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(54) Title: SELECTIVE TESTICULAR 11 β -HSD INHIBITORS AND METHODS OF USE THEREOF

(57) Abstract: Methods for increasing and decreasing male fertility using selective 11 β -HSD1-dehydrogenase, 11 β -HSD1-reductase and 11 β -HSD2 dehydrogenase modulating compounds are described.

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SELECTIVE TESTICULAR 11 β -HSD INHIBITORS AND METHODS OF USE THEREOF

Related Applications:

5 This application claims priority to U.S. Provisional Patent Application Serial No. 60/466,387, filed April 29, 2003. This application is related to U.S. Patent Application Serial No. 10/327,566, filed December 20, 2002 and U.S. Provisional Patent Application Serial No. 60/342,693, filed December 21, 2001. The entire contents of each of the aforementioned applications are hereby incorporated herein by reference.

Background:

10 Corticosteroids, also referred to as glucocorticoids are steroid hormones, the most common form of which is cortisol. Modulation of glucocorticoid activity is important in regulating physiological processes in a wide range of tissues and organs. High levels of glucocorticoids may result in excessive salt and water retention by the kidneys, producing high blood pressure.

15 Glucocorticoids (GC's) play an important role in the regulation of vascular tone and blood pressure. Glucocorticoids can bind to and activate the glucocorticoid receptor (GR) and, possibly, the mineralocorticoid receptor (MR)) to potentiate the vasoconstrictive effects of both catecholamines and angiotensin II (Ang II). Tissue glucocorticoid levels are regulated by two isoforms of the enzyme 11 β -hydroxysteroid dehydrogenase (11 β -HSD). 11 β -HSD converts glucocorticoids into metabolites that are unable to bind to MRs (Edwards C R *et al.* (1988) *Lancet* 2:986-9; Funder *et al.*, (1988) *Science* 242, 583,585).

Summary of the Invention:

25 In an embodiment, the invention pertains, at least in part, to a method for increasing male fertility, by administering an effective amount of a 11 β -HSD1 reductase inhibitor.

30 In another embodiment, the invention pertains, at least in part, to a method for decreasing male fertility, by administering an effective amount of a 11 β -HSD1 dehydrogenase inhibitor or a 11 β -HSD2 dehydrogenase inhibitor, such that said fertility is decreased.

35 In another embodiment, the invention pertains, at least in part, to a method for increasing testosterone levels in a subject, comprising administering to said subject an effective amount a 11 β -HSD1 reductase inhibitor.

In another embodiment, the invention pertains, at least in part, to a method for decreasing testosterone levels in a subject, comprising administering to said subject an effective amount a 11 β -HSD1 dehydrogenase inhibitor.

In another embodiment, the invention pertains, at least in part, to a
 5 pharmaceutical composition comprising an effective amount of a 11-keto-progesterone, 11-keto-testosterone, 11-keto-androsterone, 11-keto androstenedione, 11-keto dehydroepiandrosterone, 3 α , 5 α -reduced-11-ketoprogesterone, 3 α , 5 α -reduced-11-keto-testosterone, 3 α , 5 α -reduced-11-keto-androstenedione, 3 α , 5 α -tetrahydro-11-dehydro-corticosterone, 3 α , 5 α -reduced-11-keto-androsterone, 3 α , 5 α -reduced-11-keto
 10 dehydroepiandrosterone, 5 α -reduced-11-ketoprogesterone, 5 α -reduced-11-keto-testosterone, 5 α -reduced-11-keto-androstenedione, 5 α -reduced-11-dehydro-corticosterone, 5 α -reduced-11-keto-androsterone, 5 α -reduced-11-keto dehydroepiandrosterone, 3 α , 5 β -tetrahydro-deoxycorticosterone, 3 α , 5 α -tetrahydro-progesterone, 3 α , 5 α -tetrahydro-testosterone, 3 α , 5 α -tetrahydro-deoxycorticosterone, or
 15 a pharmaceutically acceptable salt, ester, or prodrug thereof and a pharmaceutically acceptable carrier, wherein said effective amount is effective to increase male fertility.

In another embodiment, the invention pertains, at least in part, to a pharmaceutical composition comprising an effective amount of 11 β -hydroxy testosterone, 11 β -hydroxy androstenedione, 11 β -hydroxy dehydroepiandrosterone,
 20 11 β -progesterone, chenodeoxycholic acid, 3 α , 5 α -reduced-11 β -hydroxy testosterone, 3 α , 5 α -reduced-11 β -hydroxy androstenedione, 3 α , 5 α -reduced-11 β -hydroxy dehydroepiandrosterone, 3 α , 5 α -reduced-corticosterone, 3 α , 5 α -reduced-aldosterone, 3 α , 5 α -reduced-11 β -progesterone, 5 α -reduced-11 β -hydroxy testosterone, 5 α -reduced-11 β -hydroxy androstenedione, 5 α -reduced-11 β -hydroxy dehydroepiandrosterone,
 25 5 α -reduced-11 β -progesterone, 3 α , 5 β -reduced-11 β -OH-progesterone, 3 α , 5 β -reduced-11 β -OH-testosterone, 3 α , 5 β -tetrahydro-deoxycorticosterone, 3 α , 5 β -tetrahydro-progesterone, 3 α , 5 β -tetrahydro-testosterone, 3 α , 5 α -tetrahydro-deoxycorticosterone, 3 α , 5 β -chenodeoxycholic acid or a pharmaceutically acceptable salt, prodrug, or ester thereof and pharmaceutically acceptable carrier, wherein said effective amount is
 30 effective to decrease male fertility.

Brief Description of the Drawings:

Figure 1 is a bar graph which shows that the exposure of rat aortic rings to corticosterone and 11 β -HSD2 antisense resulted in a statistically significant increase in
 35 the contractile response to phenylephrine.

Figure 2 is a bar graph which shows that in aortic rings treated with 11 β -HSD1 antisense, the contractile responses to all concentrations of phenylephrine were significantly increased compared to aortic rings treated with corticosterone and nonsense oligomers.

5 Figure 3 is a bar graph which illustrates that 11-dehydro-corticosterone amplifies the contractile responses to phenylephrine in rat aortic rings.

Figure 4 is a bar graph which shows that the conversion of corticosterone to 11-dehydrocorticosterone was lower than in aortic rings incubated with corticosterone and 11 β -HSD1 nonsense oligomers.

10 Figures 5A-5D are representative HPLC chromatograms.

Detailed Description of the Invention:

I. Glucocorticoids and 11 β -HSD1 Reductase, 11 β -HSD1 Dehydrogenase and 11 β -HSD2 Dehydrogenase

15 Glucocorticoids can affect vascular tone by modifying the actions of several vasoactive substances. Glucocorticoids amplify the vasoconstrictive actions of α -adrenergic catecholamines and angiotensin II on vascular smooth muscle cells. It has been reported that glucocorticoids decrease the biosynthesis of both nitric oxide and prostaglandin I, and attenuate the vasorelaxant actions of atrial natriuretic peptide in
20 vascular tissue. Thus, the multiple effects of glucocorticoids in vascular tissue operate to increase vascular tone. Since vascular smooth muscle cells contain both glucocorticoid (GR) and mineralocorticoid (MR) receptors it is possible that glucocorticoids could mediate their effects in vascular tissue via either or both of these
25 receptor types.

Glucocorticoids (GC's) are metabolized in vascular and other tissue by two isoforms of 11 β -hydroxysteroid dehydrogenase (11 β -HSD). 11 β -HSD2 is unidirectional and metabolizes glucocorticoids to their respective inactive 11-dehydro derivatives. 11 β -HSD1 is bi-directional, also possessing reductase activity and thus the
30 ability to regenerate active glucocorticoids from the 11-dehydro derivatives. In vascular tissue, glucocorticoids amplify the pressor responses to catecholamines and angiotensin II and may down-regulate certain depressor systems such as nitric oxide and prostaglandins. Both 11 β -HSD2 and 11 β -HSD1 are believed to regulate glucocorticoid levels in vascular tissue and are part of additional mechanisms that control vascular tone.

35 Glucocorticoids are known to play an important role in the regulation of vascular tone and blood pressure. Glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) are present in aorta, mesenteric arteries and VSM cells in culture. Glucocorticoids can bind to and activate GR (and possibly MR) to potentiate the vasoconstrictive effects

of both catecholamines and Ang II. Human and rat vascular endothelial cells contain both 11 β -HSD2 and 11 β -HSD1, 11 β -HSD2 uses NAD⁺ as a co-factor and acts only as a dehydrogenase converting glucocorticoids to their inactive 11-dehydro metabolites. It is generally understood that 11 β -HSD2 operates to protect both MR and GR from
5 excessive stimulation by glucocorticoids and we and others have shown that glucocorticoids further amplify the contractile effects of phenylephrine and Ang II when 11 β -HSD enzyme activity is inhibited.

11 β -HSD1 uses NADP⁺ as a co-factor and is bi-directional functioning as both a reductase and dehydrogenase. Using RT-PCR, it has been shown that rat vascular
10 smooth muscle (VSM) cells only contain 11 β -HSD1, which under "physiologic conditions" acts largely as a reductase (3 reductase to 1 dehydrogenase) generating active corticosterone from inactive 11-dehydro-corticosterone.

11 β -HSD1 reductase has an important role as a generator of active GC in vascular tissue. 11 β -HSD inactivates glucocorticoid molecules, allowing lower
15 circulating levels of aldosterone to maintain renal homeostasis. Human and rat vascular endothelial cells (EC) contain both 11 β -HSD1 and 11 β -HSD2. 11 β -HSD2 uses NAD⁺ as a co-factor and acts only as a dehydrogenase converting glucocorticoids to their inactive 11-dehydro metabolites.

11 β -HSD2 operates to protect both MR and GR from excessive stimulation by
20 glucocorticoids and it has been shown that glucocorticoids further amplify the contractile effects of phenylephrine (PE) and Ang II when 11 β -HSD1 or 2 dehydrogenase enzyme activity is inhibited.

