Physiology & Behavior 173 (2017) 23–29

Contents lists available at ScienceDirect

Physiology & Behavior

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

Sources of variance within and among young men in concentrations of 17β-estradiol and testosterone in axillary perspiration

Brittney Elliott [a], Cameron Muir [b], Denys deCatanzaro [a,*]

[a] Department of Psychology, Neuroscience & Behaviour, McMaster University, Hamilton, Ontario L8S 4K1, Canada
[b] Department of Psychology, Centre for Neuroscience, Brock University, St. Catharines, Ontario L2S 3A1, Canada

HIGHLIGHTS

- Estradiol and testosterone were measured in young men's axillary perspiration.
- Some men showed low concentrations like those in facial perspiration (<5 ng/mL).
- Others showed much higher concentrations, ranging up to several hundred ng/mL.
- Individual differences were stable across four repeated measures.
- Sexual experience and proximate female interactions modulated estradiol levels.

ARTICLE INFO

Article history:
Received 26 August 2016
Received in revised form 19 January 2017
Accepted 19 January 2017
Available online 21 January 2017

Keywords:
Estradiol
Testosterone
Pheromones
Axilla
Perspiration

ABSTRACT

The most potent estrogen, 17β-estradiol (E_2), and its precursor, testosterone (T), play critical roles in mammalian reproductive processes. Evidence indicates that these steroids are present in bioactive form in the excretions of many male mammals. It has been demonstrated that small lipophilic steroids such as E_2 can be absorbed by proximate females from male excretions, arriving in the uterus, brain, and other organs where there are estrogen receptors. We took repeated samples of axillary perspiration from men aged 20–30 years during vigorous exercise. Both steroids were consistently measurable, with concentrations that ranged from values comparable to those in facial perspiration and urine of both men and women to values that greatly exceeded concentrations observed in any other substrate of men and women. Inter-individual variance in axillary E_2 was positively correlated with the extent of intimate experience with women, as assessed by a questionnaire, but unrelated to subjective measures of stress, exercise habits, or phytoestrogen content of diet. In addition, higher levels of axillary E_2 were observed in participants when samples were collected by a female (as compared to a male) experimenter. These data are concordant with an hypothesis that male excretion of sex steroids could exert pro-reproductive influences on proximate females.

1. Introduction

High concentrations of unconjugated, bioactive 17β-estradiol (E_2) and testosterone (T) are often found in mammalian males' excretions. In laboratory mice, for example, urine from both sexes reliably contains these steroids [1–4]. Unlike females, male mice actively disperse their urine in very small droplets around their environment when socially stimulated, and in some circumstances they direct it toward females [4–7]. Urinary concentrations of E_2 rise progressively over 3–5 days when male mice are exposed to females across a wire-mesh grid [3,4,8]. Unconjugated sex steroids are also present in mouse feces [9] and in excretions from the feet and nasal area deposited on cage walls [3]. Sex steroids and their metabolites have also been measured in the urine and feces of diverse wild and domestic mammals, with species differences in the ratios of conjugated versus unconjugated forms [10,11]. In various mammals including humans, substantial concentrations of E_2, other estrogens, and androgens have been found in semen [12–15].

Hormones are normally assumed to act within the individual whose glands produce them; however recent data show that sex steroids can transfer among cohabiting individuals [16]. Exogenous sex steroids can be absorbed percutaneously and nasally into blood circulation, due to low molecular mass, polarity, and a lipophilic nature [17–21]. When male mice were given a dose of tritiated estradiol (^{3}H-E_2) representing just a fraction of their endogenous E_2, then housed for a few days with female mice, the females showed radioactivity in the uterus, ovaries, brain, and other tissues [18,22]. Transfer of ^{3}H-E_2 is especially rapid and intense during mating in mice, as E_2 in seminal

* Corresponding author.
E-mail address: decatanz@mcmaster.ca (D. deCatanzaro).

http://dx.doi.org/10.1016/j.physbeh.2017.01.036
0031-9384/

emissions is deposited in the reproductive tract where estrogen receptors are abundant, while also passing into the female's blood circulation [23]. Male-to-female transfer of ^{3}H-E_2 has been found during cohabitation in big brown bats, and their great phylogenetic distance from mice suggests that inter-individual E_2 transfer could occur in many mammals [24]. Evidence increasingly implicates E_2 transfer in causation of "pheromonal" effects that are observed in many mammalian species, including male-induced disruption of pregnancies sired by other males (Bruce effect) and promotion of puberty in juvenile females (Vandenbergh effect) [16]. Both of those effects can be mimicked by giving females very low exogenous doses of E_2 [3,25,26]. Both effects also depend on female exposure to male urinary E_2, but they do not depend on male urinary T [27].

