the precursor substrate P5 and the end product, the stronger the effect of competitive inhibition is on its synthesis. Therefore, as the P5 supply rate increases, the suppression of A4 synthesis occurs first, following by that of DHEA and then 17OH-P4. In turn, this increasingly drives P5 flux down the Δ^4 pathway towards the synthesis of P4. The increased flux down the Δ^4 pathway is able to overcome the effect of the inhibition of 3β-HSD by its catalytic substrates and products, allowing P4 synthesis to continue to rise with increasing P5 supply rate. When competitive inhibition of enzyme activities by substrates and products was removed from the model, the synthesis of all the steroids continues to increase with increasing P5 supply rate. These results, predicted by the model, have not been verified experimentally and we do not know the physiological range of the rate of P5. However, we postulate that competitive inhibition by substrates and products may be a mechanism that allows very sensitive and immediate regulation of steroid synthesis without the need to alter gene expression of steroidogenic enzymes. Such a mechanism complements metabolic inactivation to regulate the steady-state concentrations of steroids achieved when P5 supply rate is acutely up-regulated.

To sum up, this model allows one to predict the steady state concentration of steroids when the data on the level of the enzyme activities, the enzyme types, and substrate supply are available. It also helps to identify where in the catalytic mechanisms and in the interacting pathways are opportunities to influence the final outcome of steroid synthesis. Potential targets for therapeutic interventions may be thus, identified. One limitation of this model is that it is unable to simulate how changes in the availability of regulatory factors, such as co-factors, affect the enzymes' activities or affinities but it can help to understand how resultant changes in the enzymes' activities or affinities can affect steroid synthesis. This model does not consider the spatial distribution of 3β-HSD and P450c17 but assumes that both enzymes reside within the same compartment. As steroids have high membrane permeability, it is expected that spatial separation of the enzymes within a cell has minor effect and would not modify the qualitative behaviours of the steroidogenic pathways that are determined predominantly by the nature of the catalytic reactions and the way they are connected biochemically. The effects of spatial compartmentalization will be incorporated into future models.

Acknowledgements

We thank James L. Thomas (Mercer University School of Medicine, Georgia, USA) for references and advice on the mechanism of the 3β-HSD enzyme, Walter Miler (University of California, San Francisco, USA) for references and advice on the biochemical characteristics of the P450c17 enzyme, and Pascale Chavatte-Palmer (INRA, Jouy-en-Josas, France) for the discussion on the functioning of the whole network and Paul Shorten for help with the preparation of the manuscript. This work was supported by the New Zealand National Research Centre for Growth and Development. This work also was supported in part by 1R13DK092108-01 partially funded by the US National Institute of Environmental Health Science (NIEHS), National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), and Eunice Kennedy Shriver National Institute of Child Health & human development (NICHD).

Appendix A.

Applying the equilibrium approximation [20] for reactions catalyzed by 3β-HSD

$$\text{Reaction}\,(1): \mathrm{E} + \mathrm{P5} \underset{k_{-s_1}}{\overset{k_{s_1}}{\rightleftharpoons}} \mathrm{E}_{S_1} \xrightarrow{k'_1} \mathrm{E}_{P_1} \underset{k_{p_1}}{\overset{k_{-p_1}}{\rightleftharpoons}} \mathrm{E} + \mathrm{P4}$$

$$\text{Reaction}\,(2): \mathrm{E} + \mathrm{17OHP5} \underset{k_{-s_2}}{\overset{k_{s_2}}{\rightleftharpoons}} \mathrm{E}_{S_2} \xrightarrow{k'_2} \mathrm{E}_{P_2} \underset{k_{p_2}}{\overset{k_{-p_2}}{\rightleftharpoons}} \mathrm{E} + \mathrm{17OHP4}$$

$$\text{Reaction}\,(3): \mathrm{E} + \mathrm{DHEA} \underset{k_{-s_3}}{\overset{k_{s_3}}{\rightleftharpoons}} \mathrm{E}_{S_3} \xrightarrow{k'_3} \mathrm{E}_{P_3} \underset{k_{p_3}}{\overset{k_{-p_3}}{\rightleftharpoons}} \mathrm{E} + \mathrm{A4}$$

