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SCIENCE DIRECT
Hormones and Behavior 50 (2006) 285–292
Hormones and Behavior
www.elsevier.com/locate/yhbeh

Masculinized otoacoustic emissions in female spotted hyenas (*Crocuta crocuta*)

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Received 16 September 2005; revised 22 March 2006; accepted 23 March 2006
Available online 6 May 2006

Abstract

In humans and rhesus monkeys, click-evoked otoacoustic emissions (CEOAEs) are stronger in females than in males, and there is considerable circumstantial evidence that this sex difference is attributable to the greater exposure to androgens prenatally in males. Because female spotted hyenas are highly androgenized beginning early in prenatal development, we expected an absence of sexual dimorphism in the CEOAEs of this species. The CEOAEs obtained from 9 male and 7 female spotted hyenas confirmed that expectation. The implication is that the marked androgenization to which female spotted hyenas are exposed masculinizes the cochlear mechanism responsible for CEOAEs. The CEOAEs measured in 3 male and 3 female hyenas that had been treated with anti-androgenic agents during prenatal development were stronger than the CEOAEs of the untreated animals, in accord with the implied inverse relationship between prenatal androgen exposure and the strength of the cochlear mechanisms producing CEOAEs. The CEOAEs of three ovariectomized females and two castrated males were essentially the same as those for the untreated females and males, suggesting that there is little or no activational effect of hormones on CEOAE strength in spotted hyenas. Distortion product OAEs (DPOAEs) also were measured. Those sex differences also were generally small (as they are in humans), and the effects of the anti-androgen agents were inconsistent. Thus, prenatal androgen exposure apparently does affect OAEs, but the effects appear to be greater for the reflection-based cochlear mechanism that underlies CEOAEs than for the nonlinear cochlear mechanism underlying DPOAEs.

Keywords: Spotted hyena; Otoacoustic emissions; Sex differences; Prenatal development; Masculinization; Androgenization; Anti-androgen drugs; Flutamide; CEOAE; DPOAE

The mammalian cochlea produces sounds known as otoacoustic emissions or OAEs (Kemp, 1978; Probst et al., 1991). In humans and rhesus monkeys, these emissions are sexually dimorphic, with females having stronger OAEs than males (see below for details). There is circumstantial evidence linking this sex difference to the actions of androgens secreted by the fetal testes of male mammals (McFadden, 1998, 2002). Described here are measurements of OAEs obtained from spotted hyenas (*Crocuta crocuta*), a species in which females are exposed to substantial concentrations of androgens in utero (Licht et al., 1992; Yalcinkaya et al., 1993). If it is correct that exposure to prenatal androgens reduces the strength of OAEs, then one might anticipate the sexual dimorphism observed in the OAEs of humans and rhesus monkeys to be reduced or eliminated in spotted hyenas.

The OAEs produced by the cochlea propagate back through the middle-ear system into the external ear canal where they can be measured using small microphone systems (e.g., Kemp, 1978; Probst et al., 1991). Only three of the several known forms of OAEs will be of interest here. Click-evoked OAEs (CEOAEs) are echo-like sounds that are produced by the cochlea in response to the presentation of brief acoustic stimuli (for more details, see McFadden et al., 2006). Spontaneous OAEs (SOAEs) are weak tonal sounds that are continuously emitted by the majority of normal-hearing human ears without

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0018-506X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.yhbeh.2006.03.013

the need for eliciting stimuli from the experimenter. Distortion product OAEs (DPOAEs) are tonal signals created by certain nonlinear mechanisms of the cochlea during the simultaneous presentation of two primary tones close in frequency (see Probst et al., 1991). The DPOAE of interest here appears at the frequency $2f_{lower}–f_{higher}$ (a frequency below the two primaries). DPOAEs are believed to be produced by a nonlinear cochlear mechanism that is fundamentally different from the linear, reflection-based mechanism that is responsible for CEOAEs and SOAEs (Shera and Guinan, 1999, 2003).

In humans, CEOAEs are stronger (McFadden et al., 1996; McFadden and Pasanen, 1998; McFadden and Shubel, 2003) and SOAEs are more numerous (Bilger et al., 1990; Talmadge et al., 1993; McFadden, 1993b; McFadden and Loehlin, 1995; McFadden and Pasanen, 1999; McFadden and Shubel, 2003) in females than in males, and these sex differences exist in newborns as well as in adults (Strickland et al., 1985; Burns et al., 1992; Morlet et al., 1995, 1996). In humans (Gaskill and Brown, 1990; Lonsbury-Martin et al., 1991; Moulin et al., 1993; Cacace et al., 1996; Dhar et al., 1998; Bowman et al., 2000) and in rhesus monkeys (Torre and Fowler, 2000; McFadden et al., 2006), the sex difference in DPOAEs appears to be much smaller than those for CEOAEs and SOAEs. A parsimonious interpretation of these facts is that the linear cochlear mechanism responsible for producing CEOAEs and SOAEs can be permanently weakened by exposure to high levels of androgens during prenatal development (McFadden, 1998, 2002)–an organizational effect of androgen exposure–and that the nonlinear mechanism responsible for DPOAEs is less susceptible to the prenatal effects of androgens.[1]

In a companion paper, we report that rhesus monkeys exhibit a substantial sex difference in CEOAEs and little or no sex difference in DPOAEs (McFadden et al., 2006), just as in humans (Gaskill and Brown, 1990; Lonsbury-Martin et al., 1991; Moulin et al., 1993; Cacace et al., 1996; Dhar et al., 1998; Bowman et al., 2000). This suggests that the cochlear mechanisms underlying OAEs in these two species may share some important similarities developmentally, a suggestion that currently is difficult to evaluate because so little is known about sex differences in OAEs in other species.

