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Dehydroepiandrosterone (DHEA) is an anabolic steroid like dihydrotestosterone (DHT), the most potent natural androgen, and tetrahydrogestrinone (THG)

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Abstract

We have recently taken advantage of the unique power of DNA microarrays to compare the genomic expression profile of tetrahydrogestrinone (THG) with that of dihydrotestosterone (DHT), the most potent natural androgen, thus clearly demonstrating that THG is an anabolic steroid. In 2004, the U.S. Controlled Substances Act has been modified to include androstenedione (4-dione) as an anabolic steroid. However, despite the common knowledge that dehydroepiandrosterone (DHEA) is the precursor of testosterone, DHEA has been excluded from the list of anabolic steroids. We thus used the same DNA microarray technology to analyze the expression profile of practically all the 30 000 genes of the mouse genome modulated by DHEA and DHT in classical androgen-sensitive tissues. Daily subcutaneous injections of DHT (0.1 mg) or DHEA (3 mg) for 1 month in gonadectomized C57BL6/129 SV mice increased ventral prostate, dorsal prostate, seminal vesicle and preputial gland weight ($p < 0.01$ for all tissues). As early as 24 h after single injection of the two steroids, 878, 2681 and 14 probe sets were commonly stimulated or inhibited ($p < 0.01$, change $\geq 30\%$), in the prostate (ventral + dorsal), seminal vesicles and preputial glands, respectively, compared to tissues from gonadectomized control animals. After 7 days of daily treatment with DHEA and DHT, 629, 919 and 562 probe sets were commonly modulated in the same tissues while after 27 days of treatment, 1195, 5127 and 2883 probe sets were modulated, respectively. In analogy with the data obtained with THG, the present microarray data provide an extremely precise and unquestionable genomic signature and proof of the androgenic/anabolic activity of DHEA. Such data add to the literature showing that DHEA is transformed into androgens in the human peripheral tissues as well as in laboratory animal species, including the monkey, thus exerting potent androgenic/anabolic activity. The present microarray approach to identify anabolic compounds is applicable to all potential androgenic/anabolic compounds.

Keywords: Mouse; DHEA; THG; DHT; DNA microarrays; Gene expression profile; Androgenic/anabolic compounds

1. Introduction

Major efforts are being devoted by the World Anti-Doping Agency (WADA) in collaboration with its national counterparts in most countries in order to eliminate doping from sports. In addition to their performance-enhancing properties prohibited by the International Olympic Committee (IOC), these compounds carry serious health safety risks, even for natural anabolic substances like testosterone, 4-dione and DHEA which are used at pharmacological or supraphysiological doses by athletes and others for muscle building purposes.

Recently, the Anabolic Steroid Control Act of 2004 has amended the U.S. Controlled Substances Act to include 4-dione as an anabolic steroid. According to this bill, "the term anabolic steroid means any drug or hormonal substance chemically and pharmacologically related to testosterone (other than estrogens, progestins, corticosteroids and DHEA)". DHEA was thus excluded "a priori" from the list of anabolic steroids. This list includes androstanediol (3α-diol and 3β-diol), androstanedione (A-dione), androstenediol (5-diol), 4-dione, dihydrotestosterone (DHT), testosterone

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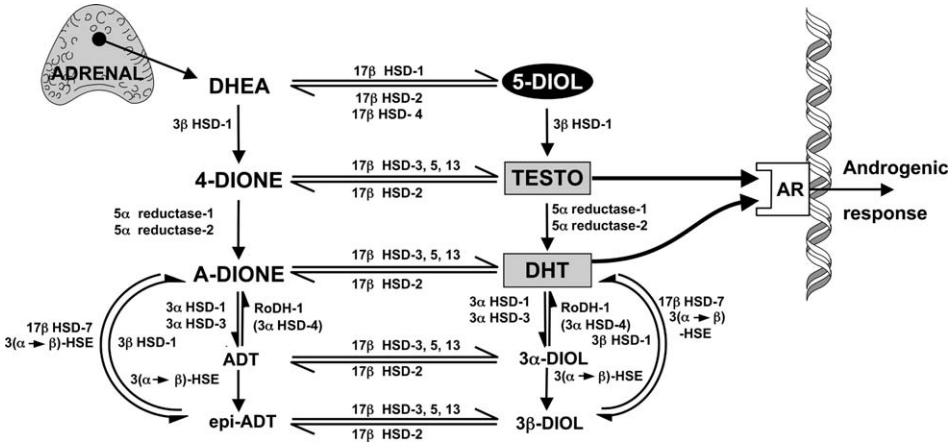

Fig. 1. Pathways of androgen formation in the human.