Working with freshly prepared rat Leydig cells, it was shown that certain 11 β -hydroxylated and 11-keto derivatives of androgens and progestogens are potent selective
25 inhibitors of 11 β -HSD1 (11 β -hydroxy steroid dehydrogenase), an enzyme present in testicular Leydig cells. These cells have been shown to regulate the effects of glucocorticoids on testosterone biosynthesis.

These substances may either inhibit the inactivation of active glucocorticoids by 11 β -HSD1 dehydrogenase or inhibit the regeneration of active glucocorticoids by 11 β -
30 HSD1 reductase. It has been shown that the testis are able to synthesize several of these substances and that inhibitors may also be locally synthesized.

Inhibitors which cause testicular levels of corticosterone in rodents or cortisol in humans to increase would decrease production of testosterone, whereas those which cause them to decrease would increase testosterone production.

II. Methods of Modulating Male Fertility

Lower concentrations of glucocorticoids stimulate spermatogenesis. Therefore, 11 β -HSD1 reductase inhibitors may be used to treat infertility. In contrast, 11 β -HSD1 dehydrogenase inhibitors and 11 β -HSD2 dehydrogenase inhibitors may be used decrease
5 fertility.

The invention includes a method for increasing male fertility. The method includes administering an effective amount of a selective 11 β -HSD1 reductase inhibitor to a subject, such that fertility is increased. The invention also includes a method for increasing testosterone levels by administering to a subject an effective amount of a 11 β -
10 HSD1 reductase inhibitor.

In another embodiment, the invention features a method for decreasing male fertility. The method includes administering an effective amount of a selective 11 β -HSD1 dehydrogenase and/or a selective 11 β -HSD2 dehydrogenase inhibitor. The invention also includes a method for decreasing testosterone levels in a subject by
15 administering to said subjects an effective amount of a selective 11 β -HSD1 dehydrogenase and/or a selective 11 β -HSD2 dehydrogenase inhibitor, such that testosterone levels are decreased in said subject.

The term "subject" includes subjects which modulation of testosterone levels is desired, such as mammals. Examples of mammals include dogs, cats, bears, rabbits,
20 mice, rats, goats, cows, sheep, horses, and, preferably, humans. The subject may be suffering from or at risk of suffering from infertility. In a further embodiment, the subject is male.

The term "effective amount" of the 11 β -HSD1 reductase, 11 β -HSD1 dehydrogenase, or 11 β -HSD2 dehydrogenase modulating compound is that amount
25 necessary or sufficient to modulate testosterone levels in a subject so that a desired effect, e.g., increasing or decreasing fertility, is obtained. The effective amount can vary depending on such factors as the size and weight of the subject, or the particular 11 β -HSD1 reductase, 11 β -HSD1 dehydrogenase, or 11 β -HSD2 dehydrogenase modulating compound, e.g., inhibiting, compound.

30 In a further embodiment, the 11 β -HSD1 reductase, 11 β -HSD1 dehydrogenase, or 11 β -HSD2 dehydrogenase modulating compound may be administered in combination with a pharmaceutically acceptable carrier.

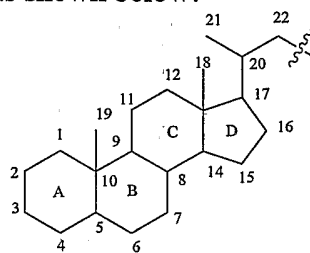
The language "in combination with" another agent includes co-administration of the compound of the invention and the agent, administration of the compound of the
35 invention first, followed by the other agent and administration of the other agent first, followed by the compound of the invention.

III. 11 β -HSD1 Reductase Modulating Compounds, 11 β -HSD1-Dehydrogenase Modulating Compounds and 11 β -HSD2 Dehydrogenase Modulating Compounds

The term "11 β -HSD1 reductase modulating compound" include compounds and agents (e.g., oligomers, proteins, etc.) which modulate or inhibit the activity of 11 β -HSD1 reductase. In an advantageous embodiment, the 11 β -HSD1 reductase modulating compound is an 11 β -HSD1 reductase inhibitor (also referred to as "11 β -HSD1 reductase inhibiting compound"). The 11 β -HSD1 reductase modulating compound may be a small molecule, e.g., a compound with a molecular weight below 10,000 daltons.

In a further embodiment, the 11 β -HSD1 reductase modulating compound is a selective inhibitor of 11 β -HSD1 reductase. The term "selective 11 β -HSD1 reductase inhibitor" includes compounds which selectively inhibit the reductase activity of 11 β -HSD1 as compared to the dehydrogenase activity. In a further embodiment, the reductase activity is inhibited at a rate about 2 times or greater, about 3 times or greater, about 4 times or greater, about 5 times or greater, about 10 times or greater, about 15 times or greater, about 20 times or greater, about 25 times or greater, about 50 times or greater, about 75 times or greater, about 100 times or greater, about 150 times or greater, about 200 times or greater, about 300 times or greater, about 400 times or greater, about 500 times or greater, about 1×10^3 times or greater, about 1×10^4 times or greater, about 1×10^5 times or greater, or about 1×10^6 or greater as compared with the inhibition of the dehydrogenase activity of 11 β -HSD1.

In a further embodiment, the 11 β -HSD1 reductase modulating compound may be a steroid or a steroid derivative. The steroid ring system is generally numbered according to IUPAC conventions, as shown below:



Examples of 11 β -HSD1 reductase modulating compounds include 11-keto steroid compounds, e.g., compounds with the steroid ring system with a carbonyl functional group at the 11-position of the steroid ring. Examples of steroid compounds with an 11-keto group include, for example, 11-keto progesterone, 11-keto-testosterone, 11-keto-androsterone, 11-keto-androstenedione, 11-keto-dehydroepiandrosterone, 3 α , 5 α -reduced-11-keto-progesterone, 3 α , 5 α -reduced-11-keto-testosterone, 3 α , 5 α -reduced-11-keto-androstenedione, and 3 α ,5 α -tetrahydro-11-dehydro-corticosterone.

Examples of 11 β -HSD1 reductase modulating compounds also include 3 α , 5 α -reduced steroid compounds. Examples of 3 α , 5 α -reduced steroid compounds include 3 α , 5 α -reduced-11-ketoprogesterone, 3 α , 5 α -tetrahydro-progesterone, 3 α , 5 α -tetrahydro-testosterone, 3 α , 5 α -tetrahydro-deoxycorticosterone, 3 α , 5 α -reduced-11-keto-
5 testosterone, 3 α , 5 α -reduced-11-keto-androstenedione, 3 α , 5 α -reduced-11-keto-dehydroepiandrosterone, and 3 α , 5 α -tetrahydro-11-dehydro-corticosterone.

Other 11 β -HSD1 reductase modulating compounds include 5 α -reduced derivatives such as 5 α -reduced 11-keto progesterone, 5 α -reduced 11-keto-testosterone, 5 α -reduced 11-keto-androsterone, 5 α -reduced 11-keto-androstenedione, 3 α , 5 β -
10 tetrahydro-deoxycorticosterone, and 5 α -reduced 11-keto-dehydroepiandrosterone.

Steroid derivatives include compounds with a steroid ring structure optionally substituted with additional substituents which allow the compound to perform its intended function. It should be noted that the steroid compounds may be converted to the active form of the modulating compound within the subject. The invention includes
15 administering compounds which are in other forms, e.g., prodrugs, and which are metabolized *in vivo* to yield the 11 β -HSD1 reductase modulating compounds described herein.

Other examples of 11 β -HSD1 reductase modulating compounds include carbenoxolone and derivatives thereof.

20 Other examples of 11 β -HSD1 reductase modulating compounds include carbenoxolone and derivatives thereof. In other embodiments, 11 β -HSD1 reductase modulating compound is a nucleic acid. In another embodiment, the 11 β -HSD1 reductase inhibitor is an antisense nucleic acid. In another embodiment, the 11 β -HSD1 reductase inhibitor is a siRNA.

25 The basic mechanism of RNA interference can be understood as a two step process (Zamore P.D., *Nature Struc. Biol.*, 8, 9, 746-750, (2001)). First, the dsRNA is cleaved to yield short interfering RNAs (siRNAs) of about 21-23nt length with 5' terminal phosphate and 3' short overhangs (~2nt). Then, the siRNAs target the corresponding mRNA sequence specific for destruction (Fire A. *et al.*, *Nature*, Vol 391,
30 (1998); Hamilton AJ *et al.* *Science*, 286, 950-952, (1999); Zamore PD. *et al.* *Cell*, 101, 25-33, (2000); Elbashir SM. *et al.*, *Genes & Development*, 15, 188-200, (2001); Bernstein E. *et al.* *Nature* 409, 363-366, 2001).

It has been demonstrated that chemically synthesized 21 nt siRNA duplexes specifically suppress expression of endogenous and heterologous genes in different
35 mammalian cell lines, including human kidney and HeLa cells (Elbashir SM. *et al.*, *Nature*, 411, 494-498, (2001)). It was discovered that no unspecific effects occurred in mammalian cells by transfection of short sequences (<30nt). It was suggested that 21 nt

siRNA duplexes provide a new tool for studying gene function in mammalian cells and may eventually be used as gene-specific therapeutics.

It was also found that siRNAs mediated RNAi in cell extracts and synthetic siRNAs can induce gene-specific inhibition of expression in *C. elegans* and in cell lines
5 from humans and mice (Caplen, N.J. *et al. PNAS* 171251798, 1-6, (2001)30). It was also shown that siRNAs can have direct effects on gene expression in *C. elegans* and mammalian cell culture in vivo.

Methods for making and using siRNAs are described in, for example, WO 01/75164, US 2002/0137210, WO 01/29058, WO 02/072762, WO 02/059300, WO
10 02/44321, WO 01/92513, WO 01/68836, US 2002/0173478, US 2002/0160393, US 2002/0162126, US 2002/0137709, US 2002/0132788, US 2002/0086356, and WO 99/32619; each of which is expressly incorporated herein by reference.

