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Research paper

Progesterone transfer among cohabitating female big brown bats (*Eptesicus fuscus*)

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ABSTRACT

Experiments using female mice and bats have demonstrated that tritium-labeled 17β-estradiol (^{3}H-E_2) can be absorbed via cutaneous and intranasal routes and distributed to reproductive and neural tissues. Radioactivity has also been measured in tissues of untreated females after 48 h cohabitation with ^{3}H-E_2 injected males. The present study was designed to quantify steroid transfer among female bats. Radioactive quantification via liquid scintillation counting revealed absorption of tritium-labeled progesterone (^{3}H-P_4) in adult females 1 h after cutaneous and intranasal application (10 μCi). Subsequently, pairs of mature females were each housed for 48 h with a single mature female that had been administered ^{3}H-P_4 (50 μCi) via intraperitoneal injection. Radioactivity was observed in all collected tissues of all non-injected females at levels significantly greater than the control group. Following the same paradigm, radioactivity was not observed in the tissues of untreated female bats that were housed with stimulus females treated with ^{3}H-E_2 (50 μCi). Enzyme immunoassays revealed measurable levels of unconjugated progesterone and estradiol in the urine of female bats, suggesting urine as a vector for steroid transfer. Given that bats of this species live in predominantly female roosts in very close contact, progesterone transfer among individuals is likely to occur in natural roosts.

1. Introduction

Physiologists have historically assumed that steroid hormones act solely within the individual producing them. However, recent studies in both mice (*Mus musculus*) and bats (*Eptesicus fuscus*) have shown that sex steroids can be excreted by individuals in bioactive form and absorbed by cohabitating conspecifics (Guzzo et al., 2010, 2012, 2013; deCatanzaro et al., 2014). Male excretions have long been implicated in changes of female sexual development and behavior, including novel-male induced disruption of pregnancy (Bruce, 1960), precocious puberty (Vandenbergh, 1967), and alterations of the estrous cycle (Whitten, 1956). When directly administered to inseminated female mice, very low doses of exogenous 17β-estradiol (E_2) can mimic these effects (Bronson, 1975; deCatanzaro et al., 2001, 2006; review by deCatanzaro, 2015). Bioactive sex steroids are reliably present in the urine of male and female mice (deCatanzaro et al., 2004, 2006, 2009; Guzzo et al., 2013). Through the use of radioisotopes, it has been shown that E_2 transfers from males to females at physiologically-relevant levels (Guzzo et al., 2010, 2012, 2013). Male mice that have been housed across a wire-mesh grid from females for a few days progressively show increased concentrations of E_2 in their urine and direct this urine at females (deCatanzaro et al., 2009). Female-to-female transfer of E_2 and progesterone (P_4) has also been observed in cohabiting mice (Guzzo et al., 2013); it has been suggested (deCatanzaro, 2015) that such transfer could explain the fact that group housing can suppress estrous cycling (van der Lee and Boot, 1955, 1956).

Very little research has investigated the hormonal regulation of reproduction in bats and the potential for conspecifics to alter breeding cycles. The big brown bat (*Eptesicus fuscus*) lives in maternal harems distributed throughout Central and North America (Kurta and Baker, 1990). Temperate insectivorous bats possess a unique mating system where copulation occurs mainly during the autumn and also during intermittent arousals throughout hibernation (Oxberry, 1979). Females store sperm, and fertilization occurs alongside ovulation after arousal from hibernation in the spring (Wimsatt, 1944; Christian, 1956; Oxberry, 1979; Racey, 1979). Big brown bats are highly social, displaying promiscuous mating patterns in laboratory settings (Mendonça et al., 1996). When male *E. fuscus* were administered levels of tritium-labeled estradiol (^{3}H-E_2) likely to represent just a small fraction of their endogenous E_2, then housed with female conspecifics during the

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autumn mating season, radioactivity was reliably observed in the females' neural and peripheral tissues (deCatanzaro et al., 2014). The highest levels of transferred E_2 were observed in the uterus and ovaries, where there are high concentrations of estrogen receptors (Kuiper et al., 1997). Given E_2's critical roles in female reproduction, such transfer suggests potential actions as a "pheromone", if this term is simply defined as a chemical excreted by one individual that affects the physiology and/or behavior of conspecifics (deCatanzaro, 2015).