(testo) which are all steroids of the androgenic steroidogenic pathway (Fig. 1). A long series of chemically modified steroids is also included. As illustrated in Fig. 1, despite the well known steroidogenic pathway where DHEA is acting as precursor of both 4-dione and 5-diol, two steroids directly transformed into testosterone [1], DHEA has been somewhat surprisingly excluded from the list along with estrogens, progestins and corticosteroids. The exclusion of these three classes of steroids is scientifically justified by their well-known lack of androgenic activity, except for some synthetic progestins [2,3]. The situation, however, is completely different for DHEA which is an obligatory precursor of both testosterone and DHT in peripheral tissues in both men and women [4–6].

We have recently taken advantage of the unique sensitivity of DNA microarrays to compare the genomic expression profile of THG with that of DHT, the most potent natural androgen, thus clearly demonstrating that THG is an anabolic steroid [7]. We use the same DNA microarray technique in the present study to clearly demonstrate that DHEA exerts an effect superimposable to that of DHT and THG, on a large series of genes, thus showing that DHEA is, without any doubt, an anabolic steroid like the other natural and chemically derived androgens already included in the list of the Anabolic Steroid Control Act of 2004.

2. Materials and methods

2.1. Animals and treatment

In the 24 h experiment, the male mice obtained from the interbreeding of C57BL6 and 129SV mice were produced in our animal facility. At the beginning of the study, mice were 27–33 weeks old. The animals were housed individually in an environmentally controlled room (temperature: 22 ± 3 °C; humidity: 50 ± 20%; 12-h light–12-h dark cycles, lights on at 07:15 h). The mice had free access to tap water and a commercial rodent feed (Harlan Teklad, #2018 (pellets), Madison, Wisconsin). The experiment was conducted in an animal facility approved by the Canadian Council on Animal Care (CCAC) and the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). The study was performed in accordance with the CCAC Guide for Care and Use of Experimental Animals.

For the 24 h study, animals weighing between 24 and 60 g (mean 42 g) were randomized according to their body weights and were assigned to three groups of eight animals each as follows: (1) gonadectomized (GDX) control; (2) GDX + DHT (0.1 mg/mouse); (3) GDX + DHEA (2 mg/mouse). On day 1 of the study, the animals were castrated (GDX) under isoflurane anesthesia. On day 7 of the study, 24 h prior to necropsy, mice received a single subcutaneous injection (0.2 mL/mouse) of the vehicle alone (5% ethanol–0.4% methylcellulose; group 1) or DHT (group 2) or DHEA (group 3) suspended in the same vehicle. DHT and DHEA were obtained from Steraloids Inc. (Newport, USA) and Scheweizerhall Inc. (New Jersey, USA), respectively. On day 8 of the study, mice under isoflurane anesthesia were exsanguinated at the abdominal aorta followed by cervical dislocation. The prostate (ventral + dorsal), seminal vesicles and preputial glands were collected and rapidly frozen in liquid nitrogen. Tissues were kept at −80 °C until RNA extraction. The tissues collected from the mice of the same group were pooled for RNA extraction and analysis.

For the 4 weeks study, the male mice used were also produced in our animal facility and were obtained from the interbreeding of C57BL6 and 129SV mice. At the beginning of the study, mice were 17–22 weeks old. The mice were housed and fed as described above. Animals weighing between 25 and 47 g (mean 36 g) were randomized according to their body weights and were assigned to four groups of 9–10 animals each as follows: (1) intact sham-gonadectomized control; (2) gonadectomized (GDX) control; (3) GDX + DHT (0.1 mg/mouse); (4) GDX + DHEA (3 mg/mouse). On day 1 of the study, animals of groups 2–4 were castrated (GDX) under isoflurane anesthesia while mice of group 1 were SHAM-operated. DHT and DHEA sus-

pended in 5% ethanol–0.4% methylcellulose, were injected subcutaneously once daily (0.2 mL/mouse) from day 2 to day 28 of the study while mice of groups 1 and 2 were injected with the vehicle alone during the same time period. On day 29 of the study, 24 h after the last injection, mice under isoflurane anesthesia were exsanguinated via cardiac puncture followed by cervical dislocation. The ventral prostate, dorsal prostate, right seminal vesicle, right preputial gland and gastrocnemius muscle were collected, emptied of fluid (when applicable) and weighed. Tissues were then rapidly frozen in liquid nitrogen and kept at −80 °C until RNA extraction. The tissues collected from the mice of the same group were pooled for RNA extraction and analysis. For the prostate, the ventral and dorsal lobes were pooled. Tissue weights were expressed as means ± S.E.M. The statistical significance was determined according to the multiple range test of Duncan–Kramer [8].

