Influence of pinealectomy on serum estrogen and progesterone levels in blind-anosmic female rats[1]

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Summary. Previous studies show that the suppression of gonadal function in blind-anosmic rats is dependent on the pineal gland. The present results demonstrate that in young female rats both the pineal gland and dual sensory deprivation have additional independent antigonadal effects.

The blind-anosmic (BA) model has been used previously to demonstrate the anti-reproductive role of the pineal gland in rats[2-4]. Blind-anosmic female rats may exhibit depressed plasma levels of luteinizing hormone while pituitary stores of this hormone are elevated[2]. The previously described reproductive effects of dual sensory deprivation are almost totally reversed by pinealectomy, indicating that the pineal gland mediates these effects. However, serum gonadal steroid levels have not yet been reported in the blind-anosmic female rat.

Materials and methods. Female rats from ARS Sprague-Dawley, Madison, Wisconsin, were housed 3 per clear plastic cage in controlled lighting (14 hL:10 hD; lights on at 06.00 h) with food and water available ad libitum. At the age of 23-24 days animals were divided into 4 groups and subjected to the following surgical procedures: sham-pinealectomy (ShPx, n=25); pinealectomy (Px, n=24); bilateral optic enucleation for blinding and olfactory bulbectomy for anosmia (BA, n=33); and blinding, olfactory bulbectomy and pinealectomy (BAPx, n=32). Pinealectomy[5] and olfactory bulbectomy[3] were performed by previously described techniques. All operations were performed using ether anesthesia.

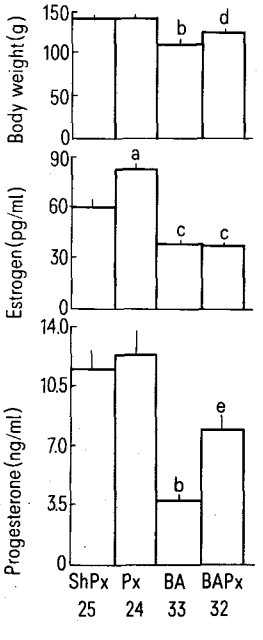
At age 50 days all animals were weighed and approximately 1 ml blood was drawn from the jugular vein under ether anesthesia between 08.30 and 13.00 h. Serum estrogen (estrone plus estradiol)[6] and progesterone[7] concentrations were measured by radioimmunoassay. The large number of animals in each group was used to avoid any possible effects of sampling in different stages of the estrous cycle. Estrous cycles were not recorded. Blood sampling was also performed alternately among the various groups to avoid any possible effect of diurnal cyclicity of hormone levels. A 1-way analysis of variance followed by Student's two-tailed t-test for several means was employed to test for statistical significance.

Results. All data are shown in the figure. Although BA animals exhibited depressed body weights compared to ShPx controls ($p < 0.001$), Px partially prevented this effect ($p < 0.001$; BAPx vs BA). Px also resulted in enhanced serum estrogen levels ($p < 0.002$; Px vs ShPx). BA depressed serum estrogen levels ($p < 0.02$; BA vs ShPx), an effect not prevented by Px. Serum progesterone concentrations were unaffected by Px alone but were significantly depressed by BA ($p < 0.001$; BA vs ShPx). Simultaneous Px partially prevented the effect of BA on serum progesterone levels ($p < 0.01$; BAPx vs BA, vs Px, and vs ShPx).

Discussion. The present results demonstrate depressed levels of circulating gonadal steroids in young female rats following BA. This is consistent with the effect of BA on uterine weights reported previously[2-4]. In addition to their small size, ovaries of female BA rats contain few vesicular follicles and corpora lutea; removal of the pineal gland from BA animals has been shown to restore ovarian size and histology to normal[3]. The literature suggests that the effect of the BA procedure is mostly, if not totally, due to the presence of an intact pineal gland[2-4]. The serum progesterone results conform to the expected pattern, in that BA depressed circulating progesterone levels while Px partially prevented this depression. However, since removal of the pineal gland resulted in increased serum estrogen concentrations, we believe estrogen levels are controlled to some degree by the pineal, even without the BA challenge.

One possible explanation for the inability of Px to prevent the effects of BA on serum estrogen levels is the length of time (4 weeks) allowed between surgery and blood sampling, since 8 weeks has often been allowed in reports of organ weight changes[2-4]. This is unlikely, because Reiter has shown that as early as 24 days following surgery, BA female rats exhibited depressed ovarian and uterine weights, whereas BAPx females at the same time point did not[4].

Another explanation for depressed estrogen levels in the BAPx group may be that BA suppresses estrogen levels via a pathway separate from the pineal gland. This possibility is strengthened, because a portion (about 50%) of the BA-induced progesterone suppression was not pineal-dependent (figure). Progesterone levels were lower in the BAPx than in the Px group ($p < 0.01$). On the other hand, the presence of BA was required for Px to influence progesterone (but not estrogen) levels. In other words, the pineal may influence estrogen levels without the presence of BA; and BA can influence levels of estrogen, and to a lesser extent progesterone, in the absence of the pineal gland. The extra-pineal effect of BA is not yet understood, but may be a result of the anosmia component. This possibility is suggested by the observation that pinealectomy completely



Body weight and plasma levels of gonadal hormones in sham-pinealectomized (ShPx), pinealectomized (Px), blind-anosmic (BA), and blind-anosmic-pinealectomized (BAPx) female rats. *a*, $p < 0.002$ vs ShPx; *b*, $p < 0.001$ vs ShPx; *c*, $p < 0.02$ vs ShPx; *d*, $p < 0.001$ vs BA; *e*, $p < 0.01$ vs BA.

reverses the anti-reproductive effect of blindness in hamsters, while in the rat anosmia magnifies the otherwise small inhibitory effect of blindness[8].
Regardless of the nature of the blinding-anosmia interaction which facilitates the response of the rat gonad to the pineal, it is apparent that both the pineal gland and dual sensory deprivation may have independent effects on the reproductive system of young female rats.

