Splenic thymidine kinase and DNA during early postnatal development of the androgenized female rat[1]

M. Litteria and C. G. Popoff

Neuroendocrine Research Laboratory (151B), Veterans Administration Medical Center, North Chicago (Illinois 60064, USA),
24 May 1984

Summary. Administration of a pharmacologic dose of testosterone propionate to neonatal female rats had no effect on either thymidine kinase activity or DNA synthesis in the spleen during the first 3 postnatal weeks.
Key words. Spleen; thymidine kinase; DNA synthesis; testosterone propionate; neonatal androgenization.

Incorporation of preformed thymidine into DNA (the salvage pathway) occurs via a sequence of phosphorylations through thymidine mono-, di- and triphosphate. The activity of thymidine kinase (EC 2.7.1.75), the initial and rate-limiting enzyme converting thymidine to the monophosphate, is specifically related to DNA synthesis during the S phase of the cell cycle[2,3]. The enzyme is characteristically elevated in a variety of proliferating tissues and is especially high in the spleen of the neonatal rat[3–5]. The high activity of splenic thymidine kinase has been correlated with the transitory and predominantly erythropoietic function of this organ during early postnatal development[4,5]. Testosterone and other androgens stimulate erythropoiesis[6–9]. This action may be mediated by direct and/or indirect effects of these steroids on the spleen[6–9]. Our study describes for the first time the effects of neonatally administered testosterone propionate (TP) on splenic thymidine kinase activity and DNA content during early postnatal development of the female rat. The pharmacologic amount of TP used in this study is followed postpuberally by the well-known persistent estrus syndrome which is characterized by anovulation and masculinization of sexual behavior[10]. This dosage also induced alterations in thymidine kinase activity and DNA synthesis in the developing cerebellum during the first 3 postnatal weeks[11].

Materials and methods. Litters bred from Sprague-Dawley rats were culled to 8 pups after delivery. 48 h after birth female littermates, paired according to body weights, were injected s.c. with either 1.5 mg testosterone propionate (TP) dissolved in sesame oil or an equivalent volume of vehicle[11]. Rats were decapitated at the ages indicated in the table and crude homogenates (5% w/v) of spleen samples were prepared in ice-cold medium consisting of 0.25 M sucrose, 0.004 M $MgCl_2$, and 0.02 M Tris-HCl buffer, pH 7.4[12]. Samples (0.05 ml) were removed for the extraction and analysis of DNA[13]. The remaining crude homogenates were centrifuged at 105,000 g for 60 min at 2 °C and the resultant supernatant served as the enzyme preparation. Protein content was analyzed in 0.01-ml samples of supernatant[14]. The reaction mixture for thymidine kinase contained in a total volume of 0.25 ml: 0.05 M Tris-HCl buffer (pH 8.0), 5 mM ATP, 2.5 mM $MgCl_2$, 40 µM methyl-[^{3}H]thymidine (specific activity 2 Ci/mmol, New England Nuclear), and 0.02 ml enzyme supernatant (0.060–0.069 mg protein). Enzyme activity was linear during a 10-min incubation at 37 °C and the rate of reaction was proportional to the protein concentration. Specific activity of thymidine kinase, i.e., pmol [^{3}H]thymidine phosphorylated per min per mg protein, was determined according to previously detailed methodology[11,15]. Significance of differences between control and TP-treated littermates was determined by the two-tailed Student's t-test for paired data; $p \leqq 0.05$ was considered significant.

Results and discussion. Neonatally administered TP had no significant effects on the parameters measured in the table. Similarly, there were no differences in the protein content of the enzyme supernatants between groups of rats at any age. Body weights, spleen weights and total DNA content per spleen progressively increased with age. The specific activity of thymidine kinase for both control and TP-treated littermates reached a maximum and plateau at ages 6 through 9 days and then declined to half this activity by 11 days. The developmental pattern for splenic thymidine kinase is in good agreement with that reported by other workers[4]. These data demonstrate that administration of a pharmacologic dose of TP to neonatal female rats does not directly or indirectly induce changes in either DNA synthesis or enzyme activity during the first 3 postnatal weeks. Thus, if TP influences erythropoiesis and/or other hematopoietic functions in the neonatal spleen the effects of this steroid do not appear to be mediated by changes in thymidine kinase activity or DNA synthesis.