In the 1-week study, C57BL6 male mice obtained from Charles River (St-Constant, Que., Canada) were used. At the beginning of the study, mice were 14 weeks old. The mice were housed and fed as described above. Animals weighing between 23 and 31 g (mean 27 g) were randomized according to their body weights and were assigned to four groups of fifteen animals each as follows: (1) intact sham-gonadectomized control; (2) gonadectomized (GDX) control; (3) GDX + DHT (0.1 mg/mouse); (4) GDX + DHEA (6.25 mg/mouse). On day 1 of the study, animals of groups 2–4 were castrated (GDX) under isoflurane anesthesia while mice of group 1 were SHAM-operated. DHT and DHEA were suspended in 5% ethanol–0.4% methylcellulose and were administered from day 2 to day 8 of the study. DHEA was administered by oral gavage once daily (0.2 mL/mouse) while DHT, considering its low oral activity, was injected subcutaneously once daily (0.2 mL/mouse) during the same time period. Mice of groups 1 and 2 received the vehicle alone orally during the same time period. On day 8 of the study, 6 h after the last treatment, mice under isoflurane anesthesia were exsanguinated via cardiac puncture followed by cervical dislocation. The prostate (ventral + dorsal), seminal vesicles and preputial glands were collected, emptied of fluid (when applicable) and rapidly frozen in liquid nitrogen. Tissues were kept at −80 °C until RNA extraction and were processed as described above.

2.2. RNA extraction and microarray analysis

Total RNA was isolated by Trizol (Invitrogen) and purified with Rneasy Mini Kit columns (Qiagen). The quality of total RNA, cDNA synthesis, cRNA amplification and cRNA fragmentation was monitored by micro-capillary electrophoresis (Bioanalizer 2100, Agilent Technologies). Twenty micrograms of total RNA were converted to cDNA and transcribed in vitro to produce biotinylated cRNA. Fifteen micrograms of fragmented cRNA were hybridized for 16 h at 45 °C with constant rotation (60 rpm). Microarrays were processed using the Affymetrix GeneChip Fluidic Station 450. Staining was made with streptavidin-conjugated phycoerythrin (SAPE) followed by an amplification with a biotinylated anti-streptavidin antibody and by a second round of SAPE before scanning using a GeneChip Scanner 3000 (Affymetrix). The signal intensities for the β-actin and GAPDH genes were used as internal quality controls. The ratio of fluorescent intensities for the 5′ end and the 3′ end of these housekeeping genes was <2. Hybridization was performed in duplicate to the GeneChip MOE 430v2.0 array (Affymetrix, Santa Clara, CA) according to the Affymetrix protocols. Scanned images were analyzed with Affymetrix GCOS v1.1 software as described [9].

The R statistical framework [10] was used to normalize the microarray data. The robust multi-array average (RMA) expression measure, as implemented in the *affy* Bioconductor package [11], was used. The identification of potentially modulated genes was carried out using the *limma* package [12]. In this approach, stabilized standard errors are estimated according to the empirical Bayes method used to calculate significant fold changes. A multiple hypothesis testing correction was used to control the false discovery rate, with a cutoff value of $p = 0.01$. For the selected transcripts, an additional condition of a minimum fold change value of 30% was applied. The chosen probe sets were clustered hierarchically with the GeneSpring program version 7.2 (Silicon Genetics, Redwood City, CA).

3. Results

As illustrated in Fig. 2, daily subcutaneous injection of 0.1 mg DHT for 1 month increased ventral prostate weight from 3.6 ± 0.7 to 16.2 ± 1.2 mg ($p < 0.01$) while 3 mg DHEA increased ventral prostate weight to 10.9 ± 0.9 mg ($p < 0.01$).

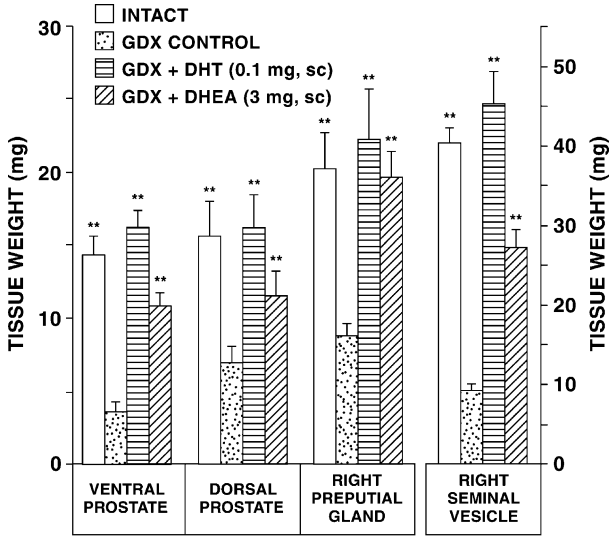

Fig. 2. Effect of 27-day daily treatment with DHT or DHEA on ventral prostate, dorsal prostate, preputial gland and seminal vesicle weight in GDX male C57BL6/129SV mice. Data are expressed as the mean ± S.E.M. of 9–10 animals per group. ** $p < 0.01$, experimental versus GDX-control mice. Intact sham-operated mice receiving the vehicle alone are added as reference.