E_2 acts in tandem with P_4 to regulate the female reproductive cycle, with both steroids needed to induce estrous behavior (Freeman, 2006). Both E_2 and P_4 also act to promote sexual development, ovulation, and endometrium preparation in females. These small (E_2, 272 Da; P_4, 314 Da), lipophilic molecules have high chemical stability, allowing them to enter circulation via cutaneous (Goldzieher and Baker, 1960; Hueber et al., 1994; Schaefer et al., 1982; Scheuplein et al., 1969) and intranasal routes (Bawarshi-Nassar et al., 1989; Guzzo et al., 2012). The bioavailability of intranasally-administered P_4 was found in rats to be 100% that of an intravenous (i.v.) dose, whereas the majority of intranasally-administered E_2 was available as bioactive E_2 in circulation, with the precise quantity varying with the given dose (Bawarshi-Nassar et al., 1989). Dermally-applied P_4 is rapidly absorbed in rats, and its distribution and metabolism are comparable to those of P_4 administered i.v. (Waddell and O'Leary, 2002).

Given close contact among female temperate bats during communal roosting, we hypothesized that E_2 and P_4 excreted by female bats could enter the circulatory system and be distributed to the tissues of cohabitating females. Previous findings have shown that sexually mature female bats can absorb exogenous E_2 via intranasal and cutaneous routes (deCatanzaro et al., 2014), and we hypothesized that P_4 would also access female circulation via intranasal and cutaneous routes. We administered a single dose of tritium-labeled-progesterone (^{3}H-P_4) to the nostrils or abdominal skin of female bats and quantified its distribution in tissues. We subsequently hypothesized that cohabitation and contact among roosting female bats could lead to inter-individual transfer of E_2 and P_4. To evaluate this, we injected female bats with ^{3}H-E_2 or ^{3}H-P_4 and housed each of them with two experimental female bats for 48 h. We focused on the ovaries and uterus due to their high concentrations of steroid receptors (Couse et al., 1997; Kuiper et al., 1997). The hypothalamus was investigated as it expresses both E_2 and P_4 receptors (Kato and Onouchi, 1977; Sar and Parikh, 1986; Warembourg et al., 1989; Simerly et al., 1990), and E_2 in the hypothalamus is critical in at least some mammals in eliciting female sexual receptivity (Pfaff, 1980). The liver and kidney were included due to their involvement in the conjugation and excretion of steroids. Other peripheral and neural tissues were also included for comparison.

As urine is a likely vector for inter-individual steroid transfer, we also undertook to quantify the presence of P_4 and E_2 in the urine of female bats. Previous studies have used blood samples to measure steroid concentrations in *Myotis lucifigus* (Buchanan and YoungLai, 1986, 1988), *Antrozous pallidus* (Oxberry, 1979), and *Eptesicus fuscus* (Mendonça, 1996), but to the best of our knowledge urinary steroid concentrations have not previously been assessed in the order Chiroptera.

2. Materials and methods

2.1. Animals and housing

Wild *E. fuscus* were caught in southern Ontario and housed in a facility that permitted animals to fly (Faure et al., 2009). Temperature and lighting varied with ambient conditions. Bats selected for experimental use were placed in stainless steel wire mesh holding cages measuring 28 × 22 × 18 (height) cm. All animals had access to mealworms (*Tenebrio molitor*) and water *ad libitum* unless otherwise stated. All experiments except Experiment 4 occurred during the species' autumn mating season. Procedures were approved by the Animal Research Ethics Board of McMaster University, conforming to guidelines of the Canadian Council on Animal Care.

