Psychoneuroendocrinology (2013) 38, 171—178

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Pharmacokinetics of testosterone and estradiol gel preparations in healthy young men

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Received 4 February 2012; received in revised form 25 May 2012; accepted 25 May 2012

KEYWORDS
Behavioral endocrinology;
Pharmacokinetics;
Testosterone;
Estradiol;
Gel

Summary The paucity of pharmacokinetic data on testosterone gel formulations and absence of such data on estradiol administration in healthy young men constitutes a fundamental gap of knowledge in behavioral endocrinological research. We addressed this issue in a double-blind and placebo controlled study in which we applied a topical gel containing either 150 mg of testosterone (N = 10), 2 mg of estradiol (N = 8) or a respective placebo (N = 10) to 28 healthy young men. We then assessed serum concentrations of estradiol and testosterone in one hour intervals up to seven hours after drug application, measured LH, SHBG and cortisol levels once at baseline and three, four as well as six hours after gel administration. Treatment with testosterone gel resulted in maximum total serum testosterone concentration three hours after administration and did not suppress LH, cortisol and SHBG levels at any time point. Administration of estradiol gel led to maximum estradiol serum concentration two hours after administration. There was no suppression of cortisol, SHBG and absolute LH levels. We report here, for the first time, pharmacokinetic data on both high dose testosterone and estradiol gel application in healthy young males. The proposed model will assist in the design of future studies that seek to establish causality between testosterone and estradiol gel administration and behavioral as well as neurophysiological effects.

1. Introduction

The medical use of sex hormone preparations has a long history and is clinically well established. Testosterone is administered as a treatment of the hypogonadal state in young and aging men (Wang et al., 2004), and is investigated

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0306-4530/$ — see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.psyneuen.2012.05.018

as a hormonal male contraceptive (Nieschlag, 2010). Estradiol administration plays a similar role in women, who use ''the pill'' worldwide on a daily basis as a contraceptive. The wide-spread use of sex hormones by the general public bears the obvious question whether there may be behavioral changes associated with their administration.

So far, a relatively large number of studies have investigated acute single-dose testosterone effects on emotion processing and social interaction and its neural correlates in healthy subjects, but these studies were performed in females exclusively (Eisenegger et al., 2011; Bos et al., 2012). Thus, an important question is whether behavioral effects following testosterone administration observed in females can be extended to males.

The well known fact that testosterone is aromatized to estradiol could raise the question whether behavioral effects of exogenously administered testosterone are in fact mostly estradiol effects. Despite this, there are surprisingly few experimental studies available that administered estradiol, and these were conducted in healthy females (Schleifer et al., 2002; Kaya et al., 2003). Thus, whether and how estradiol administration influences behavior of healthy males is unknown.

The study of the behavioral effects of exogenously applied sex hormones requires considering a number of methodological issues. First, the knowledge of the precise pharmacokinetics after an acute single dose is necessary to allow assessing the optimal time-point for behavioral testing. Second, these pharmacokinetic data should be collected in healthy subjects who have similar anthropometric characteristics [such as for example body mass index (Kornmann et al., 2009)] and educational background as those subjects who are typically studied in behavioral endocrinological experiments. Third, hormone application should be non-invasive. Injectable preparations might reduce the willingness of volunteers to participate in the study and thereby introduce a selection bias or induce psychological stress possibly interfering with the behavioral response. Finally, as there are sensitive negative feed-back loops within the male neuroendocrine system, exogenous application of high doses of testosterone might cause secondary effects, such as suppression of pituitary hormones, especially of luteinizing hormone (LH) (Wang et al., 1998), and/or adrenal hormones (cortisol) (Viau, 2002).

Therefore, a clean model for investigating behavioral effects of sex hormone administration in males consists of pharmacological preparations with a relatively short half-live at a dose that minimizes feedback effects. The two most important non-invasive ways of administering hormones is the oral and the trans-dermal route. Among these two, the trans-dermal route has the advantage that the increase in circulating hormones can be confirmed relatively easily, in each individual, using saliva sampling. Salivary concentrations correlate well with the ones obtained from serum measurements (Riad-Fahmy et al., 1982, 1987; Granger et al., 2004). However, as oral administration would confound salivary measures, the trans-dermal route is superior for large scale behavioral endocrinological studies.