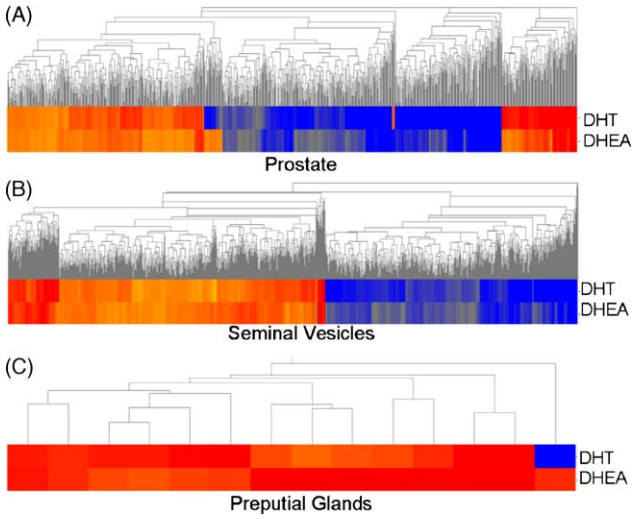

Fig. 3. Comparison of the gene expression profile 24 h following a single subcutaneous administration of 0.1 mg DHT or 2 mg DHEA on the prostate (A), seminal vesicles (B) and preputial glands (C). The probe sets selected were those identified as modulated by 30% or more (all $p < 0.01$ compared with RNA from control gonadectomized animals) in tissues from DHEA- and DHT-treated animals.

A value of 14.4 ± 1.2 mg was observed in intact control animals. Comparable results were observed on dorsal prostate, seminal vesicle and preputial gland weight (Fig. 2).

Taking advantage of the unique discriminatory power of DNA microarrays, Fig. 3 illustrates comparison of the changes of the genomic profiles in three tissues of the mice 24 h following single subcutaneous injection of DHT or DHEA. The similarity of the DHT and DHEA patterns can be seen by the genes upregulated (red) and down-regulated (blue) in the three tissues. In fact, 878 probe sets were differentially expressed in the prostate by both DHT and DHEA. In the seminal vesicles and preputial glands, the number of probe sets commonly modulated by DHT and DHEA were 2681 and 14, respectively (Table 1).

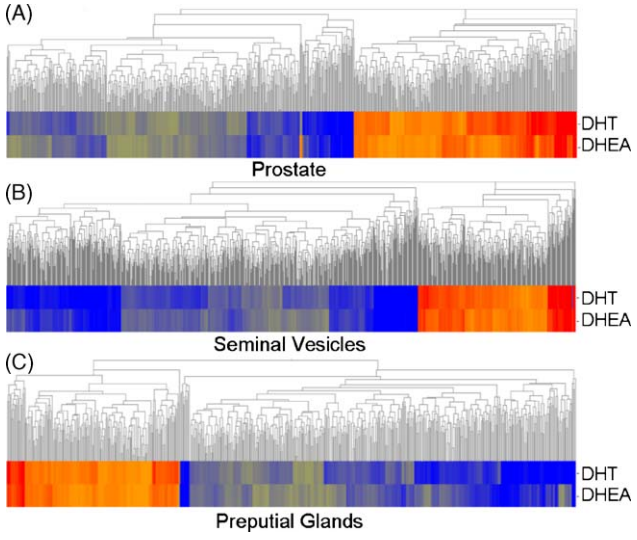

Fig. 4. Comparison of the gene expression profile 7 days following daily administration of 0.1 mg DHT or 6.25 mg DHEA in the prostate (A), seminal vesicles (B) and preputial glands (C). Data are expressed as indicated in legend to Fig. 3.