In one embodiment, the 11 β -HSD1 reductase inhibitor is a double stranded RNA oligomer, wherein the antisense strand is complementary to at least a portion of SEQ.
15 ID. No. 1. In one embodiment, the portion is 40 base pairs or less, 35 base pairs or less, 30 base pairs or less, 29 base pairs or less, 28 base pairs or less, 27 base pairs or less, 26 base pairs or less, 25 base pairs or less, 24 base pairs or less, 23 base pairs or less, 22 base pairs or less, 21 base pairs or less, 20 base pairs or less, 19 base pairs or less, or about 18 base pairs or less. In another embodiment, the oligomer has 10 or more base
20 pairs, 11 or more base pairs, 12 or more base pairs, 13 or more base pairs, 14 base pairs or more, 15 base pairs or more, 16 base pairs or more, 17 base pairs or more, 18 base pairs or more, or 19 base pairs or more. In another embodiment, the 11 β -HSD1 reductase inhibitor has an antisense strand having the sequence 5'-CAT AAC TGC CGT CCA ACA GC-3' (SEQ ID NO. 1).

25 The term "11 β -HSD1 dehydrogenase modulating compound" include compounds and agents (e.g., oligomers, proteins, etc.) which modulate or inhibit the activity of 11 β -HSD1 dehydrogenase. In an advantageous embodiment, the 11 β -HSD1 dehydrogenase modulating compound is an 11 β -HSD1 dehydrogenase inhibitor (also referred to as "11 β -HSD1 dehydrogenase inhibiting compound"). The 11 β -HSD1
30 dehydrogenase modulating compound may be a small molecule, e.g., a compound with a molecular weight below 10,000 daltons.

In a further embodiment, the 11 β -HSD1 dehydrogenase modulating compound is a selective inhibitor of 11 β -HSD1 dehydrogenase. The term "selective 11 β -HSD1 dehydrogenase inhibitor" includes compounds which selectively inhibit the
35 dehydrogenase activity of 11 β -HSD1 as compared to the reductase activity of 11 β -HSD1. In a further embodiment, the dehydrogenase activity is inhibited at a rate about 2 times or greater, about 3 times or greater, about 4 times or greater, about 5 times or greater, about 10 times or greater, about 15 times or greater, about 20 times or greater,

about 25 times or greater, about 50 times or greater, about 75 times or greater, about 100 times or greater, about 150 times or greater, about 200 times or greater, about 300 times or greater, about 400 times or greater, about 500 times or greater, about 1×10^3 times or greater, about 1×10^4 times or greater, about 1×10^5 times or greater, or about 1×10^6 or greater as compared with the inhibition of the reductase activity of 11 β -HSD1.

In one embodiment, the 11 β -HSD1 dehydrogenase inhibitor is a small molecule, such as a steroid or a derivative thereof. In a further embodiment, the steroid is 3 α , 5 β -reduced. In one embodiment, the steroid is 3 α , 5 β -reduced. Examples of 3 α , 5 β -reduced steroids include 3 α , 5 β -reduced-11 β -OH-progesterone, 3 α , 5 β -reduced-11 β -OH-testosterone, chenodeoxycholic acid, 3 α , 5 β -tetrahydro-deoxycorticosterone, 3 α , 5 β -tetrahydro-progesterone, 3 α , 5 β -chenodeoxycholic acid, and 3 α , 5 β -tetrahydro-testosterone.

In another embodiment, the 11 β -HSD1 dehydrogenase inhibitor is a 3 α , 5 α -reduced steroid. Examples of such steroids include 3 α , 5 α -reduced-11 β -OH-progesterone, 3 α , 5 α -reduced-11 β -OH-testosterone, 3 α , 5 α -reduced-11 β -hydroxy dehydroepiandrostenedione, 3 α , 5 α -reduced-11 β -OH-androstenedione, 3 α , 5 α -reduced-corticosterone, 3 α , 5 α -tetrahydro-deoxycorticosterone, and 3 α , 5 α -reduced-aldosterone.

Other examples of steroids which can be used as 11 β -HSD1 dehydrogenase inhibitors include 11 β -hydroxy steroids such as 11 β -OH progesterone, 11 β -OH testosterone, 11 β -hydroxy androstenedione, 11 β -hydroxy dehydroepiandrostenedione, 11 β -progesterone, and chenodeoxycholic acid.

In another embodiment, the steroid is 5 α -reduced. Examples of 5 α -reduced steroids include 5 α -reduced-11 β -hydroxy testosterone, 5 α -reduced-11 β -hydroxy androstenedione, 5 α -reduced-11 β -hydroxy dehydroepiandrostenedione, and 5 α -reduced-11 β -progesterone.

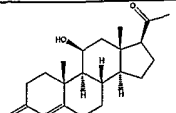
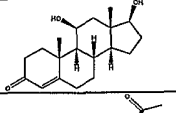
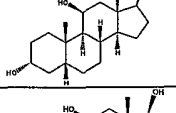
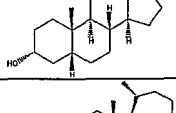
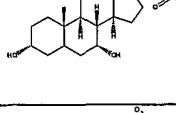
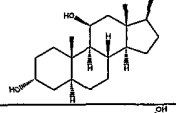
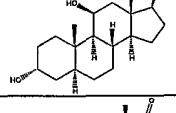
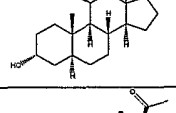
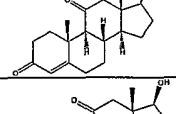
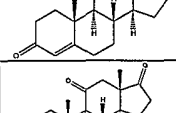
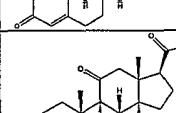
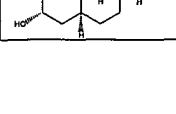
The term "11 β -HSD2 dehydrogenase inhibitor" includes agents which inhibit or decrease the dehydrogenase activity of 11 β -HSD2.

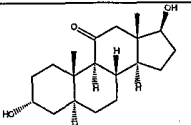
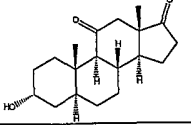
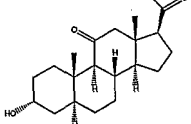
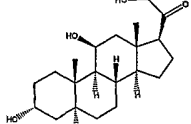
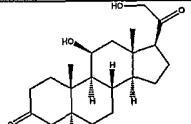
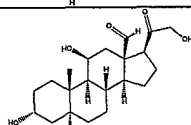
In one embodiment, the 11 β -HSD2 dehydrogenase inhibitor is a small molecule, such as a steroid or a derivative thereof. In one embodiment, the steroid is 3 α , 5 α -reduced. Examples of 11 β -HSD2 dehydrogenase inhibitors include, but are not limited to, 3 α , 5 α -reduced-11 β -OH-progesterone, 3 α , 5 α -reduced-11 β -OH-testosterone, 3 α , 5 α -reduced-11 β -OH-androstenedione, 3 α , 5 α -reduced-11-keto-progesterone, 3 α , 5 α -reduced-11-dehydro-corticosterone, 3 α , 5 α -reduced-corticosterone, or 3 α , 5 α -aldosterone. Other examples of 11 β -HSD2 dehydrogenase inhibitors include 11 β -OH-progesterone, 11 β -OH-testosterone, 11-keto-progesterone, and 5 α -dihydro-corticosterone.

Examples of 11 β -HSD1-reductase, 11 β -HSD1-dehydrogenase and 11 β -HSD2 dehydrogenase modulating compounds are described in Table 1.

TABLE 1

5

Compound Name	Structure	11 β -HSD1 Reductase	11 β -HSD1 Dehydrogenase	11 β -HSD2 Dehydrogenase
11 β -OH-progesterone		No Inhibition	Potent Inhibitor (Non-Selective)	Potent Inhibitor (Non-Selective)
11 β -OH-testosterone		No Inhibition	Inhibitor (Non-Selective)	Inhibitor (Non-Selective)
3 α ,5 β -reduced-11 β -OH-progesterone		No Inhibition	Moderate Inhibitor	No Inhibition
3 α ,5 β -reduced-11 β -OH-testosterone		No Inhibition	Moderate Inhibitor	No Inhibition
chenodeoxycholic acid (3 α ,5 β -reduced steroid)		No Inhibition	Selective inhibitor	No Inhibition
3 α ,5 α -reduced-11 β -OH-progesterone		No Inhibition	Potent Inhibitor (Non-Selective)	Potent Inhibitor (Non-Selective)
3 α ,5 α -reduced-11 β -OH-testosterone		No Inhibition	Potent Inhibitor (Non-Selective)	Potent Inhibitor (Non-Selective)
3 α ,5 α -reduced-11 β -OH-androstenedione		No Inhibition	Moderate Inhibitor	Potent Inhibitor (Non-Selective)
11-Keto-progesterone		Selective Inhibitor	No Inhibition	Potent Inhibitor
11-Keto-testosterone		Selective Inhibitor	No Inhibition	No Inhibition
11-Keto-androstenedione		Selective Inhibitor	No Inhibition	No Inhibition
3 α ,5 α -reduced-11-keto-progesterone		Selective Inhibitor	No Inhibition	Potent Inhibitor

3 α ,5 α -reduced-11-keto-testosterone		Selective Inhibitor	No Inhibition	Not tested
3 α ,5 α -reduced-11-keto-androstenedione		Selective Inhibitor	No Inhibition	Not Tested
3 α ,5 α -tetrahydro-11-dehydro-corticosterone		Potent Inhibitor	No Inhibition	Potent Inhibitor
3 α ,5 α -reduced-corticosterone		No Inhibition	Potent Inhibitor	Potent Inhibitor
5 α -dihydro-corticosterone		No inhibition	Potent Inhibitor	Potent Inhibitor
3 α , 5 α -reduced aldosterone		No Inhibition	Moderate Inhibitor	Potent Inhibitor

IV. Pharmaceutical Compositions

In yet another embodiment, the invention pertains to a pharmaceutical composition for increasing or decreasing male fertility. The composition includes an effective amount of an 11 β -HSD1 reductase, 11 β -HSD1 dehydrogenase, or 11 β -HSD2 dehydrogenase modulating, e.g., inhibiting, compound and a pharmaceutically acceptable carrier. In another embodiment, the pharmaceutical compositions may also comprise an inhibitor of 17 α -hydroxylase, 20 α -reductase or 20 β -reductase. In another embodiment, the invention also features a pharmaceutical composition comprising an effective amount of a 11 β -HSD1 reductase, 11 β -HSD1 dehydrogenase, or 11 β -HSD2 dehydrogenase modulating, e.g., inhibiting, compound, for modulating testosterone levels in a subject.