[X] is concentration of the respective steroid, enzyme, enzyme–substrate, or enzyme–product complex. e_0 is the total concentration of 3β-HSD enzyme, i.e. concentration of both free enzyme and the enzyme in complexes with substrates and products.

$$e_0 = [\mathrm{E}] + \left[\mathrm{E}_{S_1}\right] + \left[\mathrm{E}_{S_2}\right] + \left[\mathrm{E}_{S_3}\right] + \left[\mathrm{E}_{P_1}\right] + \left[\mathrm{E}_{P_2}\right] + \left[\mathrm{E}_{P_3}\right]$$

Applying the equilibrium approximation, we have:

$$[E_{S_1}] = \frac{k_{s_1}}{k_{-s_1}} \cdot [E] \cdot [P5]; \quad \left[E_{P_1}\right] = \frac{k_{p_1}}{k_{-p_1}} \cdot [E] \cdot [P4];$$

$$[E_{S_2}] = \frac{k_{s_2}}{k_{-s_2}} \cdot [E] \cdot [17OHP5]; \quad \left[E_{P_2}\right] = \frac{k_{p_2}}{k_{-p_2}} \cdot [E] \cdot [17OHP4];$$

$$[E_{S_3}] = \frac{k_{s_3}}{k_{-s_3}} \cdot [E] \cdot [DHEA]; \quad \left[E_{P_3}\right] = \frac{k_{p_3}}{k_{-p_3}} \cdot [E] \cdot [A4]$$

Then,

$$e_0 = [E] + \frac{k_{s_1}}{k_{-s_1}} \cdot [E] \cdot [P5] + \frac{k_{p_1}}{k_{-p_1}} \cdot [E] \cdot [P4] + \frac{k_{s_2}}{k_{-s_2}} \cdot [E] \cdot [17OHP5] + \frac{k_{p_2}}{k_{-p_2}} \cdot [E] \cdot [17OHP4] + \frac{k_{s_3}}{k_{-s_3}} \cdot [E] \cdot [DHEA] + \frac{k_{p_3}}{k_{-p_3}} \cdot [E] \cdot [A4]$$

Since $K_{mi} = k_{-s_i}/k_{s_i}$, $K_p = k_{-p_i}/k_{p_i}$, $i = 1,2,3$, it follows that:

$$[E] = \frac{e_0}{1 + ([P5]/K_{m1}) + ([P4]/K_{p1}) + ([17OHP5]/K_{m2}) + ([17OHP4]/K_{p2}) + ([DHEA]/K_{m3}) + ([A4]/K_{p1})}$$

Since $V_i = k_i' \cdot [E_{S_i}]$, and $V_{mi} = k_i' \cdot e_0$, the rates of reactions (1)–(3) are:

$$V_1 = \frac{V_{m1} \cdot [P5]}{K_{m1} \cdot \left(1 + ([17OHP5]/K_{m2}) + ([DHEA]/K_{m3}) + ([P4]/K_{p1}) + ([17OHP4]/K_{p2}) + ([A4]/K_{p3})\right) + [P5]}$$

$$V_2 = \frac{V_{m2} \cdot [17OHP5]}{K_{m2} \cdot \left(1 + ([P5]/K_{m1}) + ([DHEA]/K_{m3}) + ([P4]/K_{p1}) + ([17OHP4]/K_{p2}) + ([A4]/K_{p3})\right) + [17OHP5]}$$

$$V_3 = \frac{V_{m3} \cdot [DHEA]}{K_{m3} \cdot \left(1 + ([17OHP5]/K_{m2}) + ([P5]/K_{m1}) + ([P4]/K_{p1}) + ([17OHP4]/K_{p2}) + ([A4]/K_{p3})\right) + [DHEA]}$$

If the catalytic constants of 3β-HSD for P5, 17OH-P5, DHEA are similar, i.e. $k_1' = k_2' = k_3' = k_{3\beta\text{-HSD}}'$, or $V_{m1} = V_{m2} = V_{m3} = V_{m3\beta\text{-HSD}}$, then the rates of reactions (1)–(3) can be written as follows.