Female spotted hyenas are the only extant mammals that display a pseudoscrotum instead of an external vagina. They also have a hypertrophied penile clitoris, through which they urinate, mate, and give birth (Matthews, 1939). Current belief suggests that such "masculinization" of the external genitalia requires the action of androgens during fetal life (Jost, 1970). Additional findings supporting the hypothesis that female spotted hyenas have been "androgenized" in utero include: female spotted hyenas are somewhat larger than males and totally dominate adult immigrant males within clans in nature (Kruuk, 1972); the traditional sex differences in Onuf's nucleus in the spinal cord (Forger et al., 1996) and in a sexually dimorphic nucleus in the hypothalamus (Fenstemaker et al., 1999) are attenuated in spotted hyenas.

The ovaries of the pregnant spotted hyena secrete substantial quantities of androstenedione (Lindeque et al., 1986; Glickman et al., 1987), which is converted to testosterone and estradiol by the placenta and transferred to the developing fetus (Licht et al., 1992; Yalcinkaya et al., 1993). Although the essential development of masculine external genitalia in female (and male) spotted hyenas may be an androgen-*independent* phenomenon (Drea et al., 1999; Glickman et al., 2005), in utero treatment with anti-androgens does produce profound effects on genital morphology (Drea et al., 1998), reproduction (Drea et al., 2002), and endocrine function (Place et al., 2002) in both females and males (also see Glickman et al., 2005).

Given the implication that some OAEs appear to be affected by androgenic mechanisms operating early in development and the apparent blunting of certain androgen-dependent sexual dimorphisms in spotted hyenas, we predicted that the sexual dimorphism in OAEs, previously observed in humans and rhesus monkeys, would be attenuated, or eliminated, in *C. crocuta*. Furthermore, because the colony at the University of California at Berkeley contained some spotted hyenas that were treated with anti-androgens in utero and some that were gonadectomized after birth, we hoped to make a preliminary assessment of the organizational and activational effects of gonadal steroids on the cochleas and OAEs in this species. Because DPOAEs exhibit only small sex differences in humans (Gaskill and Brown, 1990; Lonsbury-Martin et al., 1991; Moulin et al., 1993; Cacace et al., 1996; Dhar et al., 1998; Bowman et al., 2000) and rhesus monkeys (Torre and Fowler, 2000; McFadden et al., 2006), we had little reason to expect a sex difference or a treatment effect on the DPOAEs of spotted hyenas.

Methods

Animals

Measurements were made on a total of 14 male and 13 female spotted hyenas. Of these, 9 males and 7 females were untreated; the others were treated as detailed below. These animals all were born and raised at the Field Station for the Study of Behavior, Ecology, and Reproduction at UC Berkeley, and all were housed singly or in small groups in outdoor enclosures. At the time of testing, all were adults, aged 4 to 16 years. The oldest animals, two 16-year-old females, were in the ovariectomized group. The mean ages (±SDs) of the untreated males and untreated females were 7.2 (±3.7) and 6.9 (±3.6) years, respectively, and all of the anti-androgen-treated animals were 9 years old, save for one 8-year-old female. Sexual maturity is typically achieved at about age 2 in males and about age 2.5–3 in females. In the wild, spotted hyenas typically live until age 15–20; in captivity, until age 25–30.

While pregnant, the mothers of 6 of the 27 animals were administered flutamide (two males) or flutamide plus finasteride (one male and three females). Flutamide blocks the androgen receptor, and finasteride blocks the enzymatic conversion of testosterone to 5α-dihydrotestosterone (DHT). Flutamide was administered daily during the final 75–89 days of pregnancy (the average gestation period is about 110 days), and finasteride was administered daily during the final 48–72 days (details of these administrations can be found in Place et al., 2002, Table 1.

[1] The reason for emphasizing prenatal androgen exposure is that, at birth and from about age 6 months until puberty, the circulating hormone levels in children are the same in both sexes. Beginning at birth, human males do exhibit a "second surge" of testosterone production that lasts about 6 months (Smail et al., 1981). Presumably, additional masculinization and defeminization is accomplished during this second surge, but details are still unknown. Male spotted hyenas also have substantially higher concentrations of testosterone than females during the immediate neonatal period. However, female spotted hyenas have high concentrations of androstenedione at birth, and that continues to be true for the first months of life while the male concentration of testosterone is declining (Frank et al., 1991; Glickman et al., 1992).