2.2. Chemicals

SOLVABLE solubilization cocktail, Ultima Gold scintillation cocktail, [2,4,6,7-[^{3}H](N)]-E_2 (dissolved in ethanol, 1.0 μCi/μl, 81.0 Ci/mmol), and 2 stock solutions of [1,2,6,7-[^{3}H](N)]-P_4 (dissolved in ethanol, 1.0 μCi/μl, 101.3 and 96.0 Ci/mmol) were obtained from PerkinElmer, Watham, MA, USA. E_2 and P_4 standards were obtained from Sigma-Aldrich, Oakville, ON, Canada. E_2 and P_4 antibodies, and HRP conjugates were obtained from the Department of Population Health and Reproduction at the University of California, Davis, CA, USA.

2.3. Experiment 1: Direct cutaneous exposure of females to ^{3}H-P_4

Experimental procedures closely followed the methods of deCatanzaro et al. (2014). On day 1, female bats (n = 5) were randomly selected from the research colony and housed overnight in a holding cage. On day 2, each female was administered 10 μCi of ^{3}H-P_4 (32.8 ng exposure per bat) to the skin of the abdomen via pipette. Animals were individually housed in a standard polypropylene mouse cage (28 × 16 × 11 cm) with a wire grid lid, without food or water. At 1 h after isolation, animals were anesthetized via isoflurane inhalation and blood was sampled via cardiac puncture. Animals were euthanized by perfusion with 20 ml of phosphate-buffered saline (PBS). Tissue samples were collected and placed in pre-weighed 8 ml scintillation vials. Reproductive tissues included the whole uterus and both ovaries. Neural tissues included samples of the olfactory bulbs, cerebellum, a section of the frontal cortex, and a section of the hypothalamus taken from the ventral surface of the brain. Peripheral tissues sampled included the heart, lung, liver, external intercostal muscle, abdominal adipose tissue, and a cross section of the kidney encompassing both cortex and medulla. Following collection, sample vials were re-weighed and wet tissue mass was recorded.

Tissue samples were solubilized by adding 1 ml of SOLVABLE to each vial. After 10 min of mechanical agitation, samples were placed into a water bath at 50 °C for 2 h. Vials were re-agitated for 10 min, then returned to sit the water bath for 2–3 h until tissues were completely dissolved. Samples were removed and permitted to cool, then 5 ml of Ultima Gold was added to each vial. Radioactivity was measured using a TriCarb 2910 TR Liquid Scintillation Analyzer with a high sensitivity option and continuous monitoring of background radiation that is automatically subtracted from sample measures (PerkinElmer, Waltham, MA). Vials were stored in the darkness chamber of this equipment for 5 min to eliminate residual heat and luminescence. The level of radioactivity from each vial was measured for 5 min, with the final adjusted estimate quantified in disintegrations per minute (DPM) calculated by QuantaSmart software. All measures of radioactivity were adjusted for wet tissue mass and are reported as DPM/mg tissue. Blood samples were centrifuged at 1500*g* for 10 min, after which 10 μl of serum was added to vials containing 5 ml of Ultima Gold. Radioactivity in blood serum was quantified as described above and reported as DPM/μl serum.

2.4. Experiment 2: Direct intranasal exposure of females to ^{3}H-P_4

As in Experiment 1, adult female bats (n = 5) were randomly selected from the colony on day 1 and housed in holding cages overnight. On day 2, animals were intranasally administered 10 μCi of ^{3}H-P_4 (32.8 ng exposure per bat), with approximately 5 μCi injected into each nostril via pipette. All other procedures, including isolation of the bats, anesthesia, perfusion, tissue collection, sample processing, and scintillation counting were identical to those of Experiment 1.