The phrase "pharmaceutically acceptable carrier" is art recognized and includes a pharmaceutically acceptable material, composition or vehicle, suitable for administering compounds of the present invention to mammals. The carriers include liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject agent from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and

potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations.

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

Examples of pharmaceutically acceptable antioxidants include: water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, α -tocopherol, and the like; and metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Formulations of the present invention include those suitable for oral, nasal, topical, transdermal, buccal, sublingual, rectal, vaginal, pulmonary and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect. Generally, out of one hundred per cent, this amount will range from about 1 per cent to about ninety-nine percent of active ingredient, preferably from about 5 per cent to about 70 per cent, most preferably from about 10 per cent to about 30 per cent.

Methods of preparing these formulations or compositions include the step of bringing into association a compound of the present invention with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a compound of the present invention with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Formulations of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of a compound of the present invention as an active ingredient. A compound of the present invention may also be administered as a bolus, electuary or paste.

10 In solid dosage forms of the invention for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the active ingredient is mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; humectants, such as glycerol; disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; solution retarding agents, such as paraffin; absorption accelerators, such as quaternary ammonium compounds; wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; absorbents, such as kaolin and bentonite clay; lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

The tablets, and other solid dosage forms of the pharmaceutical compositions of the present invention, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example,

hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

10 The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

Liquid dosage forms for oral administration of the compounds of the invention include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluent commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert dilutents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active compounds, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Formulations of the pharmaceutical compositions of the invention for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing one or more compounds of the invention with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active compound.

Formulations of the present invention which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

Dosage forms for the topical or transdermal administration of a compound of this invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound may be mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

The ointments, pastes, creams and gels may contain, in addition to an active compound of this invention, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to a compound of this invention, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane. Sprays also can be delivered by mechanical, electrical, or by other methods known in the art.

Transdermal patches have the added advantage of providing controlled delivery of a compound of the present invention to the body. Such dosage forms can be made by dissolving or dispersing the compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the active compound in a polymer matrix or gel.

Ophthalmic formulations, eye ointments, powders, solutions and the like, are also contemplated as being within the scope of this invention.

Pharmaceutical compositions of this invention suitable for parenteral administration comprise one or more compounds of the invention in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial, antiparasitic and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-administered drug form may be accomplished by dissolving or suspending the drug in an oil vehicle. The compositions also may be formulated such that its elimination is retarded by methods known in the art.

Injectable depot forms are made by forming microencapsule matrices of the subject compounds in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissue.

The preparations of the present invention may be given orally, parenterally, topically, or rectally. They are of course given by forms suitable for each administration route. For example, they are administered in tablets or capsule form, by injection, inhalation, eye lotion, ointment, suppository, etc. administration by injection, infusion or inhalation; topical by lotion or ointment; and rectal by suppositories. Oral administration or administration via inhalation is preferred.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

The phrases "systemic administration," "administered systemically," "peripheral administration" and "administered peripherally" as used herein mean the administration of a compound, drug or other material other than directly into the central nervous

system, such that it enters the patient's system and, thus, is subject to metabolism and other like processes, for example, subcutaneous administration.

These compounds may be administered to humans and other animals for therapy by any suitable route of administration, including orally, nasally, as by, for example, a
5 spray, rectally, intravaginally, parenterally, intracisternally and topically, as by powders, ointments or drops, including buccally and sublingually. Other methods for administration include via inhalation.

Regardless of the route of administration selected, the compounds of the present invention, which may be used in a suitable hydrated form, and/or the pharmaceutical
10 compositions of the present invention, are formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient,
15 composition, and mode of administration, without being toxic to the patient.

The selected dosage level will depend upon a variety of factors including the activity of the particular compound of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment,
20 other drugs, compounds and/or materials used in combination with the particular compound employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required. For
25 example, the physician or veterinarian could start doses of the compounds of the invention employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

In general, a suitable daily dose of a compound of the invention will be that
30 amount of the compound which is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above. Generally, intravenous and subcutaneous doses of the compounds of this invention for a patient will range from about 0.0001 to about 100 mg per kilogram of body weight per day, more preferably from about 0.01 to about 50 mg per kg per day, and still more
35 preferably from about 1.0 to about 100 mg per kg per day. An effective amount is that amount treats a glucocorticoid associated state.

If desired, the effective daily dose of the active compound may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms.

While it is possible for a compound of the present invention to be administered
5 alone, it is preferable to administer the compound as a pharmaceutical composition.

As set out above, certain embodiments of the present compounds can contain a basic functional group, such as amino or alkylamino, and are, thus, capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable acids. The term
10 "pharmaceutically acceptable salts" is art recognized and includes relatively non-toxic, inorganic and organic acid addition salts of compounds of the present invention. These salts can be prepared *in situ* during the final isolation and purification of the compounds of the invention, or by separately reacting a purified compound of the invention in its free base form with a suitable organic or inorganic acid, and isolating the salt thus formed. Representative salts include the hydrobromide, hydrochloride, sulfate,
15 bisulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate, mesylate, glucoheptonate, lactobionate, and laurylsulphonate salts and the like. (See, *e.g.*, Berge et al. (1977) "Pharmaceutical Salts", *J. Farm. SCI.* 66:1-19).

In other cases, the compounds of the present invention may contain one or more
20 acidic functional groups and, thus, are capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable bases. The term "pharmaceutically acceptable salts" in these instances includes relatively non-toxic, inorganic and organic base addition salts of compounds of the present invention. These salts can likewise be prepared *in situ* during the final isolation and purification of the compounds, or by
25 separately reacting the purified compound in its free acid form with a suitable base, such as the hydroxide, carbonate or bicarbonate of a pharmaceutically acceptable metal cation, with ammonia, or with a pharmaceutically acceptable organic primary, secondary or tertiary amine. Representative alkali or alkaline earth salts include the lithium, sodium, potassium, calcium, magnesium, and aluminum salts and the like.
30 Representative organic amines useful for the formation of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like.

The term "pharmaceutically acceptable esters" refers to the relatively non-toxic, esterified products of the compounds of the present invention. These esters can be
35 prepared *in situ* during the final isolation and purification of the compounds, or by separately reacting the purified compound in its free acid form or hydroxyl with a suitable esterifying agent. Carboxylic acids can be converted into esters *via* treatment with an alcohol in the presence of a catalyst. Hydroxyls can be converted into esters *via*

treatment with an esterifying agent such as alkanoyl halides. The term also includes lower hydrocarbon groups capable of being solvated under physiological conditions, e.g., alkyl esters, methyl, ethyl and propyl esters. (See, for example, Berge et al., *supra*.)

The invention also pertains to any one of the methods described *supra* further comprising administering to the subject a pharmaceutically acceptable carrier.

EXEMPLIFICATION OF THE INVENTION

Example 1: Ability of Corticosterone and 11-Dehydro-Corticosterone to Amplify the Contractile Responses of Phenylephrine

Experimental:

Male Sprague-Dawley (150-200g) rats were anesthetized with pentobarbital (50 mg/kg IP), and a median sternotomy was performed followed by the rapid removal of the thoracic aorta. The adventitia was removed, but the endothelium was left intact. The aorta was cut into 2-3 mm rings and individual rings were placed into a single well of a twenty four well culture plate and incubated at 37°C under 95% O₂- 5% CO₂. Each well contained 1 mL of DMEM/F12 containing 1% fetal bovine serum, streptomycin (100 µg/ml), penicillin (100 units/ml) and amphotericin (0.25 µg/ml). Aortic rings were incubated for 24 hours prior to contractility measurements with the following combinations of steroids, and antisense/nonsense oligonucleotides (3 µmol/L):

Corticosterone (10 nmol/L) + 11β-HSD2 antisense or 11β-HSD2 nonsense oligomer

Corticosterone (10 nmol/L) + 11β-HSD1 antisense or 11β-HSD1 nonsense oligomer

In 11-dehydrocorticosterone experiments with vehicle alone

11-dehydrocorticosterone (100 nmol/L) + 11β-HSD1 Antisense or 11β-HSD1 nonsense oligomer

Antisense phosphorothioate oligonucleotides, targeted to block either 11β-HSD2 or 11β-HSD1 gene expression, were obtained from Research Genetics, Huntsville AL. Antisense oligomers complementary to 20 bp sequences spanning the ribosome binding/translation start site were used. Oligomer sequences were: 5'-CAT AAC TGC CGT CCA ACA GC-3' (SEQ ID No.: 2) for 11β-HSD1 Antisense and 5'-AGC CCA GCG CTC CAT GAC TT- 3' (SEQ ID No. 3) for 11β-HSD2 antisense. In control experiments the corresponding sense sequence was used as the nonsense oligomer.

Antisense and nonsense oligomers were added directly to each well at 20 $\mu\text{g}/10:1$ sterile H_2O per well for a final concentration of 3 $\mu\text{mol}/\text{L}$.

For contraction measurements, aortic rings were suspended by tungsten wires with 1 g of tension and placed in a vessel bath containing serum free DMEM/F12 media at 37°C aerated with 95% O_2 -5% CO_2 , at pH 7.4. Vessels were equilibrated for 20 minutes and then tested with phenylephrine (1 nmol/L – 10 $\mu\text{mol}/\text{L}$). Although phenylephrine is structurally not a catecholamine, it is considered to be a functional catecholamine as it activates both α and β adrenoceptors. Due to its favorable stability characteristics, it is widely used as a catecholamine substitute in experiments of this nature. The intensity of contraction was assessed by use of a Narishige micromanipulator and model FT03 force transducer (Grass Instrument Co. West Warwick, RI). Measurements were recorded on computer using the Labview 4.1 Virtual Instrument System (National Instruments, Austin, TX). Adhering to this protocol, test vessel viability by demonstrating the ability of the vessel to vigorously contract when exposed to known vasoconstrictors and relax back to baseline after treatment with acetylcholine.