In sum, a hormone preparation that fulfills the above criteria is the administration of a topical gel which can be applied to chest and upper arms of the body. However, to date, there are no studies available that addressed the pharmacokinetics of high dose testosterone and estradiol gels in healthy male subjects. We therefore performed a pharmacokinetic trial of a single dose of a gel containing 150 mg of testosterone (Androgel), 2 mg of estradiol (Divigel), or a placebo gel in healthy young males. We determined sex hormone concentrations, pituitary as well as adrenal hormones at different time-points following gel application. As many of these fundamental endocrine parameters can only be assessed in blood serum, we use blood draws to establish the pharmacokinetic profile of the two gels.

2. Methods

2.1. Subjects and study design

28 Healthy young males were enrolled and randomized for our double-blind, parallel-groups and placebo controlled experiment that had been approved by the local ethics committee of the Kanton Zürich, Switzerland and the federal board for medications ''Swissmedic'', Bern, Switzerland. Subjects had been recruited from press advertisement and the volunteers database of the Economics Department at the University of Zürich, Switzerland. General health status was evaluated by medical history, physical and genital examination. Abnormal findings as well as clinical signs of hypogonadism or use of any hormones within 8 weeks prior to study entry served as an exclusion criterion. Subjects provided written informed consent and were then included in the study.

2.2. Procedure

Prior to application of study medication, body weight and height were determined and a blood sample for measuring baseline hormones of the male pituitary—testicular axis [LH, testosterone, estradiol, sex-hormone-binding globuline (SHBG)] and cortisol was taken. All baseline examinations were performed between 0800 and 1000 h. Volunteers were then allocated to one of three treatment groups in a double-blind, randomized manner.

Blood samples for determination of estradiol and testosterone were taken in one hour intervals after drug application up to seven hours post administration. LH, SHBG and cortisol measurements were taken once at baseline and repeated after three and four hours, when maximum testosterone and estradiol levels were expected [see Chik et al., 2006]. The last measurement was taken towards the study end (after six hours).

2.3. Hormone preparations

Group A received a single dose of testosterone gel, containing 150 mg testosterone [Androgel®, Bayer (Schweiz) AG, Zürich, Switzerland]. Group B received one single dose of estradiol gel, containing 2 mg of estradiol (Divigel®, Orion Pharma AG, Zug, Switzerland). Group C received a placebo gel. All gels were applied to shoulders and upper arms by a single person of the study personnel, who was not further involved in the clinical part of the study.

2.4. Hormone analysis

Serum concentrations of LH, testosterone, estradiol and cortisol were analyzed by electro-chemoluminesenz-immunoassays (Roche Diagnostics, Rotkreuz, Switzerland). The SHBG-assay was a chemoluminesenz-enzyme-immunoassay manufactured by Siemens, Baar, Switzerland). For testosterone, intra- and inter-assay coefficients were 2.1% or 3.2%, with a lower detection limit of 0.09 nmol/L. Respective assay data for estradiol were 2.1% and 2.5% and 18.4 pmol/L. Coefficients of variation were 3.7% and 7.1%. The lower detection limits for LH was 0.1 IU. Intra-assay and inter-assay coefficients of variation were 1.1% and 1.2% for LH. Lower detection limits for the cortisol- and SHBG-assays were 0.5 and 0.02 nmol/L. Coefficients of variation ranged from 1.4% (cortisol) to 6% (SHBG). All assays were conducted under strict internal and external quality control.

2.5. Statistical analysis

Our statistical analysis is based on analysis of variance (ANOVA), if not further specified. Post hoc comparisons are based on Student's *t*-tests. All tests are two-tailed tests. We examined the impact of Androgel [with a binary indicator for Androgel indicating whether the subject received Androgel (=1) or placebo gel (=0)] or Divigel [with a binary indicator for Divigel indicating whether the subject received Divigel (=1) or placebo gel (=0)], time [in hours from baseline (=0) until end of the experiment (=7)] and the impact of the interactions between time and treatment variables in a repeated measures two-way ANOVA on serum values of testosterone and estradiol serum concentrations. Maximum hormone concentrations (c_{max}) and time-point of maximum hormone concentrations (t_{max}) were determined directly from the individual serum concentration—time curves. To test for differences at specific time points, we used Student's *t*-tests. LH, SHBG and cortisol concentrations were measured at baseline and after three, four and six hours. Free testosterone concentrations were calculated from total testosterone and SHBG concentrations using the formula suggested by (Vermeulen et al., 1999).