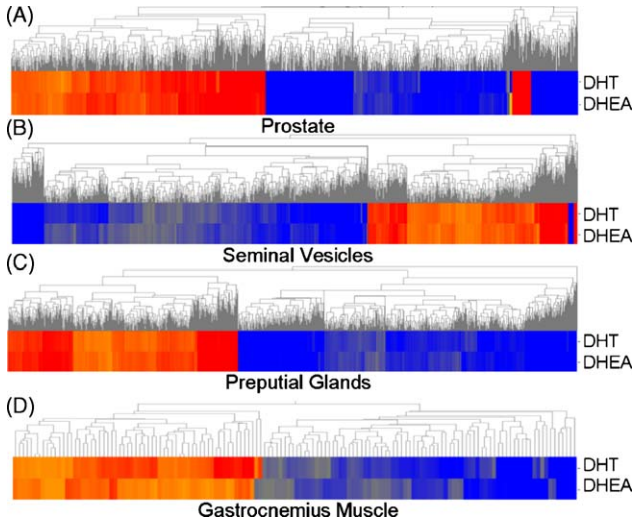

Fig. 5. Comparison of the gene expression profile 27 days following daily subcutaneous administration of 0.1 mg DHT or 3 mg DHEA in the prostate (A), seminal vesicles (B), preputial glands (C) and gastrocnemius muscle (D). Data are expressed as indicated in legend to Fig. 3.

Table 1
Probe sets commonly stimulated or inhibited by DHEA and DHT in the androgen-sensitive prostate (ventral + dorsal), seminal vesicles and preputial glands 24 h after single subcutaneous injection of DHT or DHEA or daily administration of the two steroids for 7 and 27 days ($p < 0.01$ for probe sets modulated ≥30%)

Duration of treatment (days)	Prostate	Seminal vesicles	Preputial glands
1	878	2681	14
7	629	919	562
27	1195	5127	2883

When DHT and DHEA were administered daily for 7 days, it can be seen in Fig. 4 that 629 probe sets were commonly modulated in the prostate. In the seminal vesicles and preputial glands, 919 and 562 probe sets were commonly modulated by the two steroids, respectively. After 27 days of treatment, on the other hand, 1195, 5127, 2883 and 140 probe sets were similarly modulated by both DHEA and DHT in the prostate, seminal vesicles, preputial glands and gastrocnemius muscle, respectively (Fig. 5 Table 1).

4. Discussion

The power of gene expression profiling is being well demonstrated in clinical medicine, especially in acute myeloid leukemia, diffuse large B-cell lymphoma, medulloblastoma, lung carcinoma and breast carcinoma. This tech-

nology provides a molecular classification of diseases which permits specific treatments and more accurate prognosis [13–18]. The importance of genomic profiling is particularly well demonstrated in acute myeloid leukemia which can be divided into subgroups having different responses to specific drugs [19,20]. The information obtained by genetic profiling permits the choice of the best treatment for each category of cancer while avoiding the serious side effects of inefficient treatments and the vital time lost trying inappropriate therapy while the cancer continues to progress [21,22].

Using this powerful technique of gene expression profiling, we have previously shown that the anabolic steroid THG modulated gene expression according to a pattern similar to that of the most potent natural androgen DHT [7]. Similarly, in the present study, we have shown that DHEA induces a gene expression profile similar to DHT. In addition, more than 500 genes that have been found modulated by THG and DHT in the prostate [7] are also modulated by DHEA in the present study. Moreover, most of the genes described previously [7] as modulated by both THG and DHT and validated by quantitative real-time PCR, are also found to be modulated by DHEA.

Among the 878 probe sets modulated by DHEA and DHT at 24 h in the prostate, 467 genes are classified by the Gene Ontology consortium while 411 genes are unclassified. Among the classified genes, 43.5% correspond to enzymes, 25.1% to structural proteins, 16.9% to transport proteins, 13.1% to signal transducers, 8.6% to nucleic acid binding proteins, 7.5% to proteins involved in immunity, 3.9% to cell cycle regulation, while 3.4% are chaperones, 2.6% are apoptosis regulators, 1.3% are related to cancer, 0.4% are involved in storage and 8.4% are classified by Gene Ontology as other groups. The present results clearly show that mammals possess all the necessary enzymatic machinery to convert DHEA into active androgens in peripheral target tissues. The androgenic activities exerted by DHEA correspond to the molecular functions controlled by DHT, thus clearly demonstrating that exogenous DHEA is acting as an anabolic steroid.

Humans, along with the other primates, are unique among animal species in having adrenals that secrete large amounts of the inactive precursor steroids DHEA and especially DHEA-sulfate (DHEA-S), which are converted into potent androgens and/or estrogens in peripheral tissues [23–30] (Fig. 1). In fact, plasma DHEA-S levels in adult men and women are 100–500 times higher than those of testosterone and 1000–10,000 times higher than those of estradiol, thus providing a large reservoir of substrate for conversion into androgens and/or estrogens in the peripheral intracrine tissues which naturally possess the enzymatic machinery necessary to transform DHEA into active sex steroids. DHEA, like 4-dione, is not a hormone by itself but it is an essential prehormone that is transformed into 4-dione and then into testosterone and, finally, into DHT, the most potent natural androgen (Fig. 1). This androgen precursor DHEA is secreted by the adrenals in humans and other primates in quantities larger than cortisol and is present in the blood at concentrations only second to cholesterol.