Results: Effect of 11 β -HSD Antisense on Vascular Contractile Response

Experiments were carried out to determine whether specific 11 β -HSD2 antisense oligomers affect the contractile response of vascular rings. Rat aortic rings, with endothelium intact, were incubated for 24 hours with corticosterone (10 nmol/L) and either specific 11 β -HSD2 antisense oligomers (3 $\mu\text{mol}/\text{L}$) or nonsense oligomers (3 $\mu\text{mol}/\text{L}$). Following incubation, the contractile responses to graded concentrations of phenylephrine were determined. Previously, it had been demonstrated that the incubation of aortic rings with corticosterone resulted in amplified contractile responses to graded concentrations of phenylephrine compared to controls. The exposure of rings to corticosterone together with 11 β -HSD2 antisense demonstrated a statistically significant increase in the contractile response to all concentrations (1, 10, 100 nmol/L and 1 $\mu\text{mol}/\text{L}$) of phenylephrine (Fig 1).

In the rat, both vascular endothelial and smooth muscle cells contain 11 β -HSD1. Even though this isoform operates mainly as a reductase under physiologic conditions, it was examined if 11 β -HSD1 antisense oligomers had an effect on the ability of corticosterone to amplify the contractile responses to phenylephrine in vascular tissue. Rings were incubated for 24-hours with corticosterone (10 nmol/L) and either 11 β -HSD1 antisense oligomers (3 $\mu\text{mol}/\text{L}$) or nonsense oligomers (3 $\mu\text{mol}/\text{L}$). In rings treated with 11 β -HSD1 antisense the contractile responses to all concentrations of phenylephrine (10 nmol/L, 100 nmol/L and 1 $\mu\text{mol}/\text{L}$) were significantly increased compared to rings treated with corticosterone and nonsense oligomers (Fig 2).

In rat vascular tissue, 11 β -HSD1 acts predominantly as a reductase metabolizing inactive 11-dehydro-glucocorticoid back to the active parent hormone. 11-dehydro-corticosterone (just like corticosterone) also amplifies the contractile responses to phenylephrine in rat aortic rings (Fig 3). In the rat, 11 β -HSD1 is present in both
5 vascular endothelial and smooth muscle cells and under physiological conditions this enzyme functions predominantly as a reductase.

Furthermore, the effect of 11 β -HSD1 antisense oligomers on the ability of 11-dehydro-corticosterone to amplify the contractile responses to phenylephrine was studied. Rings were incubated for 24 hours with 11-dehydro-corticosterone (100
10 nmol/L) and either 11 β -HSD1 antisense (3 μ mol/L) or nonsense (3 μ mol/L) oligomers. 11 β -HSD1 antisense oligomers attenuated the ability of 11 β -dehydro-corticosterone to amplify the contractile response to all concentrations of phenylephrine compared to 11-dehydro-corticosterone plus 11 β -HSD1 nonsense oligomers. Statistically significant decreases were observed at 100 nmol/L and 1 μ mol/L phenylephrine (Fig 3).

15 In aortic rings incubated (24-hours) with corticosterone (10 nmol/L) and 11 β -HSD2 antisense (3 μ mol/L), the contractile response to graded concentrations of phenylephrine (PE: 10 nmol/L – 1 μ mol/L) were significantly ($P < 0.05$) increased compared to rings incubated with corticosterone and 11 β -HSD2 nonsense. 11 β -HSD1 antisense oligomers also enhanced the ability of corticosterone to amplify the contractile
20 response to phenylephrine.

Discussion

Earlier experiments showed that inhibitors of 11 β -HSD dehydrogenase activity enhance the ability of corticosterone to amplify the vasoconstrictive actions of
25 phenylephrine and angiotensin II in rat aorta. The examples show that a specific 11 β -HSD2 antisense oligomer also enhances the ability of corticosterone to amplify the contractile responses of catecholamines. Since 11 β -HSD2 appears to exist only in endothelial cells, this observation supports a role for the action of glucocorticoids in affecting endothelial cell function. Although 11 β -HSD1 acts predominantly as a
30 reductase in vascular tissue, 11 β -HSD1 antisense oligomers also enhanced the ability of corticosterone to amplify the contractile effects of phenylephrine in rat aortic rings. This observation suggests that 11 β -HSD1-dehydrogenase, in addition to 11 β -HSD2, also operates to protect GR and MR from over-activation by glucocorticoids in vascular tissue. Further experiments to determine whether antisense oligomers down-regulate
35 mRNA and protein expression of their respective 11 β -HSD isoform under conditions in which they enhance contractile responses in aortic rings will be done. Using a similar protocol to the one described here, it has been shown using RT-PCR analysis, that 11 β -

HSD2 antisense and 11 β -HSD1 antisense down-regulate the expression of their respective enzyme isoforms in cultured rat vascular endothelial and smooth muscle cells.

The example confirms that 11-dehydro-corticosterone also amplifies the contractile actions of catecholamines in rat aortic rings. Since 11-dehydro-
5 glucocorticoids do not bind to GR (or MR) to any major extent, it is proposed that 11-dehydro-corticosterone is metabolized back to corticosterone by 11 β -HSD1-reductase in vascular smooth muscle and/or endothelial cells. This hypothesis is supported by the discovery that 11-keto-progesterone, a specific inhibitor of 11 β -HSD1-reductase activity (backward reaction), diminished the ability of 11-dehydro-corticosterone to amplify the
10 contractile effects of phenylephrine and decreased the metabolism of 11-dehydro-corticosterone back to corticosterone. The examples also demonstrate that 11 β -HSD1 antisense oligomer also attenuates the ability of 11-dehydro-corticosterone to amplify the contractile responses of phenylephrine indicating that the down-regulation of 11 β -HSD1 gene expression can affect the regeneration of active glucocorticoid (from 11-
15 dehydro-glucocorticoid) in vascular tissue. Indeed, the examples show that 11 β -HSD1 antisense can significantly reduce the metabolism of 11-dehydro-corticosterone back to corticosterone in aortic ring preparations.

Example 2: Metabolism of Corticosterone and 11-Dehydro-Corticosterone in

20 Vascular Tissue

Experimental:

The effects of 11 β -HSD1 and 11 β -HSD2 antisense on the inter-conversion of ³H-corticosterone and ³H-11-dehydro-corticosterone by aortic rings was also determined. Rings (2-3mm) obtained in a similar manner as those in the contraction studies, were
25 incubated in 1 ml DMEM/F12 media containing 1% FBS at 37°C under 95% O₂-5% CO₂ in 24-well culture plates. Rings were incubated for 24 hours with:

(i) ³H-corticosterone (10 nmol/L) $\pm$ 11 β -HSD2 or 11 β -HSD1 antisense (3 μ mol/L); control groups received nonsense oligomers. The amount of ³H-11-dehydro-corticosterone in the incubation medium after 24 hrs was then measured. The effects of
30 11 β -HSD1 antisense/nonsense were measured in quadruplicate (n = 6 aortic rings per well) and the effects of 11 β -HSD2 antisense/nonsense in duplicate (n = 8 aortic rings per well),

(ii) ³H-11-dehydro-corticosterone (10 nmol/L) $\pm$ 11 β -HSD1 antisense (3 μ mol/L); this experiment was performed in duplicate (n = 10 aortic rings per well).
35 Control groups were incubated with the appropriate nonsense oligomer. ³H-corticosterone in the incubation medium after 24 hrs was then measured. In this experiment, aortic rings were also analyzed for ³H-corticosterone content. Rings from

duplicate incubations (total n = 20) were blotted dry, pooled and homogenized in 50 % methanol using a Polytron. The homogenates were then centrifuged, extracted as below using Sep-Paks and injected onto a HPLC system for analysis.

5 Incubation media was collected, ran through a Sep-Pak and eluted with 3 mls of methanol, the eluate was then dried under nitrogen and reconstituted in 500:1 methanol. The aortic rings were dried and weighed. The steroids present in the eluate were separated by high-pressure liquid chromatography with a Dupont Zorbax C8 column eluted at 44°C at a flow rate of 1 mL/min using 55% methanol for 10 minutes. Steroids
10 were observed by monitoring radioactivity on-line with a Packard Radiomatic Flo-One/Beta Series A-500 counter connected to a Dell Optiflex 425 S/L computer. Corticosterone and 11-dehydro-corticosterone were identified by comparing their retention times with that of known standards.

Corticosterone and phenylephrine were obtained from Sigma (St Louis, MO),
15 11-dehydrocorticosterone from Research Plus (Bayonne, NJ) and ³H-steroids from New England Nuclear (Boston, MA). Where appropriate, data were expressed as mean ± SE and analyzed using ANOVA and the Student's *t* test with Bonferroni modification. *P* values of less than 0.05 are considered significant.

20 *Results: Effects of 11β-HSD Antisense on Steroid Metabolism*

A series of experiments were then conducted to test whether 11β-HSD2 and 11β-HSD1 antisense oligomers did affect the enzymatic conversion of corticosterone and 11-dehydrocorticosterone. In experiments in which aortae were taken from rats (n= 4) and 6 rings cut from each aorta were incubated for 24 hrs with ³H-corticosterone (10 nM)
25 plus 11β-HSD1 antisense (3 μM), the conversion of corticosterone to 11-dehydrocorticosterone was 21% lower than in aortic rings incubated with corticosterone and 11β-HSD1 nonsense oligomers (Figure 4). In a further two experiments, aortae were taken from rats (n = 2) and 8 aortic rings cut from each. Aortic ring preparations incubated for 24hrs with corticosterone and 11β-HSD2 antisense (3 μM), demonstrated a
30 24% reduction in the conversion of corticosterone to 11-dehydrocorticosterone compared to aortic rings incubated with corticosterone and 11β-HSD2 nonsense (Figure 4).