Effects of testosterone propionate (TP) on female rats

Age (days)		Body weight (g)	Spleen Wet weight (mg)	Total DNA (µg)	Specific activity of thymidine kinase*
3 (8)	C	8.49 ± 0.40	25.95 ± 1.38	454.40 ± 33.26	1156.58 ± 104.31
	T	8.29 ± 0.41	24.42 ± 1.84	415.66 ± 41.98	1183.12 ± 114.23
6 (10)	C	15.18 ± 0.30	93.12 ± 4.99	1868.09 ± 111.63	2300.62 ± 96.23
	T	15.13 ± 0.37	84.43 ± 2.64	1638.26 ± 87.96	2361.62 ± 123.97
9 (8)	C	19.59 ± 0.81	118.45 ± 4.69	2710.10 ± 115.95	2276.15 ± 142.44
	T	19.71 ± 0.65	123.60 ± 7.42	2742.48 ± 187.89	2435.12 ± 102.10
10 (12)	C	23.38 ± 0.94	120.73 ± 5.49	2565.55 ± 133.13	1491.75 ± 71.45
	T	23.13 ± 0.60	117.92 ± 6.43	2357.82 ± 236.78	1602.99 ± 69.42
11 (12)	C	24.88 ± 0.55	140.10 ± 4.62	2955.13 ± 105.21	1125.29 ± 89.75
	T	24.70 ± 0.62	138.60 ± 3.95	2991.56 ± 102.90	1238.28 ± 72.35
15 (12)	C	37.73 ± 0.60	182.99 ± 7.02	3871.59 ± 140.94	800.59 ± 51.44
	T	37.52 ± 0.58	174.46 ± 7.15	3721.30 ± 148.52	874.87 ± 42.28
22 (10)	C	54.85 ± 1.30	315.08 ± 13.15	6899.56 ± 260.53	1094.52 ± 33.91
	T	53.04 ± 1.54	304.32 ± 15.34	6461.00 ± 277.18	1145.36 ± 42.87

Each value is the mean ± SE for the number of determinations indicated in parentheses for control (C) and TP-treated (T) rats. *Specific activity of thymidine kinase = pmol [^{3}H] thymidine phosphorylated/min/mg protein.

1 Acknowledgments. We wish to thank Dr M. Breen and H.G. Weinstein, Research-in-Aging Laboratory, for their critical review of the manuscript, and L. Garbett for typing the manuscript. This study was supported by the Veterans Administration.
2 Baserga, R., N. Engl. J. Med. *304* (1981) 453.
3 Roux, J.M., Enzyme *15* (1973) 361.
4 Machovich, R., and Greengard, O., Biochim. biophys. Acta *286* (1972) 375.
5 Greengard, O., and Machovich, R., Biochim. biophys. Acta *286* (1972) 382.
6 Byron, J.W., Blood *40* (1972) 198.
7 Miranda, C., and Scaro, J.L., Revta esp. Fisiol. *34* (1978) 173.
8 Peschle, C., Magli, M.C., Cillo, C., Lettieri, F., Pizzella, F., Genovesa, A., and Soricelli, A., Life Sci. *21* (1977) 773.
9 Besa, E.C., and Bullock, L.P., Endocrinology *109* (1981) 1983.
10 Barraclough, C.A., Recent Prog. Hormone Res. *22* (1966) 503.
11 Litteria, M., Brain Res. *181* (1980) 401.
12 Yamagami, S., Mori, K., and Kawakita, Y., J. Neurochem. *19* (1972) 369.
13 Burton, K., Biochem. J. *62* (1956) 315.
14 Lowry, O.H., Rosebrough, M.J., Farr, A.L., and Randall, R.J., J. biol. Chem. *193* (1951) 265.
15 Litteria, M., and Popoff, C.G., Neurochem. Res. *6* (1981) 931.

0014-4754/85/050649-02$1.50 + 0.20/0

Plasma aldosterone in newborn and adult *Vipera aspis*

B. Uva, M. Vallarino and G. Isola

Istituto di Anatomia Comparata, Università di Genova, via Balbi 5, I-16126 Genova (Italy), 30 May 1984

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.