It is thus remarkable that man, in addition to possessing very sophisticated endocrine and paracrine systems, has largely vested in sex steroid formation in peripheral tissues [23,29–34]. The level of transformation of the adrenal precursor steroid DHEA into androgens and/or estrogens in peripheral target tissues thus depends upon the level of expression of the various steroidogenic enzymes in each cell of each tissue (Fig. 1) [5,23]. In fact, while the ovaries and testes are the exclusive sources of androgens and estrogens in lower mammals [33], the situation is very different in man and higher primates, where active sex steroids are in large part or wholly synthesized locally in peripheral tissues, thus providing target tissues with the appropriate controls which adjust the formation and metabolism of sex steroids to local requirements.

This new field of endocrinology has been called intracrinology [23,24]. In women, after menopause, all estrogens and almost all androgens are made locally in peripheral tissues from DHEA that, indirectly exerts effects, among others, on bone formation, muscle, adiposity, insulin and glucose metabolism, skin, libido and well being [5,35]. In men, where the secretion of androgens by the testicles continues for life, the contribution of DHEA to androgens has been best measured in the prostate where about 50% of androgens are made locally from DHEA [6,31]. In fact, a stimulatory effect of DHEA on muscle mass has been observed in postmenopausal women treated with DHEA [35]. Our previous study [1], as well as the present data show a large series of genes modulated by androgenic/anabolic steroids in the muscle.

The androgens testosterone and DHT made in peripheral tissues from DHEA of adrenal or exogenous origin exert their action locally in the same cells where synthesis takes place with only minimal release as active androgens in the circulation. It is thus reasonable to suggest that measurement of the serum levels of testosterone is of questionable biological and clinical significance. Following their formation and availability for local intracellular action, testosterone and DHT are inactivated and transformed in the same cells into water soluble glucuronide derivatives which diffuse quantitatively into the general circulation where they can be measured before their elimination by the kidneys (Fig. 1).

As illustrated in Fig. 2, DHEA, like DHT, causes a marked stimulation of ventral prostate, dorsal prostate, seminal vesicle and preputial gland weight. These parameters are well recognized measures of androgenic and anabolic activity [32,36,37]. Of major importance, the stimulatory effect of DHEA on prostate weight and androgen-dependent growth and gene expression is seen at plasma concentrations of DHEA and 4-dione comparable to those found in adult men and women. In fact, at a serum level of 3.4 ng DHEA/mL in the rat, intraprostatic DHT is increased to 2.7 ng/g tissue [32]. That study demonstrated that DHEA is transformed into DHT in the absence of the testicles, thus providing the evi-

dence for the formation of DHT and its action in peripheral tissues. This finding has been at the origin of intracrinology. In fact, that study has demonstrated, for the first time, that DHEA, at physiological concentrations, induces high levels of intraprostatic DHT resulting in a marked stimulation of ventral prostate weight and the increased expression of androgen-sensitive genes [32,34].