To determine the effects of 11β-HSD1 antisense on 11β-HSD1-reductase activity rat aortae were taken from rats(n= 2) and 10 aortic rings cut from each. These aortic
35 rings were then incubated for 24 hours with ³H-11-dehydrocorticosterone and either 11β-HSD1 antisense or nonsense and the production of corticosterone was measured. The production of ³H-corticosterone was markedly reduced in rings incubated with 11β-HSD1 antisense compared to rings incubated with 11β-HSD1 nonsense oligomers

(Figure 4, representative HPLC chromatograms from these experiments are also shown in Figure 5). Thus, 11 β -HSD1 antisense profoundly diminished the ability of the rat aortic rings to metabolize 11-dehydro-corticosterone back to corticosterone. The aortic ring tissue in these experiments was also pooled (n = 20) and analyzed for steroid
5 content. The amount of radioactivity in the tissue was approximately 2-3% of the total radioactivity in the incubation media. The production of ³H-corticosterone in aortic rings incubated with 11 β -HSD1 antisense was again markedly lower than that in rings incubated with 11 β -HSD1 nonsense oligomers (see HPLC chromatograms, Figure 5). The levels of ³H-11-dehydrocorticosterone metabolism measured in the incubate and in
10 the aortic tissue were very similar (Figure 5). This indicates that measuring steroid content in the media does not under-represent the level of steroid metabolism in the tissue compartment.

Discussion

15 In this example, experiments were undertaken to determine whether antisense oligomers could affect 11 β -HSD enzyme activity and, indeed, it has been demonstrated that 11 β -HSD2 and 11 β -HSD1 antisense caused moderate reductions (24 and 21% respectively) in the metabolism of corticosterone. These reductions in
20 metabolism translate to relatively small increases in residual corticosterone levels in the aortic ring tissue that would not appear to account for the relatively large increases in phenylephrine-induced vasoconstriction observed in the contractile studies. However, glucocorticoids have been reported to not only amplify the contractile effects of catecholamines in vascular tissue but to also diminish the effects of certain
25 vasorelaxation pathways (glucocorticoids decrease nitric oxide and prostaglandin I₂ synthesis); such actions would serve to further enhance the effects of glucocorticoids on increasing catecholamine-induced vasoconstriction and may explain how small changes in glucocorticoid levels can have profound effects on vascular tone.

In addition, 11 β -HSD2 and 11 β -HSD1 antisense also decreased the metabolism of corticosterone to 11-dehydro-corticosterone. 11-dehydro-corticosterone (100 nmol/L)
30 also amplified the contractile response to phenylephrine in aortic rings ($P < 0.01$), most likely due to the generation of active corticosterone by 11 β -HSD1-reductase; this effect was significantly attenuated by 11 β -HSD1 antisense. 11 β -HSD1 antisense also caused a marked decrease in the metabolism of 11-dehydro-corticosterone back to corticosterone by 11 β -HSD1-reductase. These findings underscore the importance of 11 β -HSD2 and
35 11 β -HSD1 in regulating local concentrations of glucocorticoids in vascular tissue. They also indicate that decreased 11 β -HSD2 activity may be a possible mechanism in hypertension and other blood pressure associated disorders and that 11 β -HSD1-reductase may be a possible target for anti-hypertensive therapy.

The results of these examples underscore the importance of 11 β -HSD2 in regulating the access of glucocorticoids to GR and/or MR in vascular tissue and suggest that 11 β -HSD1-dehydrogenase may also play a role in protecting GR and MR in this tissue. In addition, they suggest that the antisense oligomers used in these experiments
5 down-regulate 11 β -HSD gene expression and decrease glucocorticoid metabolism in vascular tissue, an effect leading to increased vascular responsiveness to catecholamines.

The examples also demonstrate that both 11 β -HSD2 and 11 β -HSD1 regulate local glucocorticoid concentrations in vascular tissue with 11 β -HSD2 and 11 β -HSD1-dehydrogenase working to decrease- and 11 β -HSD1-reductase increase the amount of
10 glucocorticoid that can access GR and MR in vascular smooth muscle. Physiological concentrations of both free corticosterone and 11-dehydrocorticosterone are similar over the course of the day in rodents. Therefore significant quantities of not only glucocorticoid, but also of 11-dehydro-glucocorticoid are available for conversion back to the glucocorticoid. Since glucocorticoids amplify catecholamine and angiotensin II
15 pressor responses and may inhibit the effects of some vasorelaxant pathways, a possible mechanism that may increase vascular tone and induce hypertension includes a decrease in 11 β -HSD2 activity. Interestingly, many patients with essential hypertension also demonstrate decreased 11 β -HSD2 activity as assessed by altered plasma and urinary cortisol:cortisone ratios. Moreover, the plasma half-life of 11 α -³H-cortisol is prolonged
20 in patients with essential hypertension consistent with the idea that 11 β -HSD2 activity is diminished in this condition. The present work also suggests that since 11 β -HSD1 reductase generates active glucocorticoid in vascular tissue, a possible therapeutic target in the treatment of hypertension could be the specific inhibition of 11 β -HSD1 reductase activity.

25

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments and methods described herein. Such equivalents are intended to be encompassed by the
30 scope of the following claims.

All patents, patent applications, and literature references cited herein are hereby expressly incorporated by reference.

CLAIMS

1. A method for increasing male fertility, comprising administering an effective amount of a 11β -HSD1 reductase inhibitor to a subject, such that said fertility is
5 increased.
2. A method for increasing testosterone levels in a subject, comprising administering to said subject an effective amount of a 11β -HSD1 reductase inhibitor, such that testosterone levels in said subject are increased.
- 10 3. The method of claim 1 or 2, wherein said 11β -HSD1 reductase inhibitor is selective for testicular 11β -HSD1 reductase.
4. The method of claim 2, wherein said 11β -HSD1 reductase inhibitor is a steroid
15 or a derivative thereof.
5. The method of claim 4, wherein said steroid is an 11-keto steroid.
6. The method of claim 5, wherein said 11-keto steroid is 11-keto-progesterone, 11-
20 keto-testosterone, 11-keto-androsterone, 11-keto androstenedione, or 11-keto dehydroepiandrostenedione.
7. The method of claim 4, wherein said steroid is 3α , 5α -reduced or 3α , 5β -reduced.
- 25 8. The method of claim 7, wherein said steroid is 3α , 5α -reduced-11-ketoprogesterone, 3α , 5α -reduced-11-keto-testosterone, 3α , 5α -reduced-11-keto-androstenedione, 3α , 5α -tetrahydro-11-dehydro-corticosterone, 3α , 5α -reduced-11-keto-androsterone, 3α , 5α -tetrahydro-progesterone, 3α , 5α -tetrahydro-testosterone, 3α , 5α -tetrahydro-deoxycorticosterone, 3α , 5β -tetrahydro-deoxycorticosterone or 3α , 5α -
30 reduced-11-keto dehydroepiandrostenedione.
9. The method of claim 4, wherein said steroid is 5α -reduced.
10. The method of claim 9, wherein said steroid is 5α -reduced-11-ketoprogesterone,
35 5α -reduced-11-keto-testosterone, 5α -reduced-11-keto-androstenedione, 5α -reduced-11-dehydro-corticosterone, 5α -reduced-11-keto-androsterone, or 5α -reduced-11-keto dehydroepiandrostenedione.

11. A method for decreasing male fertility, comprising administering to a subject an effective amount of a 11β -HSD1 dehydrogenase inhibitor, such that said fertility is decreased.
- 5 12. A method for decreasing testosterone levels in a subject, comprising administering to a subject an effective amount of a 11β -HSD1 dehydrogenase inhibitor, such that testosterone levels in said subject are decreased.
13. The method of claim 11 or 12, wherein said 11β -HSD1 dehydrogenase inhibitor
10 is selective for testicular 11β -HSDI dehydrogenase.
14. The method of claim 11 or 12, wherein said steroid is 11β -hydroxy testosterone, 11β -hydroxy androstenedione, 11β -hydroxy dehydroepiandrosterone, 11β -progesterone, or chenodeoxycholic acid.
- 15 15. The method of claim 11 or 12, wherein said 11β -HSD1 dehydrogenase inhibitor is a 3α , 5β -reduced steroid, a 3α , 5α -reduced steroid, or a 5α -reduced steroid.
16. The method of claim 15, wherein said steroid is 3α , 5α -reduced- 11β -hydroxy
20 testosterone, 3α , 5α -reduced- 11β -hydroxy androstenedione, 3α , 5α -reduced- 11β -hydroxy dehydroepiandrosterone, 3α , 5α -reduced-corticosterone, 3α , 5α -reduced-aldosterone, 3α , 5β -tetrahydro-deoxycorticosterone, 3α , 5β -tetrahydro-progesterone, 3α , 5β -tetrahydro-testosterone, 3α , 5α -tetrahydro-deoxycorticosterone, or 3α , 5α -reduced- 11β -progesterone.
- 25 17. The method of claim 15, wherein said steroid is 5α -reduced- 11β -hydroxy testosterone, 5α -reduced- 11β -hydroxy androstenedione, 5α -reduced- 11β -hydroxy dehydroepiandrosterone, or 5α -reduced- 11β -progesterone.
- 30 18. The method of claim 15, wherein said steroid is 3α , 5β -reduced- 11β -OH-progesterone, or 3α , 5β -reduced- 11β -OH-testosterone.
19. The method of any one of claims 1-18, wherein said subject is a human.
- 35 20. The method of any one of claims 1-19, further comprising administering a pharmaceutically acceptable carrier.