2.5. Experiment 3: Direct exposure of untreated females to females injected with ^{3}H-P_4, ^{3}H-E_2, or ethanol

Transfer of ^{3}H-P_4 and ^{3}H-E_2 from stimulus female bats to cohabiting conspecific females was evaluated and compared to data from control females exposed to stimulus females that received 70% ethanol. On day 1, adult female bats were randomly selected and housed in holding cages. On day 2, females of each condition were randomly divided into groups of three. One female from each group was injected with 50 μCi of ^{3}H-P_4 (155.2 or 163.8 ng exposure), ^{3}H-E_2 (169.2 ng exposure), or ethanol via i.p. injection. All injected animals were isolated for 1 h to prevent accidental transfer from the injection site. Each injected female was then placed in a cage with two subject females, with 4 replicates (n = 8 subject females) for P_4, 3 replicates for E_2 (n = 6 subject females), and 3 replicates for the ethanol control condition (n = 6 subject females). After 48 h of cohabitation, animals were anesthetized, perfused with 20 ml of PBS, and blood and tissue samples were collected using methods described above. Water and food were sampled from all holding cages and measured for radioactivity to ensure that there was no contamination from ^{3}H-steroids. Dry swipes of the dissection table, surgical tools, and other equipment were taken between dissections to ensure that there was no contamination.

2.6. Experiment 4: Quantifying unconjugated steroids in female urine

Urine was collected non-invasively from captive female bats during the non-reproductive season. Animals were selected from the colony and hand-held over a wax-paper-lined work surface. Urine from each animal was collected separately in 70 μl hematocrit tubes (Fisherbrand, Pittsburgh, PA, USA) and kept frozen (−20 °C) until time of analysis.

Urine analysis of unconjugated P_4 and E_2 was completed using modified enzyme immunoassay protocols previously outlined (deCatanzaro et al., 2003, 2004). Approximately 50 urine samples from non-reproductive females of varying ages were pooled to develop standard curves and validate the assay. Serial dilutions of pooled urine were used to obtain optical densities and generate standard curves. A regression line was fit to the data, and samples were interpolated into the equation to obtain an estimate of hormone in pg/well. Data were plotted with a serially-diluted standard for each steroid against logarithmically-transformed doses. This test indicates whether measurable levels of steroid were present in the urine of female bats, and whether the steroid molecules react to the antibodies in a predictable manner (Kemeny, 1991).

2.7. Data analysis

Shapiro-Wilk and Bartlett tests revealed that the data did not meet assumptions for parametric statistics of normality and homogeneity. Therefore, a Kruskal-Wallis H test was performed on each tissue in Experiment 3 to evaluate differences among conditions, followed by Holm-Bonferroni adjustment to keep family-wise α at $p < 0.05$ (Holm, 1979). For tissues showing significance, post hoc multiple comparisons were conducted using the Kruskal-Conover test. Statistical analysis focused on differences between treatments rather than those among tissues because of potential differential impacts of perfusion (Guzzo et al., 2013). Analyses were performed using the R software environment (R Core Team, 2016).

3. Results

3.1. Experiment 1: Direct cutaneous exposure to ^{3}H-P_4

Direct cutaneous exposure of ^{3}H-P_4 resulted in measurable radioactivity 1 h later in all 5 subject bats (Fig. 1). Three subjects displayed radioactivity in all of their sampled tissues and blood serum, whereas one subject had no measurable radioactivity in the olfactory bulb, frontal cortex, and hypothalamus, and another lacked detectable radioactivity in the olfactory bulb and blood serum. Large ranges of radioactivity were observed among subjects in the muscle, 8.3–86.5; adipose, 6.6–613.7; uterus, 19.9–92.5; and ovary, 13.3–90.2 DPM/mg. Measurements from peripheral tissues (heart, lung, muscle, abdominal adipose, uterus, ovary, liver, kidney) were in a completely non-overlapping range from the neural tissues (olfactory bulb, cerebellum, frontal cortex, hypothalamus). This experiment demonstrates that ^{3}H-P_4 can be directly absorbed across the ventral skin surface of female bats.

3.2. Experiment 2: Direct intranasal exposure to ^{3}H-P_4

Direct intranasal administration of ^{3}H-P_4 yielded radioactivity 1 h later in all tissues sampled from all subject bats (Fig. 1). The highest values were observed in the liver, kidney, uterus, abdominal adipose, and ovaries, in that order, and values from these tissues were in completely non-overlapping ranges of all other