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2 D.E. Blask and R.J. Reiter, Neuroendocrinology *17*, 362 (1975).
3 R.J. Reiter, S. Sorrentino and E.M. Ellison, Gen. comp. Endocr. *15*, 326 (1970).
4 R.J. Reiter, Endocr. Exp. *6*, 3 (1972).
5 R.A. Hoffman and R.J. Reiter, Anat. Rec. *153*, 19 (1965).
6 C. Wu, L.E. Lundy and S.G. Lee, Am. J. Obstet. Gynecol. *115*, 169 (1973).
7 S. Furuyama and C.A. Nugent, Steroids *17*, 663 (1971).
8 R.J. Reiter, in: Handbook of Physiology, Endocrinology IV; part 2, p.519. Ed. E. Knobil and W.H. Sawyer. American Physiological Society, Washington 1974.

Presence of ecdysone and ecdysterone in the tick *Amblyomma hebraeum* Koch

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Summary. Ecdysone and ecdysterone, the moulting hormones of insects and crustaceans, are also present in nymphs of the tick *Amblyomma hebraeum*. They were demonstrated by means of radioimmunoassay and of gas-liquid chromatography combined with mass fragmentography or mass spectrometry of their trimethylsilyl-derivatives.

The occurrence of ecdysteroids as moulting hormones (MH) in insects and crustaceans is now well established[2,3]. In contrast, reports on the chemistry of MH in other groups of the phylum arthropoda are very scarce. The MH of *Limulus polyphemus* (Xiphosura) behaved like ecdysone, ecdysterone and inokosterone in thin layer chromatography (TLC)[4]. In nymphs of the spider *Pisaura mirabilis*, ecdysterone was detected by TLC and radioimmunoassay (RIA)[5]. However, these techniques do not allow precise chemical identification.
The few reports on ecdysteroids in ticks (Acarina, Ixodoidea) deal only with the physiological effects of exogenous hormones. Ecdysteroids ingested with the blood meal induce supermoulting in adults[6,7]. Applied topically, they break larval diapause[8,9]. Injected into the hemocoel, they inhibit oogenesis[10]. We report here the presence of ecdysone and ecdysterone in nymphs of the tick *Amblyomma hebraeum* Koch.
Materials and methods. The nymphs were fed on cattle. After dropping from the host, they were kept at 27 °C and 70% relative humidity. Moulting to adults occurred about 28 days after dropping. In order to determine the chemical nature of ecdysteroids, 2 samples (62 and 110 g each) of nymphs at day 17 after dropping from the host were homogenized in methanol-water (3:2). After centrifugation, the residue was extracted 3 times with 60% methanol or methanol. The combined supernatants were purified by precipitation at −18 °C followed by centrifugation[13], and then taken to dryness. The residue was reextracted with methanol. 60 ml of isoamyl-acetate was added and the methanol evaporated. The extracts were submitted to chromatography on a small column of silicic acid which was first eluted with 20 ml of isoamylacetate followed by 20 ml of methylethylketone. The ecdysteroids were eluted with 40 ml of methanol. The eluates were concentrated and subjected to TLC on precoated plates (MERCK, silica gel F_{254}, thickness: 0,5 mm). Delipidation was achieved by an initial development in diisopropylether. The ecdysteroids were then separated with chloroform-methanol (7:3). Bands of 1 cm width were scraped off the plates and the substances eluted with methanol and ethylacetate. After

Relative abundance of characteristic ions detected by MF in a purified extract of *Amblyomma*, at retention time of fully silylated derivative prepared from authentic ecdysone (Simes, Italy). Intensity is given as percent of the main peak, m/e 171, which is characteristic of the side chain. Ions at m/e 567, 582 and 636 are the most abundant ions characterizing ecdysone nucleus and OTMS group on C-22

m/e	171	567	582	636
Biological compound	100	10.7	2.1	6.0
Standard compound	100	13.1	2.2	6.2

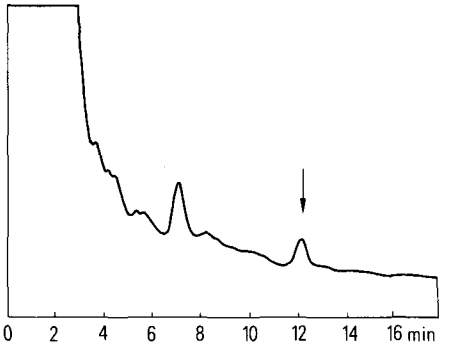

Fig. 1. Chromatogram (total ionization current of the mass spectrometer) of a purified extract of *Amblyomma*. A prominent peak has the same retention time (arrow) as the fully silylated derivative prepared from authentic ecdysterone (Simes, Italy). Unidentified peak at about 7 min retention time does not appear to be an ecdysteroid. Chromatographic conditions: LKB 9000 GLC-MS apparatus; column: 2 m×2 mm OV.1 (1%) on Gas Chrom P. Temperatures: column 280 °C; flash heater and separator 295 °C. Flow rate of carrier gas (helium): 30 ml/min.