21. A pharmaceutical composition comprising an effective amount of 11-keto-progesterone, 11-keto-testosterone, 11-keto-androsterone, 11-keto androstenedione, 11-keto dehydroepiandrosterone, 3 α , 5 α -reduced-11-ketoprogesterone, 3 α , 5 α -reduced-11-keto-testosterone, 3 α , 5 α -reduced-11-keto-androstenedione, 3 α , 5 α -tetrahydro-11-dehydro-corticosterone, 3 α , 5 α -reduced-11-keto-androsterone, 3 α , 5 α -reduced-11-keto dehydroepiandrosterone, 5 α -reduced-11-ketoprogesterone, 5 α -reduced-11-keto-testosterone, 5 α -reduced-11-keto-androstenedione, 5 α -reduced-11-dehydro-corticosterone, 5 α -reduced-11-keto-androsterone, 5 α -reduced-11-keto dehydroepiandrosterone, 3 α , 5 β -tetrahydro-deoxycorticosterone, 3 α , 5 α -tetrahydro-progesterone, 3 α , 5 α -tetrahydro-testosterone, 3 α , 5 α -tetrahydro-deoxycorticosterone, or a pharmaceutically acceptable salt, ester, or prodrug thereof and a pharmaceutically acceptable carrier, wherein said effective amount is effective to increase male fertility.
22. A pharmaceutical composition comprising an effective amount of 11 β -hydroxy testosterone, 11 β -hydroxy androstenedione, 11 β -hydroxy dehydroepiandrosterone, 11 β -progesterone, chenodeoxycholic acid, 3 α , 5 α -reduced-11 β -hydroxy testosterone, 3 α , 5 α -reduced-11 β -hydroxy androstenedione, 3 α , 5 α -reduced-11 β -hydroxy dehydroepiandrosterone, 3 α , 5 α -reduced-corticosterone, 3 α , 5 α -reduced-aldosterone, 3 α , 5 α -reduced-11 β -progesterone, 5 α -reduced-11 β -hydroxy testosterone, 5 α -reduced-11 β -hydroxy androstenedione, 5 α -reduced-11 β -hydroxy dehydroepiandrosterone, 5 α -reduced-11 β -progesterone, 3 α , 5 β -reduced-11 β -OH-progesterone, 3 α , 5 β -reduced-11 β -OH-testosterone, 3 α , 5 β -tetrahydro-deoxycorticosterone, 3 α , 5 β -tetrahydro-progesterone, 3 α , 5 β -tetrahydro-testosterone, 3 α , 5 α -tetrahydro-deoxycorticosterone, 3 α , 5 β -chenodeoxycholic acid or a pharmaceutically acceptable salt, prodrug, or ester thereof and pharmaceutically acceptable carrier, wherein said effective amount is effective to decrease male fertility.