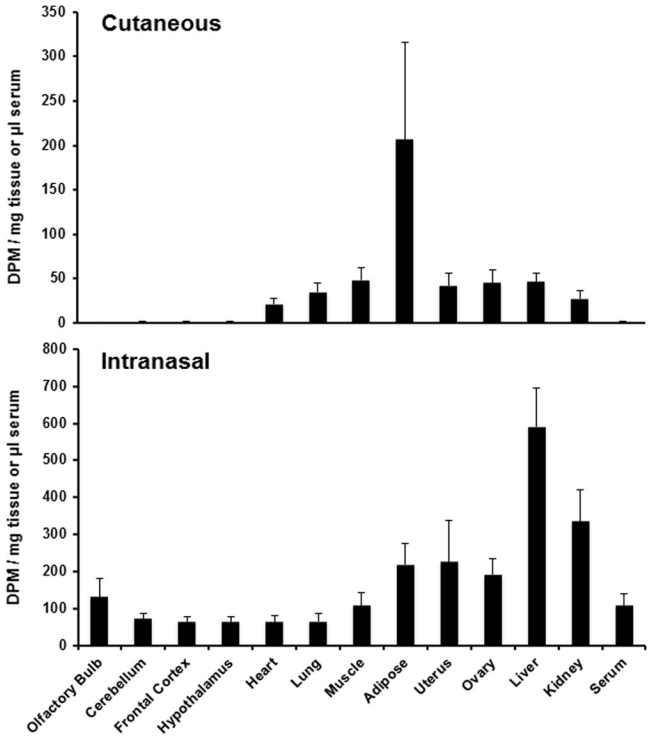

Fig. 1. Radioactivity (mean DPM + SEM) measured in solubilized tissues and blood serum of adult female bats 1 h after direct (*upper panel*) cutaneous administration (n = 5) and (*lower panel*) intranasal administration of (n = 5) of 10 μCi (32.8 ng) ^{3}H-P_4 in Experiments 1 and 2.

samples from the cerebellum, frontal cortex, and hypothalamus. Large ranges of measurements among subjects were observed in the kidney, 125–542; liver, 345–910; and uterus, 82–671 DPM/mg. This experiment demonstrates that ^{3}H-P_4 can be directly absorbed after intranasal administration in female bats.

3.3. Experiment 3: Direct exposure of untreated females to females treated with ^{3}H-P_4, ^{3}H-E_2, or ethanol

The mean radioactivity counts measured in subject female bats following 48 h cohabitation with a ^{3}H-P_4, ^{3}H-E_2, or ethanol-treated female are reported (Fig. 2). A Kruskal-Wallis H test followed by Holm-Bonferroni adjustment produced significant effects of treatment in the olfactory bulbs, $H(2) = 10.2$, $p = 0.024$); cerebellum, $H(2) = 9.9$, $p = 0.024$; frontal cortex, $H(2) = 13.1$, $p = 0.007$; hypothalamus, $H(2) = 17.5$, $p = 0.002$); heart, $H(2) = 16.0$, $p = 0.003$; lung, $H(2) = 11.3$, $p = 0.017$; muscle, $H(2) = 14.9$, $p = 0.005$; adipose, $H(2) = 16.5$, $p = 0.003$; uterus, $H(2) = 14.9$, $p = 0.005$; ovaries, $H(2) = 17.5$, $p = 0.002$; liver, $H(2) = 8.7$, $p = 0.026$; and kidney, $H(2) = 12.0$, $p = 0.015$. There was no significant effect of treatment on serum, $H(2) = 4.2$, $p = 0.123$. Multiple comparisons revealed a significant difference between the P_4 and control groups in all tissues but not in serum, and between P_4 and E_2 in all substrates except the liver and serum (Fig. 2). No differences were found between the E_2 and control groups.

Following 48 h exposure to a ^{3}H-P_4-treated stimulus female, radioactivity was measured in the tissues of all 8 subject females across four replicates. The olfactory bulbs, ovaries, uterus and lung contained the highest mean levels, in that order. A larger range of values was observed in tissues of subject females exposed to ^{3}H-P_4-treated females; olfactory bulbs, 0–4.3; lung, 0–4.1; uterus, 0.3–4.5; and ovaries, 0.3–5.0. Tissues samples from the four stimulus ^{3}H-P_4-treated bats showed mean DPM ± SEM/mg as follows: olfactory bulb, 129 ± 20; cerebellum, 125 ± 19; frontal cortex, 130 ± 17; hypothalamus, 119 ± 14; heart, 89 ± 26; lung, 164 ± 34; muscle, 150 ± 4; adipose, 1703 ± 1025; uterus, 177 ± 15; ovary, 250 ± 37; liver, 453 ± 46; and kidney, 216 ± 27; the value for serum was 224 ± 48 DPM/μl.