3. Results

3.1. Subject sample characteristics

Two of the 28 subjects enrolled in the study were excluded: the first one had a baseline testosterone value above normal (46.7 nmol/L). The normal range of total testosterone values in healthy men are assumed to lie between 10 and 35 nmol/L (Wang, 2007). Biochemical analyses could not be performed accurately for the second one, due to severe blood contamination of the centrifuged blood serum samples. Thus, 26 subjects were evaluable for analysis, eight subjects who received Divigel, eight subjects who received Androgel and ten subjects who received a placebo gel. All volunteers tolerated the different gel preparations without any objective or subjective side effects. Table 1 shows baseline hormonal and anthropometric parameters prior to gel application.

3.2. Androgel effects

Fig. 1(A) shows the changes in testosterone levels in the placebo and the Androgel group. Average levels (over all eight time points) were 16.0 nmol/L in the placebo group and were significantly higher in the Androgel group (27. 8 nmol/L, $p < 0.01$, Student's *t*-test). A repeated measures ANOVA revealed a significant main effect of time ($p < 0.05$) and a significant interaction effect Androgel $\times$ time ($p < 0.01$) on testosterone levels, suggesting that levels change differentially over time in the Androgel compared to the placebo group. Subsequent analysis revealed that after Androgel application testosterone concentrations covered a wide range with a minimum of 18 nmol/L and a maximum of 113 nmol/L compared to placebo (min. 6.9; max. 23 nmol/L). c_{max} was observed three hours after Androgel administration (39.7 nmol/L), and levels at that time point were significantly higher than those obtained three hours after placebo administration (14.7 nmol/L, $p < 0.05$, Student's *t*-test).

Fig. 1(B) shows the corresponding changes in estradiol levels. Repeated-measures ANOVA revealed a significant effect of time ($p < 0.05$), but not Androgel ($p > 0.50$) on estradiol levels. The interaction effect time $\times$ Androgel was not significant ($p > 0.10$), suggesting that Androgel vs. placebo treatment did not affect estradiol levels differentially over time.

3.3. Free testosterone levels

Following Androgel administration, we observed a trend for a significant main effect of time on free testosterone levels ($p < 0.1$). Furthermore, we found a significant interaction effect of Androgel $\times$ time on free testosterone levels ($p < 0.05$), suggesting that Androgel vs. placebo

Table 1 Baseline characteristics of 26 healthy men prior to application of Androgel® (Group A), Divigel® (Group B) or Placebo gel (Group C). Values are given as mean (SEM). Statistics are *F*-tests based on ANOVA, *p*-values corrected for multiple comparisons.

	Group A 150 mg of testosterone	Group B 2 mg of estradiol	Group C Placebo	Statistics
Age (years)	22.4 (0.6)	21.4 (0.6)	22.1 (0.6)	$F = 0.65$, $p = 1.00$
BMI (kg/m^2)	22.5 (0.6)	23.6 (0.6)	21.7 (0.4)	$F = 3.30$, $p = 0.44$
LH (IU/L)	5.5 (0.7)	5.5 (0.2)	3.9 (0.4)	$F = 4.76$, $p = 0.15$
Testosterone (nmol/L)	18.8 (1.9)	18.0 (1.21)	18.1 (1.4)	$F = 0.09$, $p = 1.00$
SHBG	23.6 (2.4)	25.4 (4.4)	22.9 (2.3)	$F = 0.17$, $p = 1.00$
Free testosterone (pmol/L)	481.6 (40.1)	450.5 (30.7)	470.6 (37.1)	$F = 0.17$, $p = 1.00$
Estradiol (pmol/L)	123.0 (8.7)	108.1 (11.9)	115 (5.0)	$F = 0.68$, $p = 1.00$
Cortisol (nmol/L)	451 (24.9)	437.0 (32.0)	471.0 (64.0)	$F = 0.19$, $p = 1.00$

