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Plasma aldosterone in newborn and adult *Vipera aspis*

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Summary. In the venomous viviparous snake *Vipera aspis*, plasma aldosterone concentration shows significant seasonal changes mainly related to temperature-dependent behavior. A difference is also present between newborns and adults. A correlation between sodium and potassium status and aldosterone plasma level in active and inactive life is suggested.
Key words. Plasma aldosterone; seasonal changes; reptiles; venomous snakes.

The highly sensitive radioimmunological methods now available have made it possible to measure aldosterone levels in circulating blood in spite of its very low concentration. Plasma aldosterone levels have been estimated in small reptiles like *Uromastix acanthinurus* and *Tiliqua rugosa*[1] as well as bigger animals like tortoises[2]. Venomous snakes are a very poorly known group among reptiles in spite of their world wide distribution; plasma aldosterone has been measured only in the sea snake *Hydrophys cyanocinctus*[3].

In the present investigation plasma aldosterone was determined by radioimmunoassay in the viviparous venomous snake *Vipera aspis* during active life in temperate climate (*Vipera aspis* breeds in June and in September), during inactive life and in the young immediately after birth.

Material and methods. Adult male and female *Vipera aspis* ranging in length from 60 to 75 cm were captured from May to September (active life) and from November to January (inactive life) in Liguria (North Italy). The animals were housed in the laboratory for a short period (not exceeding 24 h) at room temperature in summer, and at 4 °C in winter to maintain their torpid state. In September one of them gave birth to 9 young snakes about 10 cm long which were immediately killed. All the animals were killed by decapitation, starting at approximately 11.00 h, and blood was collected directly into lithium-heparinized tubes. After centrifugation at 4 °C (700 × g) separated plasma was analyzed for aldosterone and electrolytes. Na and K were determined by ion-selective electrodes (Electrolyte II Beckman). Plasma aldosterone concentration was determined using a RIA kit (Aldoctk-125-M CEA SORIN, Italy): bound and free hormones were separated using antibody-coated tubes. Competitive cross reactions with other steroids were 6.4×10^{-3}% for corticosterone, 5.5×10^{-3}% for desoxycorticosterone and less than 8×10^{-4}% for testosterone and other steroids (calculated after Abraham et al.[4]). To each tube containing 200 µl of sample 700 µl of 8-anilino-naphtalene sulphonic acid, sodium salt was added, dissolved in 6 ml distilled water. Radioactivity was measured in a Gamma Counter (Beckman 5500). Serial dilutions of unlabeled aldosterone were treated in the same way to obtain a standard curve. Intra-assay variability was 7.8% and inter-assay variability was 12.4%. Sensitivity was 10.5 ± 2.2 pg/ml.

Results. During active life in the adult *Vipera aspis* aldosterone plasma level was 93.3 ± 2.5 ng/100 ml and Na and K concentrations were not significantly different (168 ± 5.2 and 4.06 ± 0.05 mmol/l, respectively). Aldosterone concentration was significantly lower in the newborn snakes than in the adults (56.0 ± 4.6 ng/100 ml). In winter, during inactive life, Na and K plasma levels were significantly lower (161.0 ± 3.9 and 3.4 ± 0.5 mmol/l, respectively). Aldosterone dropped to a very low level (22.5 ± 3.5 ng/100 ml). The data are summarized in the table.

Discussion. The aldosterone concentration found in *Vipera aspis* during active life is approximately in agreement with the plasma levels measured by RIA in *Uromastix acanthinurus* and in *Tiliqua rugosa* (36.04 ± 4.7 and 31.74 ± 5.6 ng/100 ml, respectively) by Bradshaw et al.[1]. The same authors underline the variability which may exist between different reptilian species. However in *Vipera aspis* plasma aldosterone level is considerably lower than that measured in the snake *Hydrophys cyanocinctus* (1270 ± 340 ng/100 ml)[3]. This difference observed in the two species of venomous snakes may be correlated with the animal's behavior in different ecological situations. In *Hydrophys cyanocinctus* aldosterone plasma concentration does not fluctuate according to the salinity of the environment, and this sea snake is considered to be an osmoconforming euryhaline reptile. In *Vipera aspis* plasma aldosterone concentration is low at birth an increases after birth in correlation with the effort exerted by the animals to maintain their ion-water balance in the aerial environment. The fall of aldosterone level

Electrolytes and aldosterone levels in adult and newborn *Vipera aspis*. Seasonal changes. Values are means ± SD

	n	Na mmol/l	K mmol/l	Aldosterone ng/100 ml
Active life: (from May to Sept.)	15	175.1 ± 2.9	4.05 ± 0.3	93.3 ± 2.5
Inactive life: (from Nov. to Jan.)	9	161.0 ± 3.9*	3.4 ± 0.5**	22.5 ± 3.5*
Newborns	9	168.0 ± 5.2	4.06 ± 0.4	56.0 ± 4.6*

*$p < 0.01$ versus values in adults during active life; **$p < 0.05$ versus values in adults during active life.

from active to inactive life is in agreement with the clearly defined seasonal cyclic changes in the adrenocortical tissue observed in other snakes by histological and histochemical methods; in the winter phase only little signs of activity are shown[5]. Besides, in winter the lowered temperature induces a torpid state in *Vipera aspis* with a complete absence of food uptake. Consequently the potassium level in the extracellular fluids decreases. At least in mammals studies in vivo suggest that following potassium deficiency aldosterone secretion is decreased and after potassium loading it is increased[6-8]. The low plasma potassium seen in inactive adult snakes could therefore be a cause of the low plasma aldosterone and the low plasma sodium concentration could be a consequence of the low aldosterone level.