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References

[1] F. Labrie, V. Luu-The, A. Bélanger, S.-X. Lin, J. Simard, C. Labrie, Is DHEA a hormone? Starling review, J. Endocrinol. 187 (2005) 169–196.
[2] C. Labrie, L. Cusan, M. Plante, S. Lapointe, F. Labrie, Analysis of the androgenic activity of synthetic "progestins" currently used for the treatment of prostate cancer, J. Steroid Biochem. 28 (1989) 379–384.
[3] J.P. Raynaud, C. Bonne, M.M. Bouton, M. Moguilewsky, D. Philibert, G. Azadian-Boulanger, Screening for anti-hormones by receptor studies, J. Steroid Biochem. 6 (1975) 615–622.
[4] F. Labrie, V. Luu-The, S.X. Lin, C. Labrie, J. Simard, R. Breton, A. Bélanger, The key role of 17β-HSDs in sex steroid biology, Steroids 62 (1997) 148–158.
[5] F. Labrie, V. Luu-The, C. Labrie, A. Bélanger, J. Simard, S.-X. Lin, G. Pelletier, Endocrine and intracrine sources of androgens in women: inhibition of breast cancer and other roles of androgens and their precursor dehydroepiandrosterone, Endocr. Rev. 24 (2003) 152–182.
[6] F. Labrie, A. Bélanger, B. Candas, L. Cusan, J.L. Gomez, C. Labrie, V. Luu-The, J. Simard, GnRH agonists in the treatment of prostate cancer, Endocr. Rev. 26 (2005) 361–379.
[7] F. Labrie, V. Luu-The, E. Calvo, C. Martel, J. Cloutier, S. Gauthier, P. Belleau, H. Boucher, J. Morissette, M.H. Lévesque, C. Labrie, Tetrahydrogestrinone (THG) induces a genomic signature typical of a potent anabolic steroid, J. Endocrinol. 184 (2) (2005) 427–433.
[8] C.Y. Kramer, Extension of multiple range tests to group means with unique numbers of replications, Biometrics 12 (1956) 307–310.
[9] S. Vasseur, C. Malicet, E.L. Calvo, C. Labrie, P. Berthezene, J.C. Dagorn, J.L. Iovanna, Gene expression profiling by DNA microarray analysis in mouse embryonic fibroblasts transformed by rasV12 mutated protein and the E1A oncogene, Mol. Cancer 2 (2003) 19.
[10] R.D.C. Team, A language and environment for statistical computing, R Foundation for Statistical Computing, 2005, ISBN 3-900051-07-0.
[11] R.A. Irizarry, L. Gautier, B. Milo Bolstad, C. Miller, M. Astrand, L.M. Cope, R. Gentleman, J. Gentry, W. Huber, B. MacDonald, I.P. Rubenstein, C. Workman, J. Zhang, affy: Methods for Affymetrix Oligonucleotide Arrays, Package Version, 2004, 1.5.8-1.
[12] G.K. Smyth, Linear models and empirical Bayes methods for assessing differential expression in microarray experiments, Stat. Appl. Genet. Mol. Biol. 3 (2004) 3.
[13] D.G. Beer, S.L. Kardia, C.C. Huang, T.J. Giordano, A.M. Levin, D.E. Misek, L. Lin, G. Chen, T.G. Gharib, D.G. Thomas, M.L. Lizyness, R. Kuick, S. Hayasaka, J.M. Taylor, M.D. Iannettoni, M.B. Orringer, S. Hanash, Gene-expression profiles predict survival of patients with lung adenocarcinoma, Nat. Med. 8 (2002) 816–824.
[14] Y. Wang, J.G. Klijn, Y. Zhang, A.M. Sieuwerts, M.P. Look, F. Yang, D. Talantov, M. Timmermans, M.E. Meijer-van Gelder, J. Yu, T. Jatkoe, E.M. Berns, D. Atkins, J.A. Foekens, Gene-expression profiles to predict distant metastasis of lymph-node-negative primary breast cancer, Lancet 365 (2005) 671–679.
[15] T.J. MacDonald, K.M. Brown, B. LaFleur, K. Peterson, C. Lawlor, Y. Chen, R.J. Packer, P. Cogen, D.A. Stephan, Expression profiling of medulloblastoma: PDGFRA and the RAS/MAPK pathway as therapeutic targets for metastatic disease, Nat. Genet. 29 (2001) 143–152.
[16] M.J. Korenberg, Gene expression monitoring accurately predicts medulloblastoma positive and negative clinical outcomes, FEBS Lett. 533 (2003) 110–114.
[17] H. Dai, L. van't Veer, J. Lamb, Y.D. He, M. Mao, B.M. Fine, R. Bernards, M. van de Vijver, P. Deutsch, A. Sachs, R. Stoughton, S. Friend, A cell proliferation signature is a marker of extremely poor outcome in a subpopulation of breast cancer patients, Cancer Res. 65 (2005) 4059–4066.
[18] Y. Pawitan, S. Michiels, S. Koscielny, A. Gusnanto, A. Ploner, False discovery rate, sensitivity and sample size for microarray studies, Bioinformatics 21 (2005) 3017–3024.
[19] L. Bullinger, K. Dohner, E. Bair, S. Frohling, R.F. Schlenk, R. Tibshirani, H. Dohner, J.R. Pollack, Use of gene-expression profiling to identify prognostic subclasses in adult acute myeloid leukemia, N. Engl. J. Med. 350 (2004) 1605–1616.
[20] P.J. Valk, R.G. Verhaak, M.A. Beijen, C.A. Erpelinck, S. Barjesteh van Waalwijk van Doorn-Khosrovani, J.M. Boer, H.B. Beverloo, M.J. Moorhouse, P.J. van der Spek, B. Lowenberg, R. Delwel, Prognostically useful gene-expression profiles in acute myeloid leukemia, N. Engl. J. Med. 350 (2004) 1617–1628.
[21] A.K. Burnett, Current controversies: which patients with acute myeloid leukaemia should receive a bone marrow transplantation?—an adult treater's view, Br. J. Haematol. 118 (2002) 357–364.
[22] A.R. Mistry, E.W. Pedersen, E. Solomon, D. Grimwade, The molecular pathogenesis of acute promyelocytic leukaemia: implications for the clinical management of the disease, Blood Rev. 17 (2003) 71–97.
[23] F. Labrie, Intracrinology, Mol. Cell. Endocrinol. 78 (1991) C113–C118.
[24] F. Labrie, A. Bélanger, J. Simard, V. Luu-The, C. Labrie, DHEA and peripheral androgen and estrogen formation: intracrinology, Ann. NY Acad. Sci. 774 (1995) 16–28.
[25] F. Labrie, J. Simard, V. Luu-The, A. Bélanger, G. Pelletier, Y. Morel, F. Mebarki, R. Sanchez, F. Durocher, C. Turgeon, Y. Labrie, E. Rhéaume, C. Labrie, Y. Lachance, The 3β-hydroxysteroid dehydrogenase/isomerase gene family: lessons from type II 3β-HSD congenital deficiency, in: V. Hansson, F.O. Levy, K. Taskén (Eds.), Proceedings of the Ernst Schering Research Foundation Workshop on Signal Transduction in Testicular Cells, Suppl. 2, Springer-Verlag, Berlin, 1996, pp. 185–218.
[26] F. Labrie, V. Luu-The, S.-X. Lin, J. Simard, C. Labrie, M. El-Alfy, G. Pelletier, A. Bélanger, Intracrinology: role of the family of 17β-hydroxysteroid dehydrogenases in human physiology and disease, J. Mol. Endocrinol. 25 (2000) 1–16.
[27] F. Labrie, V. Luu-The, C. Labrie, J. Simard, DHEA and its transformation into androgens and estrogens in peripheral target tissues: intracrinology, Front Neuroendocrinol. 22 (2001) 185–212.
[28] V. Luu-The, Analysis and characteristics of multiple types of human 17beta-hydroxysteroid dehydrogenase, J. Steroid Biochem. Mol. Biol. 76 (2001) 143–151.
[29] F. Labrie, A. Bélanger, L. Cusan, B. Candas, Physiological changes in dehydroepiandrosterone are not reflected by serum levels of active androgens and estrogens but of their metabolites: intracrinology, J. Clin. Endocrinol. Metab. 82 (1997) 2403–2409.