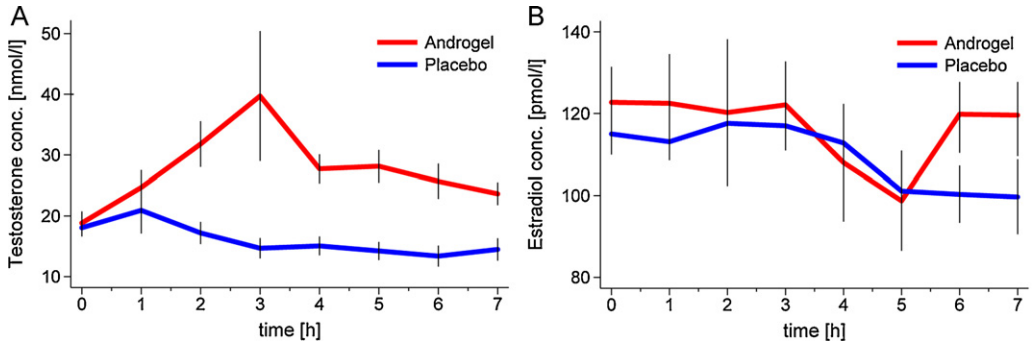

Figure 1 Sex hormone concentrations following Androgel application at different time-points. Whiskers indicate standard errors of the mean. (A) Topical administration of a gel containing 150 mg of testosterone results in maximum average testosterone concentrations of 39.7 nmol/L three hours after application (red line). Testosterone levels in the placebo group are significantly lower than in the Androgel group two hours after administration (blue line). (B) Estradiol levels in the Androgel group (red line) are not significantly different from the placebo group (blue line).

Table 2 Free testosterone levels calculated from SHBG levels and total testosterone levels in the three different treatment groups. Values are given as mean [pmol/L] (SEM).

Time (h)	Group A 150 mg of testosterone	Group B 2 mg of estradiol	Group C Placebo
0 h	481.6 (40.1)	450.5 (30.7)	470.6 (37.1)
3 h	1230.0 (417.4)	539.5 (38.0)	382.5 (47.0)
4 h	765.7 (71.7)	470.6 (44.3)	382.6 (38.1)
6 h	736.6 (92.7)	515.0 (21.9)	334.8 (40.0)

administration influenced free testosterone levels differentially over time (Table 2). For Divigel, no significant main or interaction effects were observed (all $p > 0.1$).

3.4. Divigel effects

Fig. 2(A) depicts estradiol levels over time following Divigel administration. Average levels were 109.6 pmol/L in the placebo group and were significantly higher in the Divigel group (213.0 pmol/L, $p < 0.01$, Student's t-test). The main effect of time was significant ($p < 0.01$) as was the interaction effect Divigel $\times$ time ($p < 0.01$). Estradiol concentrations in the Divigel group covered a range with a minimum of 63.0 pmol/L and a maximum of 514.0 pmol/L compared to placebo (min. 50.0, max. 155.0 nmol/L). Levels reached their maximum (290.0 pmol/L) after two hours. These levels were significantly higher than those measured two hours after placebo administration (117.6 pmol/L, $p < 0.01$, Student's t-test).

Fig. 2(B) shows the corresponding changes in testosterone levels. Statistical analysis revealed a significant main effect of time ($p < 0.05$), but neither a significant main effect of Divigel ($p > 0.15$) nor a significant interaction effect time $\times$ Divigel ($p > 0.20$).