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Maturation of the pineal melatonin rhythm in long- and short-day reared Djungarian hamsters[1]

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Summary. Male Djungarian hamsters, reared under long (16L/8D) or short (10L/14D) days, were sacrificed at various ages during the day or night, or at night following a 30-min light pulse. The pineal melatonin rhythm matured similarly under long and short days by 20 days of age. The results are discussed in context of the hypothesis that melatonin mediates the photoperiod effects which forestall puberty in short-day reared hamsters.
Key words. Hamster, Djungarian; melatonin rhythm, pineal; long-day rearing; short-day rearing.

The pineal gland is known to mediate the photoperiodic regulation of adult reproductive function in a variety of seasonal breeding mammals[4-6]. The putative pineal hormone, melatonin, appears to be involved in the mediation of these photoperiod influences. Increased pineal melatonin production is restricted to and appears proportional to the hours of darkness[7]. Moreover, timed melatonin treatments in pinealectomized hamsters have been employed to mimic the inductive and suppressive effects of both long and short days on reproductive function[8-10]. In the Djungarian hamster, the mature adult rhythm in pineal melatonin content is characterized by low daytime and high nighttime concentrations[4,11]; exposure to light suppresses nocturnal production of melatonin and its rate-limiting enzyme, N-acetyl transferase, to low daytime values[12,13]. Previous studies have implicated the daily melatonin rhythm in the photoperiodic mechanism controlling sexual maturation in the Djungarian hamster[5,14]. In hamsters raised under long days, a day/night rhythm in pineal melatonin content develops by the end of the 2nd week of life[12]. By 3 weeks of age, the mechanism which allows light to suppress the nocturnal increase in pineal melatonin is also functional. However, in contrast with the normal reproductive development that occurs under long days, exposure to short days from soon after birth forestalls gonadal maturation within 20 days of age. Discrimination of long from short days may be hypothesized to depend upon the presence of a mature melatonin rhythm. The paucity of information on short-day reared hamsters led to the present study which determined when maturation of the pineal melatonin rhythm occurred in Djungarian hamsters reared under long or short photoperiods, respectively.

Materials and methods. Djungarian hamsters (*Phodopus sungorus*) were born in a long-day breeding colony (16L:8D, light on 02.00–18.00 h; original stock provided by Dr Klaus Hoffmann, Max-Planck-Institut für Verhaltensphysiologie, Erlings-Andechs, FRG). Litters were either maintained under long days or transferred to short days (10L:14D, lights on 05.00–15.00 h) on the day of birth. Weaning occurred at 18 days of age and males were group housed (4–5 per cage) for later use. Food and water were available ad libitum with a once a week sunflower seed supplement as previously described[15]. All nighttime sacrifices were performed under dim red illumination which does not interfere with pineal production of melatonin[15]. Males were sacrificed at various ages (fig.) by decapitation during the day (2–4 h before lights off) or night (5 h after lights off) or at night immediately following exposure to a 30-min pulse of light (daytime fluorescent lights, about 500 lux, 4:5–5 h after lights off). In addition, two groups of 12 adults were born and reared in under either long a short days and sacrificed in the same protocol at 60–80 days of age. After decapitation, pineal glands were rapidly removed, stored frozen ($-60\,^{\circ}C$) in individual vials and then shipped frozen to Dr Tamarkin (NIH, NICHHD, Bethesda, MD) for radioimmunoassay of melatonin[16,17]. The limit of assay sensitivity was 2 pg/tube; intra- and interassay variation was 2% and 10%, respectively. Data was analyzed by a two-way ANOVA followed by selected t-tests comparing age versus photoperiod treatments. Significant differences between comparisons were noted at $p < 0.05$.

Results and discussion. At 60–80 days of age a mature pineal melatonin rhythm was present (fig., lower panel); high nighttime levels were suppressed to low daytime concentrations by a brief exposure to light. Differences among the three photoperiod treatments (day, night and night + 30 min light) were statistically significant. A similar melatonin pattern occurred in 20-day-old males; however, the nocturnal increase 5 h after lights off was significantly less in both long and short days as compared with adult hamsters. This result suggests that the pineal melatonin rhythm had matured within three weeks of birth. This confirms previous results for long-day reared hamsters[12]. Amplitude differences in pineal melatonin content between 20- and 60–80-day-old hamsters are difficult to assess with only a single nighttime sample. In long-day reared Syrian