All three replicates investigating ^{3}H-E_2 transfer from treated stimulus females to untreated subject females yielded zero or near-zero levels of radioactivity in subject tissues. In one replicate, radioactivity was not measured in any tissue or serum sample of either subject female. In a second replicate, both subject females had low levels of radioactivity measured in two tissue samples (cerebellum 0.06 and heart 0.03; lung 0.06 and muscle 0.57 DPM/mg) but no measurable radioactivity in other tissues including the serum. In a third replicate, radioactivity was measured in 5 sampled tissues (lung, 0.2; adipose, 0.04; uterus, 0.3; liver, 0.6; kidney, 0.5 DPM/mg) of one subject female and in 10 tissues of the other subject female (olfactory bulb, 0.5; cerebellum, 0.4; frontal cortex, 0.6; heart, 0.1; lung, 0.3; muscle, 0.07; uterus, 0.7; liver, 1.0; kidney, 1.1; serum, 1.2 DPM/mg). Tissues samples from the three ^{3}H-E_2-treated females contained the following mean DPM ± SEM/mg: olfactory bulb, 633 ± 115; cerebellum, 591 ± 88; frontal cortex, 604 ± 68; hypothalamus, 575 ± 81; heart, 343 ± 61; lung, 492 ± 81; muscle, 494 ± 37; adipose, 3273 ± 139; uterus, 2865 ± 1359; ovary, 1070 ± 384; liver, 1137 ± 446; and kidney, 781 ± 143; the value for serum was 1245 ± 297 DPM/μl.

All tissues of control females showed zero radioactivity, with the exception of the frontal cortex in one subject (0.125 DPM/mg) and the cerebellum in another (0.035 DPM/mg). Collectively, these results demonstrate that ^{3}H-P_4 consistently transfers from female bats to the tissues of cohabitating females, but that ^{3}H-E_2 does not show consistent transfer among females.

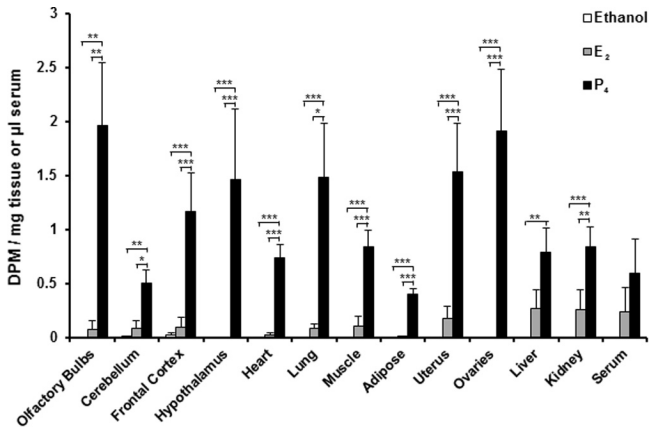

Fig. 2. Radioactivity (mean DPM + SEM) measured in solubilized tissues and blood serum of adult female subject bats after 48 h of cohabitation with either a ^{3}H-P_4, ^{3}H-E_2, or ethanol-treated adult stimulus female in Experiment 3 (n = 8, n = 6, and n = 6 subject females respectively). Asterisks denote significant differences between different treatments within a tissue at: $^*p = 0.05$, $^{**}p = 0.01$, and $^{***}p = 0.001$.

3.4. Experiment 4: Quantifying unconjugated steroids in female urine

Both P_4 and E_2 were present at measurable levels in female urine, as the samples were diluted in parallel with their standard curves. The dose-response curves and parallelisms for both P_4 and E_2 are given for pooled urinary samples (Fig. 3). This experiment demonstrates that P_4 and E_2 are present in the urine of female bats in bioactive, unconjugated form.