$$V_1 = \frac{V_{m3\beta\text{-HSD}} \cdot [P5]}{K_{m1} \cdot \left(1 + ([17OHP5]/K_{m2}) + ([DHEA]/K_{m3}) + ([P4]/K_{p1}) + ([17OHP4]/K_{p2}) + ([A4]/K_{p3})\right) + [P5]}$$

$$V_2 = \frac{V_{m3\beta\text{-HSD}} \cdot [17OHP5]}{K_{m2} \cdot \left(1 + ([P5]/K_{m1}) + ([DHEA]/K_{m3}) + ([P4]/K_{p1}) + ([17OHP4]/K_{p2}) + ([A4]/K_{p3})\right) + [17OHP5]}$$

$$V_3 = \frac{V_{m3\beta\text{-HSD}} \cdot [DHEA]}{K_{m3} \cdot \left(1 + ([17OHP5]/K_{m2}) + ([P5]/K_{m1}) + ([P4]/K_{p1}) + ([17OHP4]/K_{p2}) + ([A4]/K_{p3})\right) + [DHEA]}$$

Appendix B.

Applying equilibrium approximation for reactions catalyzed by P450c17

$$\text{Reaction (4)}: E^1 + P5 \underset{k_{-s_4}}{\overset{k_{s_4}}{\rightleftharpoons}} E_{S_4} \xrightarrow{k_4'} E^1 + 17OHP5$$

$$\text{Reaction (5)}: E^1 + 17OHP5 \underset{k_{-s_5}}{\overset{k_{s_5}}{\rightleftharpoons}} E_{S_5} \xrightarrow{k_5'} E^1 + DHEA$$

$$\text{Reaction (6)}: E^1 + P4 \underset{k_{-s_6}}{\overset{k_{s_6}}{\rightleftharpoons}} E_{S_6} \xrightarrow{k_6'} E^1 + 17OHP4$$

[X] is concentration of the respective steroid, enzyme, enzyme–substrate, or enzyme–product complex. e_0^1 is the total concentration of P450c17 enzyme.

$$e_0^1 = \left[E^1\right] + \left[E_{S_4}\right] + \left[E_{S_5}\right] + \left[E_{S_6}\right]$$

Applying the equilibrium approximation, we have

$$\left[E_{S_4}\right] = \frac{k_{s_4}}{k_{-s_4}} \cdot \left[E^1\right] \cdot [P5]; \quad \left[E_{S_5}\right] = \frac{k_{s_5}}{k_{-s_5}} \cdot \left[E^1\right] \cdot [17OHP5];$$

$$\left[E_{S_6}\right] = \frac{k_{s_6}}{k_{-s_6}} \cdot \left[E^1\right] \cdot [P4]$$

Then,

$$e_0^1 = \left[E^1\right] \left(1 + \frac{k_{s_4}}{k_{-s_4}} \cdot [P5] + \frac{k_{s_5}}{k_{-s_5}} \cdot [17OHP5] + \frac{k_{s_6}}{k_{-s_6}} \cdot [P4]\right)$$

and thus, since $K_{mi} = k_{-s_i}/k_{s_i}$, $i = 4,5,6$, it follows that

$$\left[E^1\right] = \frac{e_0^1}{1 + ([P5]/K_{m4}) + ([17OHP5]/K_{m5}) + ([P4]/K_{m6})}$$

Since, $V_i = k_i' \cdot [E_{S_i}]$, and $V_{mi} = k_i' e_0^1$, $i = 4,5,6$, it follows that the rates of reactions (4)–(6) are:

$$V_4 = \frac{V_{m4} \cdot [P5]}{K_{m4} \cdot \left(1 + ([P4]/K_{m6}) + ([17OHP5]/K_{m5})\right) + [P5]}$$

$$V_5 = \frac{V_{m5} \cdot [17OHP5]}{K_{m5} \cdot \left(1 + ([P4]/K_{m6}) + ([P5]/K_{m4})\right) + [17OHP5]}$$

$$V_6 = \frac{V_{m6} \cdot [P4]}{K_{m6} \cdot \left(1 + ([P5]/K_{m4}) + ([17OHP5]/K_{m5})\right) + [P4]}$$