[30] E.R. Simpson, S.R. Davis, Minireview: aromatase and the regulation of estrogen biosynthesis—some new perspectives, Endocrinology 142 (2001) 4589–4594.
[31] F. Labrie, A. Dupont, A. Bélanger, Complete androgen blockade for the treatment of prostate cancer, in: V.T. de Vita, S. Hellman, S.A. Rosenberg (Eds.), Important Advances in Oncology, J.B. Lippincott, Philadelphia, 1985, pp. 193–217.
[32] C. Labrie, A. Bélanger, F. Labrie, Androgenic activity of dehydroepiandrosterone and androstenedione in the rat ventral prostate, Endocrinology 123 (1988) 1412–1417.
[33] B. Bélanger, A. Bélanger, F. Labrie, A. Dupont, L. Cusan, G. Monfette, Comparison of residual C-19 steroids in plasma and prostatic tissue of human, rat and guinea pig after castration: unique importance of extratesticular androgens in men, J. Steroid Biochem. 32 (1989) 695–698.
[34] C. Labrie, J. Simard, H.F. Zhao, A. Bélanger, G. Pelletier, F. Labrie, Stimulation of androgen-dependent gene expression by the adrenal precursors dehydroepiandrosterone and androstenedione in the rat ventral prostate, Endocrinology 124 (1989) 2745–2754.
[35] P. Diamond, L. Cusan, J.L. Gomez, A. Bélanger, F. Labrie, Metabolic effects of 12-month percutaneous DHEA replacement therapy in postmenopausal women, J. Endocrinol. 150 (1996) S43–S50.
[36] R. Neri, M.D. Monahan, J.G. Meyer, B.A. Afonso, I.A. Tabachnick, Biological studies of an antiandrogen (SH-714), Eur. J. Pharmacol. 1 (1967) 438–444.
[37] F.J. Ebling, E. Ebling, V. Randall, J. Skinner, The effects of hypophysectomy and of bovine growth hormone on the responses to testosterone of prostate, preputial, Harderian and lachrymal glands and of brown adipose tissue in the rat, J. Endocrinol. 66 (1975) 401–406.