4. Discussion

These data demonstrate that female big brown bats can absorb exogenous ^{3}H-P_4 via percutaneous and intranasal routes. They also show that ^{3}H-P_4 can transfer among female conspecifics during 48 h of cohabitation during the mating season. The transfer of ^{3}H-P_4 was replicated in all eight untreated females across four

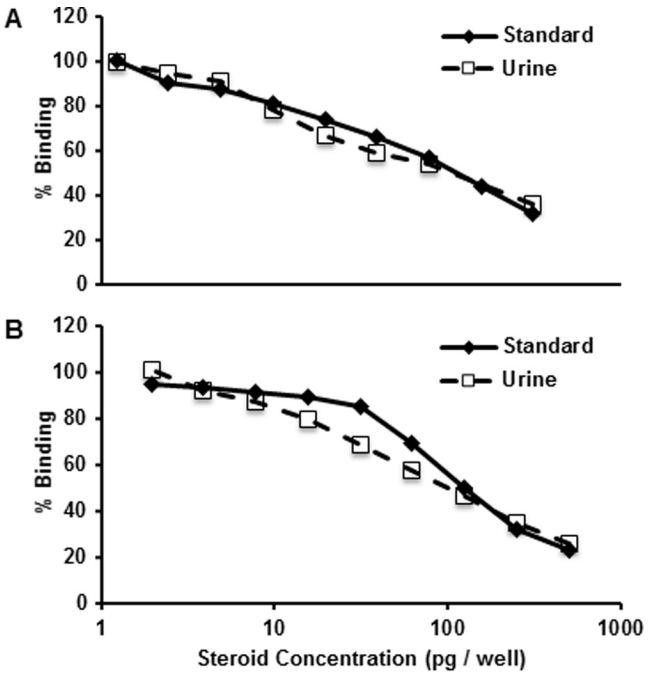

Fig. 3. Serially diluted urinary samples binding to antibody in parallel with progesterone (Panel A) or estradiol (Panel B) serially diluted standards.

replicates in which two untreated females were housed with a ^{3}H-P_4-treated female. The highest levels of radioactivity were found in the ovaries, uteri, lungs, and olfactory bulbs of untreated females; this generally corresponds to P_4 receptor densities in mammalian female tissues (Uotinen et al., 1999). As the lungs have consistently shown high levels of radioactivity in steroid transfer experiments in bats (deCatanzaro et al., 2014), we suggest that nasal exposure to the excretions of conspecifics leads to steroid absorption via the lungs as well as the vasculature of the nasal mucosa.

Steroid transfer among females could have ecological implications for big brown bats. Living in harems, female *E. fuscus* occupy maternal colonies with their offspring, while the males typically stay solo or form separate bachelor colonies (Kurta and Baker, 1990). Maternal colonies mitigate the thermoregulatory costs of reproduction in bats as cool roost temperatures delay the development of prenatal offspring and the occurrence of parturition (Racey and Swift, 1981). The close confines of female conspecifics within the roost provide sufficient conditions for steroid transfer via absorption from urine and other excretions. Grooming and allogrooming could enhance absorption of steroids from conspecific excretions, as temperate bats spend a significant portion of their daily energy meticulously grooming fur and wings (Burnett and August, 1981). In the wild, female bats would potentially be exposed to excretions from hundreds of other females in a similar reproductive state, and the summation of absorbed steroids from multiple conspecifics could reach levels sufficient to influence the reproductive state of any given female bat.

Sharing of P_4 could promote synchronicity of reproduction, blastocyst implantation, and maintenance of pregnancy. A high P_4:E_2 ratio is critical for the success of blastocyst implantation after insemination (deCatanzaro, 2015; Gidley-Baird et al., 1986; Ma et al., 2003). P_4 promotes decidualization (Clarke and Sutherland, 1990; Kurita et al., 2001; Lim et al., 1999; Ma et al., 1998; Taylor et al., 1998; Wetendorf and DeMayo, 2012), and high P_4 levels are necessary to sustain gestation (Allen and Corner, 1930; Csapo and Wiest, 1969; Milligan and Finn, 1997). In contrast, a relative lack of E_2 transfer among females (as observed in Experiment 3) during the *peri*-implantation or gestational period would help maintain the high P_4:E_2 ratio, as exogenous E_2 can prevent blastocyst implantation and disrupt gestation (deCatanzaro et al., 2001, 2006; Gidley-Baird et al., 1986; Ma et al., 2003).