FIGURE 1

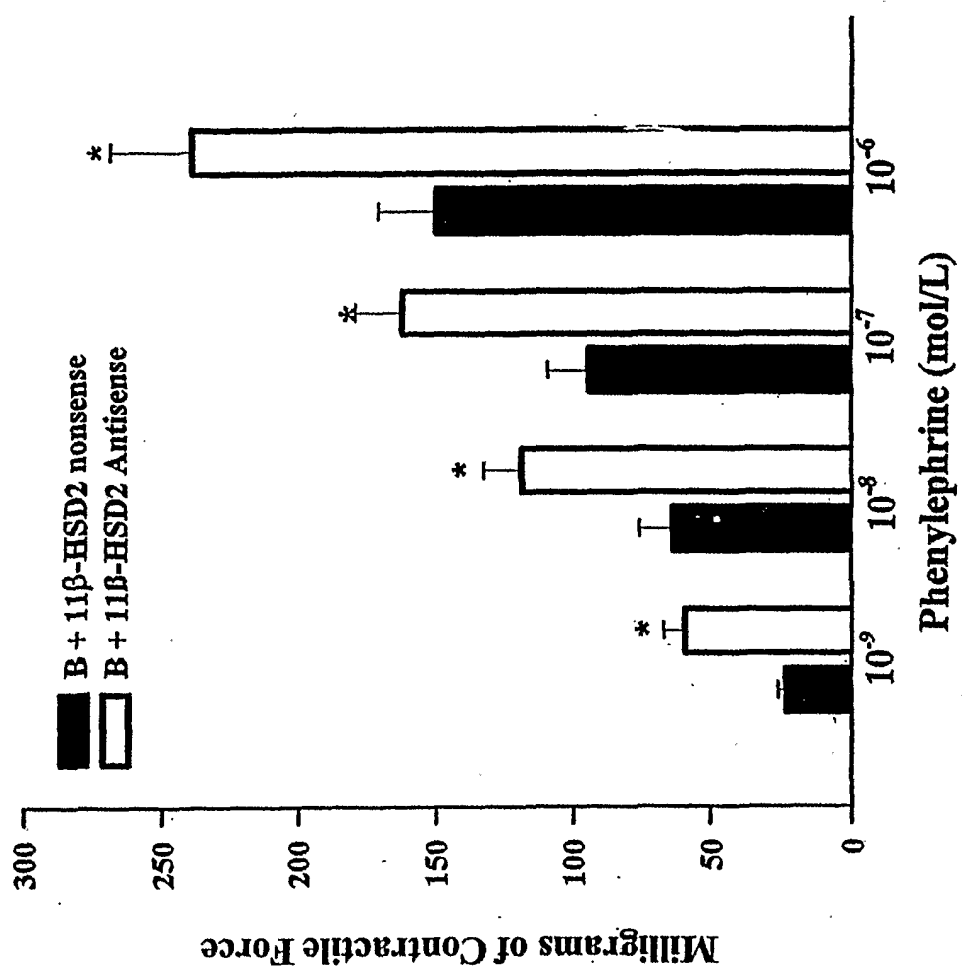


FIGURE 2

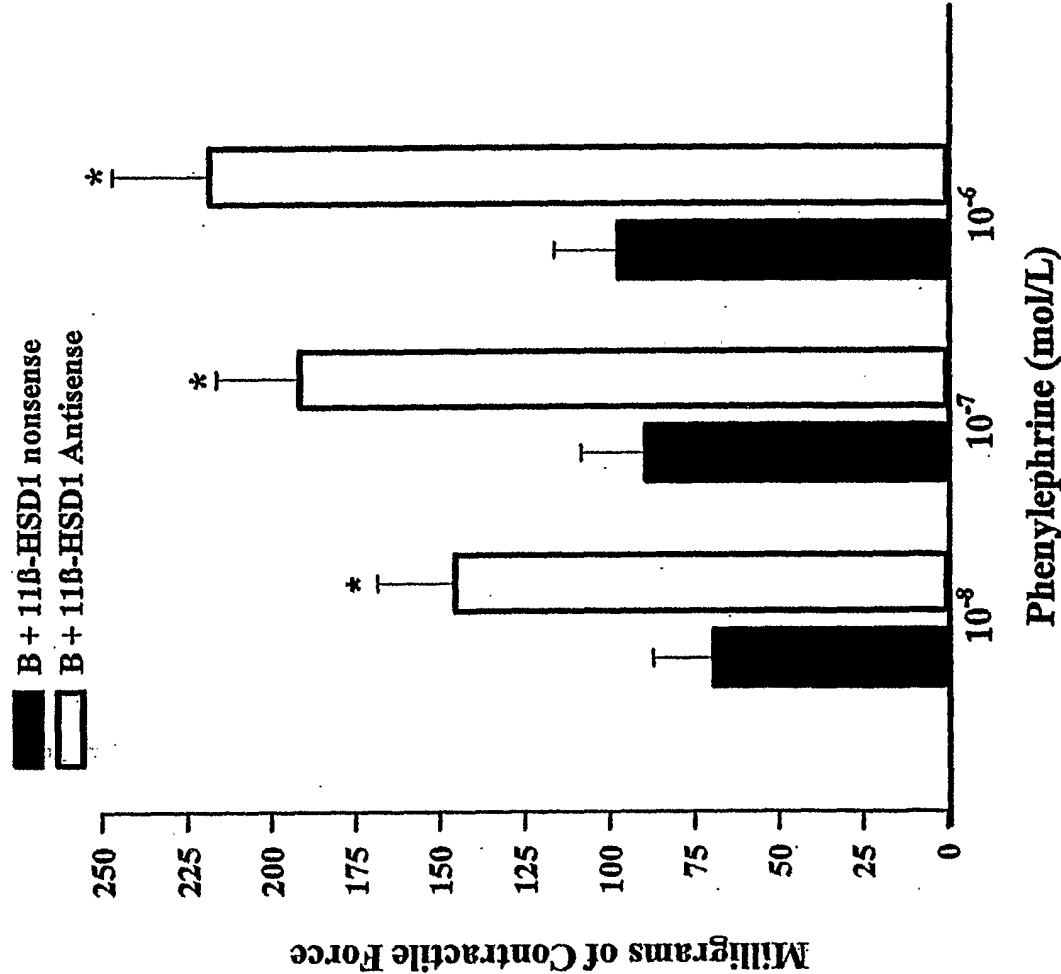


FIGURE 3

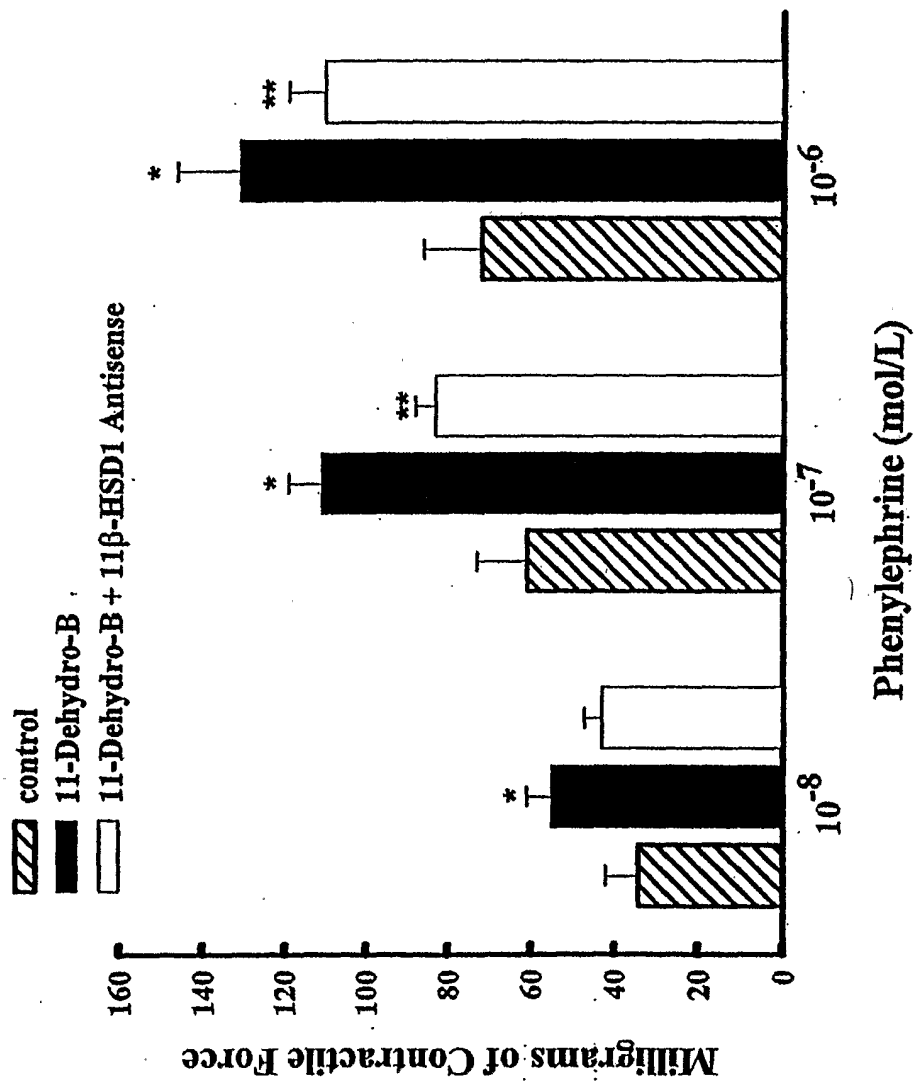


FIGURE 4

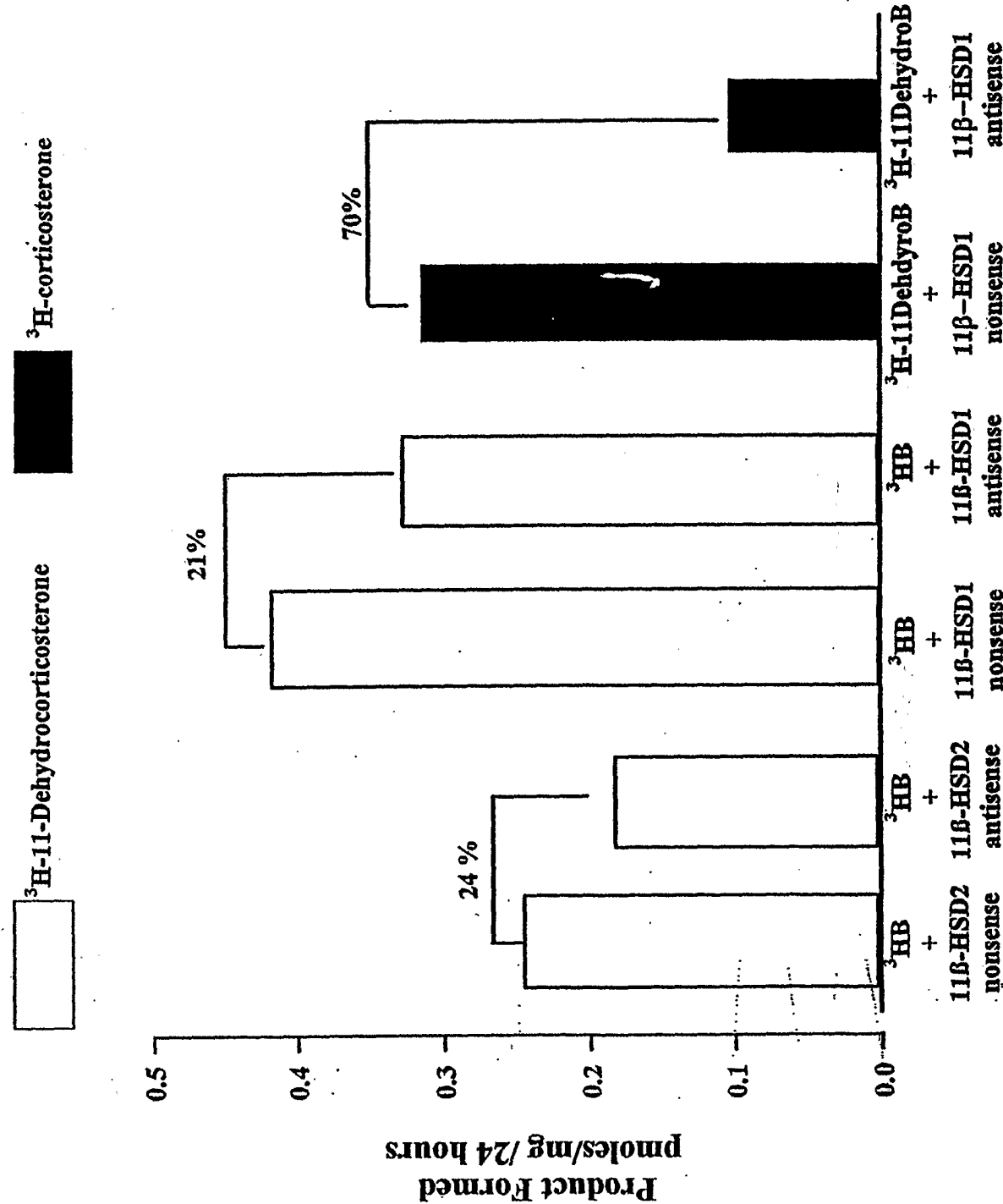


FIGURE 5A

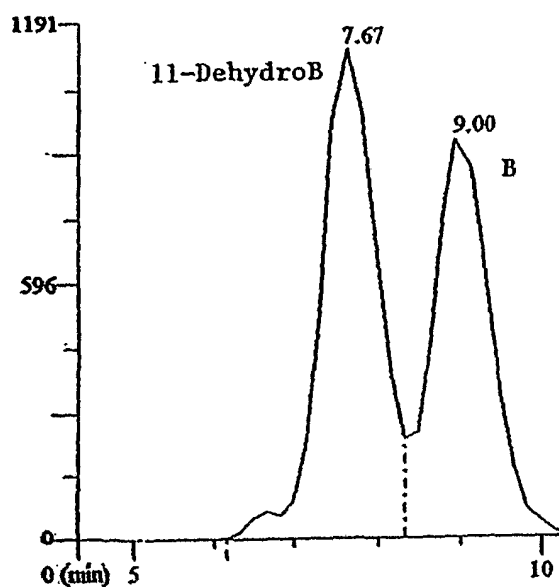


FIGURE 5B

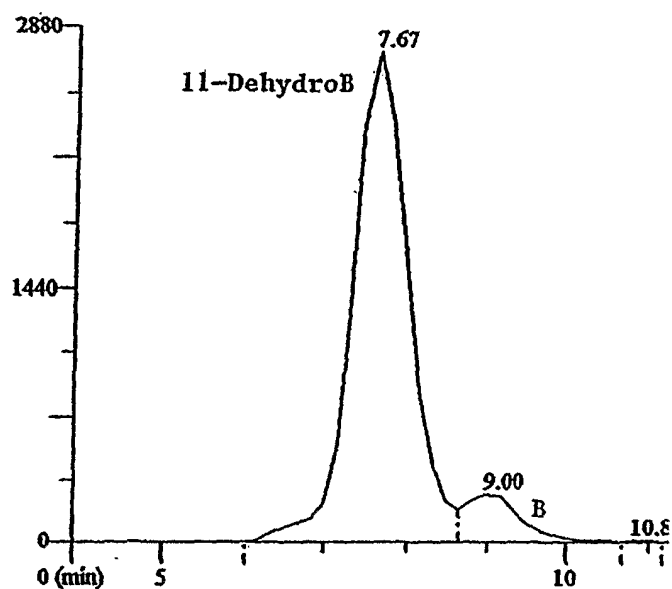


FIGURE 5C

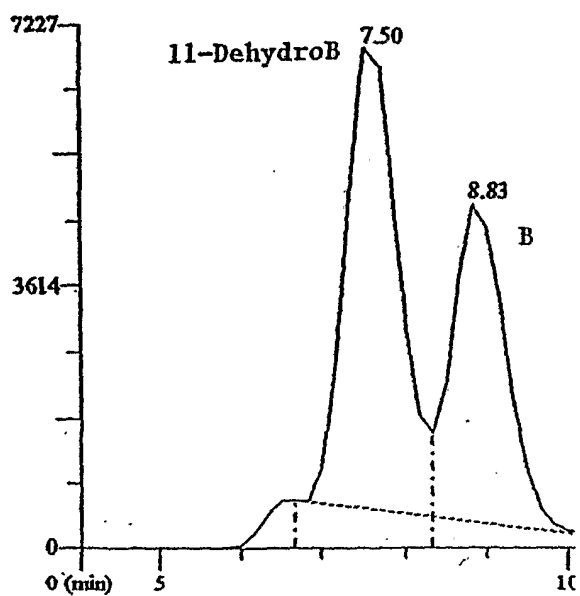
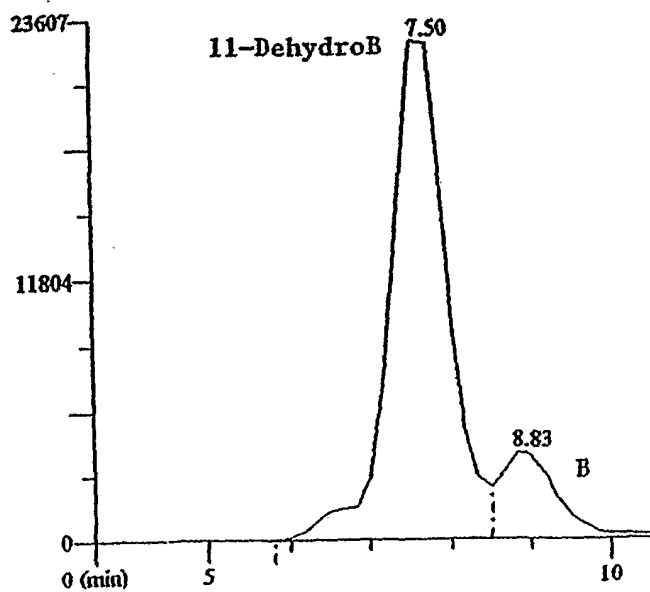


FIGURE 5D



SEQUENCE LISTING

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<120> SELECTIVE TESTICULAR 11 -HSD INHIBITORS AND METHODS OF
USE THEREOF

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