3.5. Cortisol

We found a significant main effect of time on cortisol levels, in both the Androgel ($p < 0.01$) and the Divigel group ($p < 0.01$). This reflects the well-known circadian fluctuation in cortisol secretion. However, the interaction effects

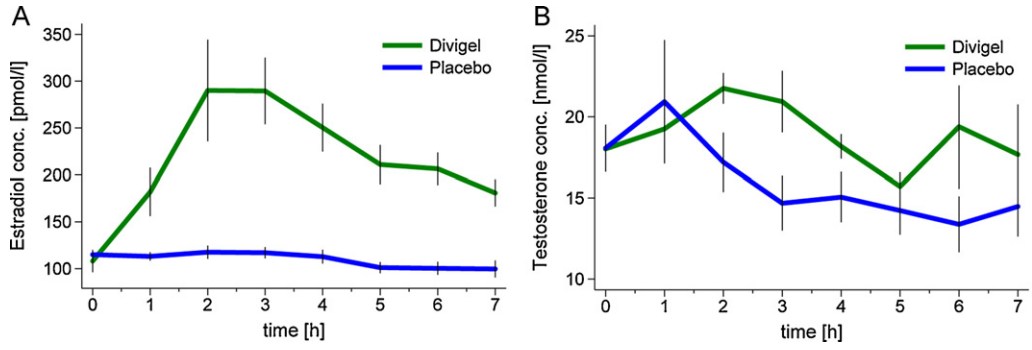

Figure 2 Sex hormone concentrations following Divigel application at different time-points. Whiskers indicate standard error of the mean. (A) Topical administration of a gel containing 2 mg of estradiol results in maximum average estradiol concentrations of 290 pmol/L two hours after application (green line). Estradiol levels in the placebo group are significantly lower than in the Divigel group already one hour after administration (blue line). (B) Testosteron levels in the Divigel group (green line) are not significantly different from the placebo group (blue line).

for both Androgel (time × Androgel, $p > 0.80$), and Divigel (time × Divigel, $p > 0.90$) were not significant, suggesting that cortisol secretion was not affected by any of the gel applications.

3.6. LH

We found no significant main effect of Androgel, time, nor a significant interaction effect Androgel × time on LH secretion (all $p > 0.20$). For Divigel, we observed a significant main effect of time ($p < 0.01$) and a significant interaction effect time × Divigel ($p < 0.05$) on LH levels. As the interaction effect might be driven by potential differences in baseline LH levels in the Divigel vs. placebo groups, we computed normalized LH levels by dividing concentrations at time points three, four and six hours by each individual's LH concentration at baseline. Using these baseline-normalized LH levels in the same ANOVA, we found neither a significant main effect of time ($p > 0.30$) nor a significant interaction effect of Divigel × time ($p > 0.20$). However, we observed a significant main effect of Divigel, with lower normalized LH levels in the Divigel compared to the placebo group ($p < 0.05$).

3.7. SHBG

We found no significant main or interaction effects on SHBG levels following either Androgel or Divigel administration (all $p > 0.10$).

3.8. Testosterone-to-estradiol ratio

To differentiate testosterone from estradiol effects, we plotted the testosterone-to-estradiol ratio (T/E2 ratio) for all three treatment groups (Fig. 3). For Androgel, we observed a significant main effect of time ($p < 0.05$), Androgel ($p < 0.01$) and a significant interaction effect time × Androgel on the T/E2 ratio ($p < 0.01$). For Divigel, we observed a significant effect of time ($p < 0.01$), Divigel ($p < 0.05$) and a marginally significant interaction effect time × Divigel on this ratio ($p = 0.0505$). The mean T/E2 ratios (SEM) in the Androgel group at baseline and three and six hours after application were 0.155 (0.013), 0.322 (0.074) and 0.218 (0.026), respectively. In the Divigel group the corresponding mean ratios (SEM) were 0.177 (0.017), 0.085 (0.019) and 0.102 (0.023), respectively.

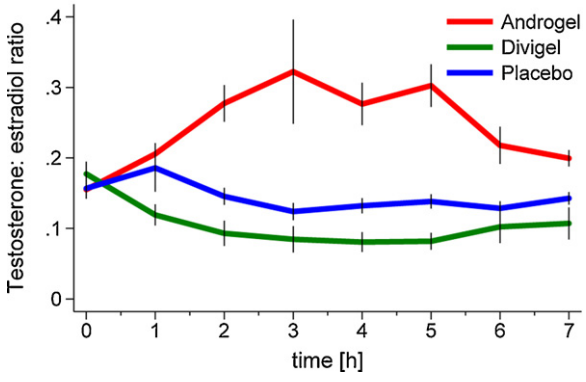

Figure 3 Topical administration of Androgel significantly elevates the T:E2 ratio (red line) above values of the placebo group (blue line) until seven hours post administration. Divigel administration significantly suppresses this ratio (green line) below values of the placebo group (blue line) until five hours post administration.