Progesterone is a precursor to androgens and estrogens (Hanukoglu, 1992; Payne and Hales, 2004). Radioactivity was observed in all females exposed to stimulus female given ^{3}H-P_4 in Experiment 3. While much of the transferred radioactivity is likely to represent bioactive P_4, the extent remains to be determined. Cutaneous absorption of P_4 is faster than that of E_2 upon dorsal cutaneous administration in the guinea pig (Goldzieher and Baker, 1960). Waddell and O'Leary (2002) found that cutaneously administered ^{3}H-P_4 was rapidly absorbed into the circulatory system of the rat. ^{3}H-P_4 and water soluble metabolites were measured in the plasma after 15 min and lipid soluble metabolites after 30 min. Water and lipid soluble metabolites were measured in high quantities in rat urine, whereas radioactivity in the organs was attributed mostly to P_4. Specifically, ^{3}H-P_4 accounted for 93% of radioactivity measured in the uterus, 83% in the lung, and 60% in the salivary glands (Waddell and O'Leary, 2002).

We previously demonstrated that female bats readily absorb exogenous E_2 during cohabitation with stimulus males during the mating season (deCatanzaro et al., 2014). In contrast, E_2 transfer among female conspecifics was absent or negligible when measured during the same season in the current study, with means of 0–0.27 DPM/mg depending on the tissue. Only one of the three replicates showed radioactivity just slightly above background levels in the two untreated subject females. In females paired with male stimulus bats previously (deCatanzaro et al., 2014), radioactivity was observed in all replicates and mean levels ranged from 0.5–5 DPM/mg depending on the tissue. One possible reason for greater and more reliable male-to-female E_2 transfer is that E_2 can come from seminal fluids during mating. Saksena et al. (1978) found that E_2 levels in the seminal vesicle fluid of male rats increased after a subcutaneous dose of exogenous E_2. The quantity of estrogens present in semen is less than P_4, however much of the P_4 is in the form of metabolites (Cooper and Waites, 1975). A recent study (deCatanzaro and Pollock, 2016) demonstrated substantial radioactivity in the reproductive and other tissues of female mice immediately following mating with a ^{3}H-E_2 injected male. The presence of estrogens and androgens in semen has also been observed in diverse mammals including rodents, lagomorphs, ungulates, and primates (Claus et al., 1983; Eiler and Graves, 1977; Reiffsteck et al., 1982; Saksena et al., 1977; Waites and Einer-Jensen, 1974). Although steroid levels in the seminal plasma of male bats have not yet been studied, exceptionally high levels of radioactivity were measured in the epididymides of male *E. fuscus* after they received ^{3}H-E_2 injections (deCatanzaro et al., 2014).

To the best of our knowledge, we have also presented the first evidence that bioactive unconjugated E_2 and P_4 are present in the urine of non-pregnant female bats, as shown in the parallelisms of Fig. 3. The presence of natural E_2 and P_4 suggests that urine may act as a vector for steroid transmission in bats. In addition to potential importance for pheromonal activity, urinary measurement could allow experimenters to quantify steroids reliably in bats via non-invasive methods in the laboratory and field.

These data concur with data showing transfer of ^{3}H-P_4 among adult female mice (Guzzo et al., 2013). Transfer of ^{3}H-E_2 from males to cohabiting females has been clearly demonstrated in mice (Guzzo et al., 2012, 2013) and bats (deCatanzaro et al., 2014). Given the long evolutionary separation of bats and rodents (Murphy et al., 2004), the similarity of the data in mice and bats suggests that sex steroid transfer between conspecifics may occur in many mammalian species.

Acknowledgments

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