If the catalytic constants of the 17α-hydroxylase activity of P450c17 for P5 and P4 are similar, i.e. $V_{m4} = V_{m6} = V_{mHyd}$, and V_{m5} is written as V_{mLyase}, these reaction rates become:

$$V_4 = \frac{V_{mHyd} \cdot [P5]}{K_{m4} \cdot \left(1 + ([P4]/K_{m6}) + ([17OHP5]/K_{m5})\right) + [P5]}$$

$$V_6 = \frac{V_{\mathrm{mHyd}} \cdot [\mathrm{P4}]}{K_{\mathrm{m6}} \cdot \left(1 + ([\mathrm{P5}]/K_{\mathrm{m4}}) + ([17\mathrm{OHP5}]/K_{\mathrm{m5}})\right) + [\mathrm{P4}]}$$

$$V_5 = \frac{V_{\mathrm{mLyase}} \cdot [17\mathrm{OHP5}]}{K_{\mathrm{m5}} \cdot \left(1 + ([\mathrm{P4}]/K_{\mathrm{m6}}) + ([\mathrm{P5}]/K_{\mathrm{m4}})\right) + [17\mathrm{OHP5}]}$$

References

[1] A.J. Conley, I.M. Bird, The role of cytochrome P450 17α-hydroxylase and 3β-hydroxysteroid dehydrogenase in the integration of gonadal and adrenal steroidogenesis via the Δ^5 and Δ^4 pathways of steroidogenesis in mammals, Biol. Reprod. 56 (1997) 789–799.

[2] W.L. Miller, R.J. Auchus, The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders, Endocr. Rev. 2 (1) (2011) 81–151.

[3] J.L. Thomas, E.A. Berko, A. Faustino, R.P. Myers, R.C. Strickler, Human placental 3β-hydroxy-5-ene-steroid dehydrogenase and steroid 5 → 4-ene-isomerase: purification from microsomes, substrate kinetics, and inhibition by product steroids, J. Steroid Biochem. 31 (5) (1988) 785–793.

[4] S.G. Raimondi, N.S. Oliver, L.C. Patrito, A. Flury, Kinetic analysis of 3β-hydroxysteroid dehydrogenase activity in microsomes from complete hydatidiform mole, J. Steroid Biochem. 36 (1990) 149–152.

[5] M.S. Breen, D.L. Villeneuve, M. Breen, G.T. Ankley, R.B. Conolly, Mechanistic computational model of ovarian steroidogenesis to predict biochemical responses to endocrine active compounds, Ann. Biomed. Eng. 35 (6) (2007) 970–981.

[6] M.S. Breen, M. Breen, N. Terasaki, M. Yamazaki, R.B. Conolly, Computational model of steroidogenesis in human H295R cells to predict biochemical response to endocrine-active chemicals: model development for metyrapone, Environ. Health Perspect. 118 (2) (2010) 265–272.

[7] W. Gibb, Substrate and nucleotide specificity of placental microsomal 3β-hydroxysteroid dehydrogenase, Steroids 37 (1) (1981) 23–31.

[8] S.G. Raimondi, N.S. Olivier, L.C. Patrito, A. Flury, Regulation of the 3β-hydroxysteroid dehydrogenase activity in tissue fragments and microsomes from human term placenta: kinetic analysis and inhibition by steroids, J. Steroid Biochem. 32 (3) (1989) 413–420.

[9] M. Wiener, S.H.G. Allen, Inhibition of placental steroid synthesis by steroid metabolites II: inhibitor specificity, Steroids 12 (1968) 261–273.

[10] J.L. Thomas, W.L. Duax, A. Addlagatta, S. Brandt, R.R. Fuller, W. Norris, Structure/function relationships responsible for coenzyme specificity and the isomerase activity of human type 1 3β-hydroxysteroid dehydrogenase/isomerase, J. Biol. Chem. 278 (37) (2003) 35483–35490.

[11] A.H. Payne, D.B. Hales, Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones, Endocr. Rev. 25 (6) (2004) 947–970.

[12] J.D. Townsley, Inhibition of placental 3β-hydroxysteroid dehydrogenase by naturally occuring steroids. A potential mechanism regulating oestrogen synthesis from unconjugated precursors, Acta Endocr. Copenh. 79 (1975) 740–748.