4. Discussion

We show, for the first time, the time-course of serum testosterone and estradiol concentrations following high dose, acute topical application of 15 g of Androgel (containing 150 mg testosterone), 2 g of Divigel (containing 2 mg estradiol) or a placebo gel in healthy male subjects. Treatment with Androgel resulted in maximum total testosterone concentration of 39.7 nmol/L three hours after administration, which declined thereafter, but remained significantly elevated until six hours post administration. Administration of Divigel resulted in maximum total estradiol concentration of 290 pmol/L two hours after administration. These levels are slightly above the normal range for males and approach those measured in women during the luteal phase of the menstrual cycle.

Previous pharmacokinetic studies in hypogonadal males reported a high range for t_{max} across individuals. Following administration of a gel containing 50 mg of testosterone t_{max} was observed between one and 24 h (Marbury et al., 2003) or later than 24 h (Wang et al., 2000). In another gel study that used a dose of 100 mg testosterone, t_{max} occurred between 16 and 22 h (Swerdloff et al., 2000). However, because hypogonadal men have much lower baseline values of testosterone and a partially incompetent hypothalamic feedback system (Nieschlag, 2006), such pharmacokinetic data are not directly comparable to our results. So far, there is only one study available that examined effects of testosterone gel in healthy young men showing that one single dose of Androgel containing 50 mg of testosterone raises serum testosterone levels to an average maximum of 20.8 nmol/L four hours after administration (Chik et al., 2006). Qualitatively, these pharmacokinetic data are much in line with our findings. Thus, our results show that pharmacokinetic studies performed in hypogonadal males do not provide an estimate of t_{max} that can be readily used to gauge the optimal time-point for behavioral testing in studies of sex hormones in healthy men. For instance, Zak et al. (2009) performed an investigation of testosterone gel effects on economic bargaining behavior in healthy young males and conducted behavioral testing 16 h after administration, based on pharmacokinetic data assessed in hypogonadal males (Swerdloff et al., 2000). Thus, the present data and (Chik et al., 2006) suggest that (Zak et al., 2009) performed testing approximately 13 h after that testosterone levels had reached their peak in their healthy study participants.

With regards to the optimal time-point for behavioral testing, previous studies in women established that neurophysiological effects appear with a delay of about four hours following application of 0.5 mg of sublingual testosterone (Tuiten et al., 2000), despite that peak serum levels are reached already 15 min after application (van Rooij et al., 2012). Following the findings of (Tuiten et al., 2000), many behavioral and neurophysiological studies successfully

applied this particular time-lag to investigate causal testosterone effects on social-emotional behavior and economic interactions in women (Postma et al., 2000; van Honk et al., 2001, 2011; Hermans et al., 2007; Bos et al., 2010; Eisenegger et al., 2010). Although the precise mechanism underlying these delayed effects remains elusive, theoretical and empirical accounts suggest that the delay might be due to the genomic pathway of steroid action. However, others have reported modulatory effects of a single intra-nasal dose of testosterone on amygdala activity at t_{max} (van Wingen et al., 2010), which might suggest that testosterone effects may influence human social behavior also via the non-genomic pathway. To date, it is unclear how the genomic or the non-genomic pathways differ in terms of their modulatory effects on human behavior (Cornil and Charlier, 2010). Based on this, we tentatively suggest two possible time-points for behavioral testing following trans-dermal application of 150 mg of testosterone. The first one is at t_{max}, that is, three hours after gel application and the second one at t_{max} + four hours, that is, approximately seven hours after administration. Although speculative, we propose similar time-points for behavioral studies of Divigel effects.