In mice, urine is known to be a vector of chemical transfer among individuals [5,7,16], whereas in humans perspiration is a more plausible vector [16,28]. In previous work from our laboratories, we undertook measures of bioactive sex steroids in perspiration [28]. Unconjugated E_2, T, and progesterone were present in the facial perspiration of men and women, but not of juveniles. Moreover, axillary perspiration of young men often contained extraordinary levels of these bioactive steroids, with average concentrations exceeding those in facial perspiration of men or women by about 90-fold for T and 45-fold for E_2. Axillary steroid concentrations showed no correlation with salivary concentrations. There was no correlation between axillary and urinary T, while E_2 showed no correlation between axillary and urinary concentrations in one set of participants and just a modest correlation ($r = 0.37$) in another set. Notably, while the axillary glands can excrete steroids carried in the blood [29], they also contain enzymes that can convert one steroid hormone to another [30,31]. There is also evidence that axillary glands have the capacity to synthesize steroids from their precursors de novo [32,33]. Muir et al. [28] observed substantial variance among young men in axillary perspiration concentrations of E_2 (range of 1.2 to 285.4 ng/mL) and T (range of 10.5 to 1141.5 ng/mL).

The current study was designed to shed light on the sources of this inter-individual variance. We recruited young men who would each provide axillary perspiration samples on four occasions, in order to determine whether the high versus low steroid content was a stable trait of individuals as opposed to being subject to fluctuations. As gonadal steroids in men and other male mammals can be attenuated by stressors [34] and stimulated by interactions with females [3,35,36], we took measures of stress and contact with young unrelated women and correlated these with axillary steroid measures. We also asked participants about phytoestrogen content of diet, which can affect E_2 [37]. We also varied the experimenter who interacted with participants, with half of the measures for each participant being taken by a young woman and half by a young man, with order counterbalanced across participants.

2. Methods

2.1. Participants

The methodology was approved by the McMaster University Research Ethics Board. Males aged 20–30 years were recruited at a running track and a fitness and weight room in the McMaster University David Braley Athletic Centre. Each was asked to give four perspiration samples with approximately 1- to 2-week intervals between samples. Of 81 men recruited, 73 gave at least one usable perspiration sample, while 49 provided four usable samples. All participants read and signed a consent form and were assured of the anonymity of their data. Participants received $12 CAD compensation for the first session, and $5 for each additional session, regardless of whether they were able to produce a usable perspiration sample during the session.

2.2. Assessment of environmental factors

Each participant completed a questionnaire on the same day as the first perspiration sample. The questionnaire was designed to assess potential exclusion criteria as well as environmental factors which could influence E_2 and/or T levels in axillary perspiration. Participants were asked to specify any prescription or non-prescription medication that they had taken in the last month and to report the number of standard alcoholic drinks that they typically consumed in a week. Exclusion criteria included whether subjects reported taking anabolic steroids or other medications that would clearly affect steroid hormone levels, but no participants reported taking such medications. They were asked 5 yes-no questions concerning recent consumption of soy milk, edamame, tofu, flax seed, or other soy or flax products. For statistical purposes, a phytoestrogen composite score was created by adding the responses for the 5 categories (0 = no, 1 = yes) for each participant. Participants were also asked to give a rating on two 0–5 Likert-scale questions asking about subjective stress (0 = not at all, 5 = unbearable), the first rating the last week and the second rating the last month. For statistical purposes, a stress composite was formed for each participant as the sum of these two answers. They were asked to specify the number of hours that they spent in vigorous exercise such as jogging, weight lifting, or playing sports in a typical week.

There were also several questions concerning romantic and intimate relations. Participants were asked to rank their sexual preference on a 5-point Likert scale from "exclusively men" to "exclusively women". The questionnaire also asked four questions concerning intimate experience with women: whether the participant had sexual relations with any female in the past week; whether the participant was in a romantic relationship with a female; whether the participant, if in such a relationship, had regular sexual relations with his partner; and whether the participant, if in a relationship, lived with his partner. A score of 0 was applied when the answer was no, and a score of 1 was given when the answer was yes. For statistical purposes, answers to these four questions were added into a single composite score for each participant indicating intimate experience with women, producing a single number ranging from 0 to 4. If participants indicated that they had had sexual relations, they were also asked to indicate whether the partner was on hormonal birth control.

Two researchers, one female and one male from the same age range as the participants, collected perspiration samples after carefully standardizing their procedures. One collected the first two perspiration samples, and the other collected the last two; the order was counterbalanced, with half of the participants seeing the female experimenter first and the others seeing the male experimenter first.

2.3. Perspiration collection

Participants were asked not to wear deodorant or antiperspirant on the days that measures were to be conducted. All participants cleaned their underarms with hypoallergenic, unscented baby wipes, and then with alcohol swabs, in case any residue of antiperspirants or other toiletries were present. Participants were then asked to wear an unused polyethylene trash bag to promote perspiration. They were permitted to wear a shirt or sweater on top of the bag if they desired. Most participants chose to exercise for approximately 20–30 min before donating a perspiration sample. After sufficient exercise had been performed, axillary perspiration was collected by gently scraping the underarm with a centrifuge tube. Samples were placed on ice packs inside a styrofoam case, before being transferred to a freezer where they were stored at −20 °C until assayed.

2.4. Chemical analysis

Perspiration samples were analyzed using enzyme immunoassay procedures previously validated by Muir et al. [28]. Estradiol and