[13] P. Soucy, V. Luu-The, Conversion of pregnenolone to DHEA by human 17α-hydroxylase/17,20-lyase (P450c17), Eur. J. Biochem. 267 (11) (2000) 3243–3247.

[14] D.M. Duffy, D.L. Hess, R.L. Stouffer, Acute administration of a 3β-hydroxysteroid-dehydrogenase inhibitor to rhesus monkeys at the midluteal phase of the menstrual cycle: evidences for possible autocrine regulation of primate corpus luteum by progesterone, J. Clin. Endocrinol. Metab. 79 (1994) 1587–1597.

[15] Y.S. Weems, P.J. Bridges, B.R. Lea Master, D.L. Vincent, C.W. Weems, Effect of trilostane on prostaglandins (PG) E and $F_{2\alpha}$ (PGE; $PGF_{2\alpha}$), estradiol-17β and progesterone secretion and pregnancy of 90 days ovariectomized pregnant ewes, Prostaglandin Other Lipid Mediators 58 (1999) 149–158.

[16] Y.S. Weems, D.L. Vincent, C. Lemme, C.W. Weems, Trilostane but not prostaglandin $F_{2\alpha}$($PGF_{2\alpha}$) or cortisol abort 90-day lutectomized pregnant sheep, Prostaglandin Other Lipid Mediators 58 (1999) 77–86.

[17] W.L. Miller, Molecular biology of steroid hormone synthesis, Endocr. Rev. 9 (3) (1988) 295–318.

[18] J.L. Thomas, J.I. Mason, S. Brandt, B.R. Spencer, W. Norris, Structure/function relationships responsible for the kinetic differences between human type 1 and type 2 3β-hydroxysteroid dehydrogenase and for the catalysis of the type 1 activity, J. Biol. Chem. 277 (45) (2002) 42795–42801.

[19] http://www.uniprot.org/uniprot/Q86TX2, the last access is on 10.09.11.

[20] J. Keener, J. Sneyd, Biochemical reactions, in: Mathematical Physiology, Springer, New York, 2009, 1-47.

[21] R.J. Auchus, T.C. Lee, W.L. Miller, Cytochrome b_5 augments the 17,20-lyase activity of human P450c17 without direct electron transfer, J. Biol. Chem. 273 (6) (1998) 3158–3165.

[22] T. Yamazaki, T. Ohno, T. Sakaki, M. Akiyoshi-Shibata, Y. Yabusaki, T. Imai, S. Kominami, Kinetic analysis of successive reactions catalyzed by bovine cytochrome P450-$_{17\alpha,\mathrm{lyase}}$, Biochemistry 37 (1998) 2800–2806.

[23] S. Tsumagari, J. Kamata, K. Takagi, K. Tanemura, A. Yosai, M. Takeishi, 3β-hydroxysteroid dehydrogenase activity and gestagen concentrations in bovine cotyledons and caruncles during gestation and parturition, J. Reprod. Fertil. 102 (1) (1994) 35–39.

[24] D. Naville, W.E. Rainey, L. Milewich, J. Ian, Regulation of 3β-hydroxysteroid dehydrogenase $\Delta^{5\rightarrow4}$-isomearse expression by adrenocorticotropin in bovine adrenocortical cells, Endocrinology 128 (1) (1991) 139–145.

[25] W.L. Miller, Minireview: Regulation of steroidogenesis by electron transfer, Endocrinology 146 (6) (2005) 2544–2550.

[26] A.J. Conley, C.J. Corbin, J.L. Thomas, N. Gee, B.L. Lasley, B.C. Moeller, S.D. Stanley, T. Berger, Costs and consequences of cellular compartmentalization and substrate competition among human enzymes involved in androgen and estrogen synthesis, Biol. Reprod., doi:10.1095/biolreprod.111.094706, in press.

[27] C.E. Fluck, D.C. Yaworsky, W.L. Miller, Effects of anticonvulsants on human P450c17 (17α-hydroxylase/17,20 lyase) and 3β-hydroxysteroid dehydrogenase type 2, Epilepsia 46 (2005) 444–448.