Androgel did not affect LH levels, our measure of HPG-axis activity. For Divigel, we observed no differences in absolute LH levels, but found lower normalized LH levels at the late time-points. This may indicate an estradiol mediated suppression of the HPG-axis, which was also reported by others (de Ronde et al., 2009). Potential differences between testosterone and estradiol gel effects on HPG-axis activity might be related to dose differences, but also to a functional difference of the two hormones on gonadotropin-secretion inhibition at the level of the pituitary (de Ronde et al., 2009). For instance, it was shown that pharmacologically raising plasma testosterone levels while co-administering an aromatase inhibitor has no significant effect on LH levels, suggesting that aromatization of testosterone is important for LH suppression in males (Finkelstein et al., 1991). However, as we report slight in LH levels at baseline in the Divigel vs. placebo group, future studies with larger number of participants, different doses of estradiol gel and/or cross-over designs might elucidate this topic further.

Neither testosterone nor estradiol gel challenge influenced cortisol levels. This is intriguing, as others have proposed a functional cross-talk between the HPA- and the HPG axis, that is, that high testosterone levels might suppress cortisol secretion from the adrenals (Viau, 2002). One explanation for the absence of cortisol modulation in our study is that acute single-dose administration of sex hormones does not induce sustained high levels of sex hormones, which might be necessary to trigger a suppression of the HPA-axis.

A critical node in the hormonal network is the enzyme aromatase, which converts testosterone to estradiol. The protein is expressed in high concentrations in fat tissue and the brains of all mammals. Ligand PET studies in vivo revealed high binding affinity of radiolabeled 11[C]-vorozole to aromatase in the amygdala of rhesus monkeys (Biegon et al., 2010b) and humans (Biegon et al., 2010a), which places the enzyme in a key position for the modulation of social—emotional behavior (Adolphs, 2010). Hence, testosterone to estradiol conversion might be an important factor that shapes emotion processing and social interaction in humans (Trainor et al., 2006). Two studies in males propose a prosocial effect of estradiol, which was investigated using estrogen patches, but these data were gathered in patients with psychiatric disorders or dementia (Kyomen et al., 1999; Hall et al., 2005) and thus need confirmation in healthy men. The importance of aromatase in the neuroendocrine network is further substantiated by observations in men with genetically determined aromatase deficiency. These men show a delayed epiphyseal closure, are overweight and have incidental impairments of sexual desire, despite having normal testosterone levels. Replacement with estrogens was reported to induce bone maturation (Rochira et al., 2000) and improve glucose metabolism (Herrmann et al., 2005). Such findings support the idea that the ratio of testosterone to estradiol (T/E2 ratio) rather than the absolute circulating amount of sex hormones is important for biological functions (Jones et al., 2007). Thus, future studies of male behavior may include the T/E2 ratio as a potential biomarker for sex hormone effects on the brain.

In summary, we report that the application of a single dose of a gel containing 150 mg of testosterone or 2 mg of estradiol produce serum plasma peaks of the respective hormone two and three hours after administration. Androgel raises testosterone levels into the upper normal range, but does not induce any secondary changes in LH, SHBG or cortisol levels. For Divigel, the obtained peak estradiol values slightly exceed those of older men, who have relatively higher levels than young men (Orwoll et al., 2006). We observed no effect on absolute LH levels, but lower baseline-normalized LH values at the late time-points in the Divigel compared to the placebo group. Furthermore, we observed no secondary effects on SHBG and cortisol levels. These fundamental pharmacokinetic data may assist in the design of future studies that seek to establish causal relationships between testosterone and estradiol gel administration and behavior in healthy young males.

Role of the funding source

This project was supported by the Hartmann-Müller Stiftung for medical research (Application number 1275). Christoph Eisenegger was also supported by the Swiss National Science Foundation (PBEZP3-131154). The funding sources had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication.

Conflict of interest statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Acknowledgements

We thank the Hartmann-Müller Stiftung (Application number 1275) for financial support. Christoph Eisenegger was also supported by the Swiss National Science Foundation (PBEZP3-131154). Monika Seiler and Isabelle Peereboom performed the laboratory analysis. Michael Naef provided helpful comments on the paper. Manuela Simoni has long supported the idea of applying estrogens to men.

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